ISSN 1124-0490 Volume 36 N. 2 - 2019 Pages 91-174
http://www.sarcoidosis.it

SARCOIDOSIS

VASCULITIS AND DIFFUSE LUNG DISEASES

OFFICIAL JOURNAL OF WASOG

Mattioli 1885 for: AIPO - SIMER

SARCOIDOSIS

VASCULITIS AND DIFFUSE LUNG DISEASES

FORMERLY "SARCOIDOSIS" (UP TO 1995) FOUNDED 1984 BY GIANFRANCO RIZZATO

PROPERTY AND COPYRIGHT

AIPO - Associazione Italiana Pneumologi Ospedalieri
Via Antonio da Recanate, 2
20124 Milano
Tel. +39 02 36590367
Fax +39 02 67382337
www.aiponet.it
aiposegreteria@aiporicerche.it

S.I.Me.R. - Società Italiana di Medicina Respiratoria
Via Privata A. Antonelli, 3
20139 Milano
Tel. +39 02 87387209
Fax +39 02 87387219
www.simernet.it
segreteria@simernet.eu

PUBLISHER
Mattioli 1885 - Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (Parma)
Tel. +39 0524 530383 - Fax +39 0524 82537 - www.mattioli1885.com - edit@mattioli1885.com

Bibliographic Indices:
This journal is regularly listed in bibliographic services, including Current Contents/Clinical Medicine, the Science Citation Index, Sci Search, Research Alert and EMBASE/Excerpta Medica (Priority Journals)

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 92-107

Lung transplantation for pulmonary sarcoidosis

Keith C. Meyer
Department of Medicine, Section of Pulmonary and Critical Care Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, United States

Abstract. Although relatively few patients with pulmonary sarcoidosis develop advanced disease that progresses to respiratory insufficiency despite receiving best practice pharmacologic interventions, lung transplantation may be the only therapeutic option for such patients to both prolong survival and provide improved quality of life. Lung transplant can be successfully performed for patients with end-stage pulmonary sarcoidosis, and post-transplant survival is similar to that for other transplant indications such as idiopathic pulmonary fibrosis. However, appropriate timing of referral, comprehensive assessment of potential candidates for lung transplant, placement of patients on the lung transplant waiting list when within the transplant window as appropriate, choosing the best procedure (bilateral versus single lung transplant), and optimal peri-operative and post-transplant management are key to successful lung transplant outcomes for patients with sarcoidosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 92-107)*

Key words: lung transplantation, sarcoidosis, interstitial lung disease, pulmonary fibrosis

Introduction

Sarcoidosis is a granulomatous, multi-system disease characterized by a wide variety of clinical presentations and phenotypes (1-4). While sarcoidosis has a tendency to spontaneously remit, its clinical course is highly variable. Although up to 95% of patients with sarcoidosis develop some form of lung disease over the course of their lives, only approximately one-third of patients develop chronic or progressive disease. Advanced lung disease in sarcoidosis can be characterized by extensive fibrosis, vascular remodeling with pulmonary hypertension, cyst formation, airway involvement with loss of patency/stricture or dilatation due to bronchiectasis, or combinations thereof (1-3, 5). However, only approximately 5% of patients diagnosed with sarcoidosis will develop advanced lung disease due to pulmonary fibrosis (5). The majority of these patients, however, eventually succumb to respiratory complications of chronic pulmonary sarcoidosis, although some patients can remain clinically stable for long periods of time (5, 6).

Lung transplantation is a treatment option that can improve quality of life and prolong survival for patients with advanced lung disease refractory to other therapeutic interventions (7). Indeed, end-stage sarcoidosis with severe fibrocystic lung disease and/or the presence of World Health Association Group 5 pulmonary hypertension (PH) remains a difficult-to-treat form of advanced lung disease for which lung transplantation may be the only intervention that can improve survival and quality of life. Although the total number of lung transplants reported to the International Society for Lung Transplantation (ISHLT) for patients with sarcoidosis is

Received: 6 March 2018
Accepted after revision: 4 February 2019
Correspondence: Keith C. Meyer, MD, MS, FCCP, FACP
Department of Medicine, Section of Allergy,
Pulmonary and Critical Care Medicine
University of Wisconsin School of Medicine and Public Health
Madison, Wisconsin, United States
Tel. 608 263-6363 (office); 608 263-3035 (secretary)
Fax: 608 263-3104
E-mail: kcm@medicine.wisc.edu

relatively low (approximately 2.5% of all transplants performed from 1995 through 2014) (8), actuarial post-transplant survival has been reported to be comparable to that for patients with other forms of pulmonary fibrosis (9-11), and median survival according to recent ISHLT data is 6.1 years following primary transplantation (8). An examination of United Network for Organ Sharing (UNOS) data for patients listed for transplant from 1995 through 2000 showed that waitlisted patients with sarcoidosis had a mortality rate that was similar to the high risk of mortality observed for patients diagnosed with idiopathic pulmonary fibrosis (IPF) (12). Additionally, Shorr et al. (13) reported that African Americans appeared to face a significantly increased risk of death (odds ratio of 2.5) while waitlisted, even when the data were adjusted for potential confounding factors. Determining the right time for referral and transplantation of sarcoidosis patients with advanced lung disease presents a considerable challenge.

Respiratory tract manifestations and complications of chronic sarcoidosis

Many risk factors that are associated with worse outcomes in patients with sarcoidosis have been identified (Table 1). Additionally, advanced pulmonary sarcoidosis has many manifestations and characteristics, and patients can develop a variety of complications (Table 2). Imaging of advanced pulmonary sarcoidosis with high-resolution computed tomography (HRCT) reveals a variety of patterns with extensive adenopathy, parenchymal fibrosis, and/or airway disease (Figure 1). While no specific risk factors for the development of advanced pulmonary sarcoidosis have been identified, a number of factors have been linked to increased risk of developing progressive and/or chronic disease (6), and some of these individuals will go on to develop advanced disease despite non-transplant therapeutic interventions. These risk factors include involvement of multiple organ systems, higher Scadding stage at diagnosis or progressing to higher Scadding radiographic stage, need for systemic therapy, lack of lymphadenopathy, female gender, older age, and black race. Nonetheless, the clinical course of sarcoidosis is highly variable, and many patients can remain stable despite symptomatic and/or persistent disease, even in the absence of chronic pharmacologic therapies.

Table 1. Risk factors for worse outcomes in sarcoidosis

- Scadding Stage III/IV disease
- Pulmonary hypertension
- Involvement of >3 organ systems
- Myocardial disease
- Older age & age of disease onset >40 years
- African American race
- Presence of neurosarcoidosis
- Chronic renal dysfunction (e.g. nephrocalcinosis)
- Chronic hypercalcemia
- Upper airway mucosal involvement (e.g. lupus pernio)
- Splenomegaly
- Skeletal involvement (e.g. extensive cystic bone lesions)
- Progressive and/or sustained respiratory symptoms
- Supplemental oxygen requirement (especially if increasing)

Table 2. Manifestations and complications of pulmonary sarcoidosis

Parenchymal disease
- Extensive/confluent granulomatous disease (e.g. alveolar sarcoidosis)
- Pulmonary fibrosis
  - can be extensive & progressive
  - most common cause of respiratory failure
  - can mimic usual interstitial pneumonia (UIP) pattern
- Bullous emphysema

Airway involvement
- Airflow obstruction (may display reactive component with partial reversibility)
- Bronchiectasis
- Bronchial stenosis (can be extensive and severe)

Vascular disease & pulmonary hypertension
- Capillary obliteration due to parenchymal fibrosis
- Plexiform arteriopathy
- Vascular compression by enlarged lymph nodes
- Cardiac dysfunction due to cardiac sarcoidosis
- Granulomatous angiitis (arterial or venous)

Pulmonary infection
- Aspergilloma
- Bacterial infections linked to bronchiectasis

Hemoptysis

Pleural disease
- Pleural thickening
- Pleural effusion (rare)
- Pneumothorax (rare)

Proximal airway disease

Sarcoidosis can involve the nasal passages, paranasal sinuses, mouth, larynx, trachea, or bronchi (14). Severe stenosis of the trachea or cartilaginous bronchi may occur, but this is estimated to occur in less

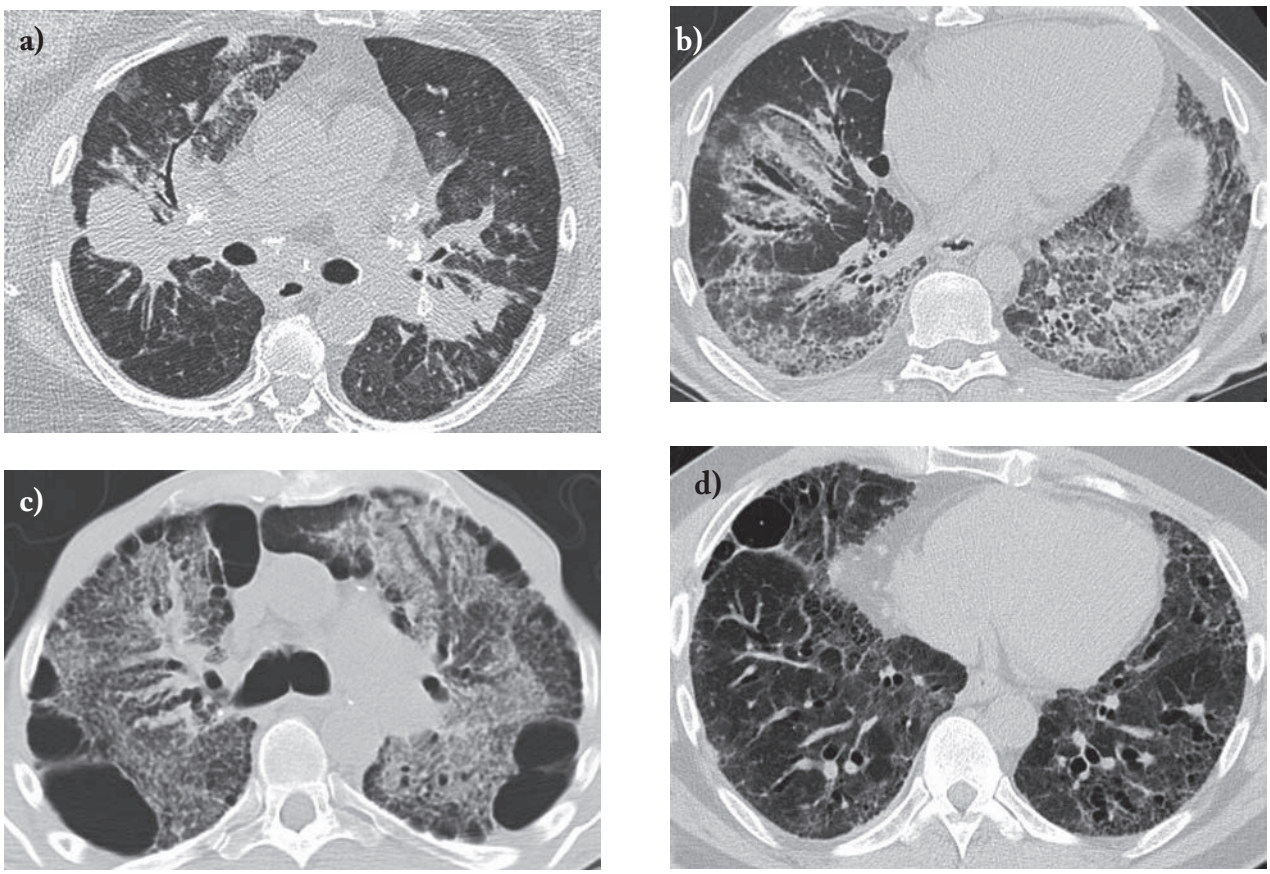


Fig. 1 a-d. Transverse high-resolution computed tomography images of potential lung transplant candidates with advanced pulmonary sarcoidosis and severe lung function impairment. a) Long-standing sarcoidosis with bulky adenopathy, diffuse granulomatous infiltrates, and mild sarcoidosis-associated pulmonary hypertension (SAPH); b) Scadding Stage 4 sarcoidosis with extensive fibrosis and traction bronchiectasis, initially interpreted as a usual interstitial pneumonia pattern; explanted lung showed diffuse granulomata typical of sarcoidosis plus extensive fibrosis; c) Stage 4 sarcoidosis with extensive diffuse fibrosis, peripheral bleb formation, and bronchiectasis; d) Stage 4 sarcoidosis with extensive bronchiectasis but without evidence of chronic infection.

than one percent of patients (15). Although patients with extensive airway narrowing may be quite symptomatic, they usually do not have enough functional impairment to qualify them for lung transplant candidacy.

Intrathoracic lymphadenopathy

Intrathoracic lymphadenopathy is observed in approximately 80% of patients during the course of their illness. Hilar adenopathy is bilateral in most cases, although unilateral hilar adenopathy can be seen in up to 5% of patients (16). Lymph node calcification can be seen at the time of diagnosis, and the likelihood of calcification increases later during the course of the disease (17).

Large nodules and alveolar consolidation

Nodules that follow a perilymphatic distribution and predominate in the mid to upper lung zones are seen in approximately 90% of patients (18). Sarcoid nodules can occasionally aggregate and form larger pulmonary nodules (up to 3 cm diameter) or large masses. Large nodules can remain stable for long periods of time, show partial or complete regression, or cavitate. Massive consolidation may occur with coalescent interstitial granulomas compressing alveoli (19).

Pulmonary fibrosis

Approximately 5% of patients have evidence of fibrotic changes on routine chest radiography at

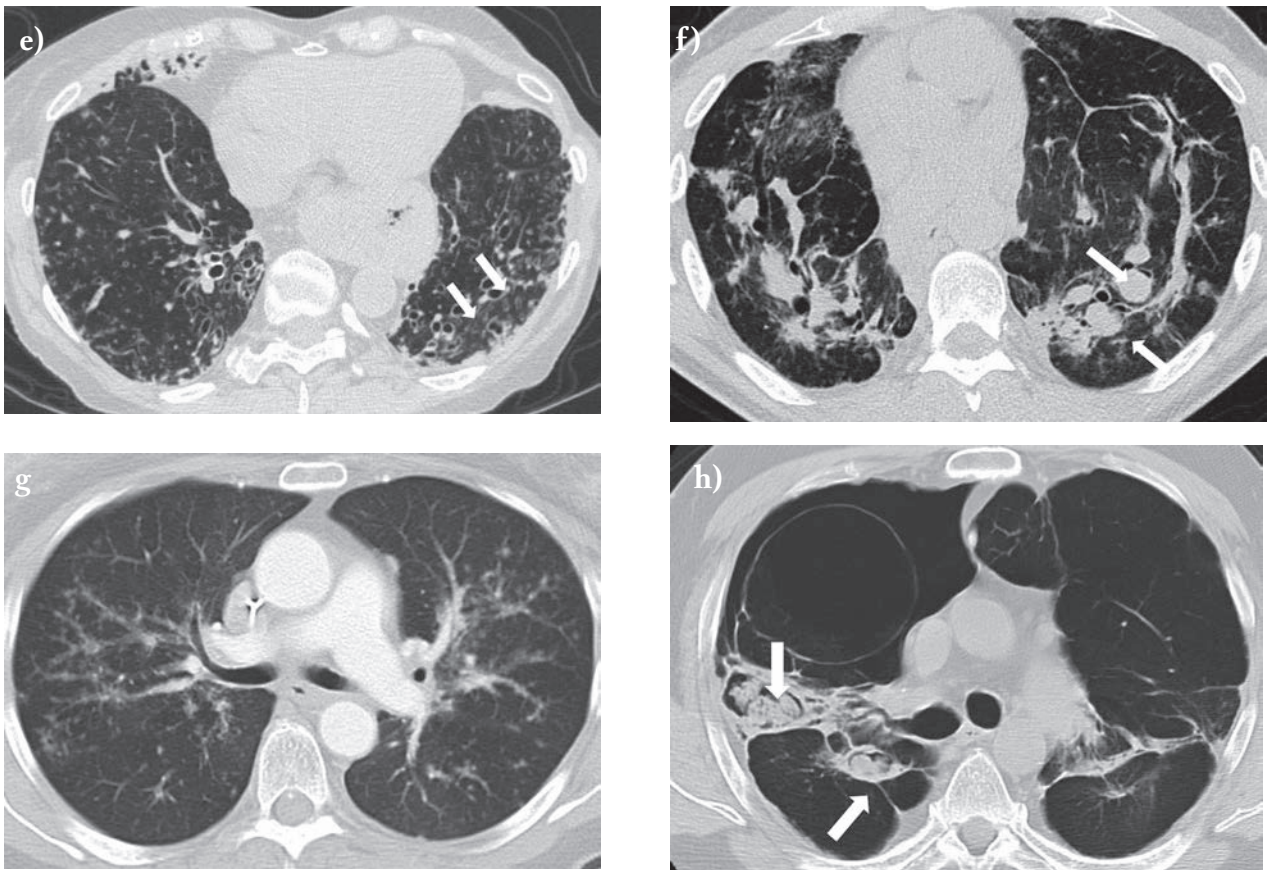


Fig. 1 e-h. Transverse high-resolution computed tomography images of potential lung transplant candidates with advanced pulmonary sarcoidosis and severe lung function impairment. e) Stage 4 sarcoidosis with extensive bronchiectasis (arrows) and chronic bacterial infection with P. aeruginosa; f) Stage 4 sarcoidosis with parenchymal fibrosis and severe SAPH with diffuse bilateral pulmonary artery aneurysm formation (arrows); g) Stage 3 sarcoidosis with severe SAPH; h) Stage 4 fibrobullous sarcoidosis with multiple aspergillomas (arrows), SAPH, and recurrent hemoptysis.

presentation (5, 20). Fibrosis tends to predominate in the upper and mid lung regions, and conglomerate masses that surround and encompass vessels and bronchi with associated bronchial distortion is seen in over half of patients with fibrotic pulmonary sarcoidosis (21). Advanced fibrotic sarcoidosis is characterized by the presence of fibrotic cysts, bullae, traction bronchiectasis, and paracicatricial emphysema, and cystic abnormalities are commonly seen in the upper lobes (22). Honeycomb change can be seen, and honeycomb-like cysts are usually found in an upper lobe distribution, but lower lobe honeycomb change that mimics a usual interstitial pneumonia (UIP) pattern that is typical of idiopathic pulmonary fibrosis (IPF) can also be seen (20, 23).

Pulmonary hypertension

The estimated prevalence of PH in sarcoidosis ranges from one to 28% (defined as mean pulmonary arterial pressure [mPAP] ≥25 mm Hg) at rest and as high as 43% if measured during exercise (24-28). Most patients with sarcoidosis-associated PH (SAPH) have radiographic changes of advanced disease (Scadding stages III or IV), although extensive parenchymal abnormalities are not always present (24-26). One case series reported that 60% of patients with SAPH lacked evidence of significant fibrosis on chest radiography (27), and the extent of parenchymal lung involvement may not correlate with the degree of PH as reflected by right heart catheterization measurements. Up to 75% of patients who are

listed for lung transplantation meet criteria for SAPH when subjected to right heart catheterization, and its presence is associated with a poor prognosis (26,29). Shorr and colleagues examined a cohort of 363 patients listed for lung transplantation for sarcoidosis and found that 66% had mPAP ≥25 mm Hg and 36% had mPAP≥40 mm Hg (26). Furthermore, nearly 70% of patients with mPAP ≥40 mm Hg needed at least some if not total assistance with functional activities, and patients with severe PH had a nearly 7-fold increase in need for supplemental oxygen.

Bronchiectasis

Sarcoidosis-related bronchiectasis is usually diffuse but can occasionally be localized. Bronchiectasis in patients with Scadding stage 4 disease has been reported to range from 18-40% on high-resolution computed tomographic (HRCT) scanning, and bronchiectasis is present in nearly 100% of patients listed for lung transplantation (21, 30, 31). Diffuse cystic bronchiectasis is perceived as being caused from either traction due to surrounding parenchymal fibrosis or direct airway damage caused by granulomatous inflammation, whereas localized bronchiectasis can be post-obstructive, caused by external compression by enlarged lymph nodes, or due to persistent endobronchial sarcoidosis (32). Suppurative bronchiectasis with recurrent infectious exacerbations can be seen in some patients (30).

Pleural disease

Granulomas can infiltrate both visceral and parietal pleura, and pleural involvement plus lymphatic channel compromise can cause pleural effusions to form. However, pleural effusion is an unusual finding in sarcoidosis and may be caused by comorbidities such as pneumonia or congestive heart failure (33). Chylothorax has also been described in sarcoidosis but is an exceedingly rare complication (34). Finally, spontaneous pneumothorax has been reported and attributed to rupture of subpleural blebs, especially when advanced fibrocystic disease is present (35).

Other complications

Aspergillus species are ubiquitous in the environment and can be commonly found in the both the oral and lung mycobiomes of normal humans (36). Both aspergillomas and other aspergillosis syndromes have been reported in patients with sarcoidosis. Mycetoma formation, which usually occurs in pre-existing cysts that are colonized by fungi (usually *Aspergillus* spp), occurs in approximately 2-5 percent of patients with sarcoidosis, and life-threatening pulmonary hemorrhage can occur (37, 38). Mycetoma formation does not have a predilection for right or left lung, but they occur most commonly in the upper lobes and can be multiple. No specific consensus recommendations currently exist for management of aspergillomas in patients with sarcoidosis. While anecdotal reports of poor outcomes in lung transplant recipients when pre-transplant mycetomas have been published, successful lung transplantation has been reported with a combination of careful native lung explantation and post-operative antifungal pharmacologic therapy (39).

Acute exacerbations of pulmonary sarcoidosis are not uncommon, but the definition of an acute exacerbation (AE) and information regarding diagnostic criteria and management are sparse. Panselinas and Judson (40) have proposed the combination of (1) worsened pulmonary symptoms in patients with known sarcoidosis that cannot be explained by alternative causes, (2) a ≥10% decline in forced expiratory volume in one second (FEV1) and/or forced vital capacity (FVC), and (3) the presence of symptoms for at least one month as diagnostic criteria for an episode of an AE of pulmonary sarcoidosis. Risk factors for AE include tapering corticosteroid therapy, administration of interferon-alpha, initiation of antiretroviral therapy, and treatment with tumor necrosis factor-alpha (TNF-α) antagonists (40).

Pharmacologic management of pulmonary sarcoidosis

Although pulmonary disease is the most common manifestation of sarcoidosis, not all patients with pulmonary disease will require drug therapy. Major indications for treating pulmonary sarcoidosis include cough, dyspnea, declining lung function, or radiologic evidence of worsening lung disease, and it is estimated that about half of patients in the US with pulmonary disease receive systemic therapy (38). Additionally, systemic therapy may be required

for significant involvement of other organ systems even though pulmonary disease appears to be stable. Asymptomatic lung disease accompanied by stable lung function does not require therapy. If indicated, pharmacologic therapies can range from inhaled corticosteroids and/or non-steroidal anti-inflammatory drugs for minimal symptoms with stable lung function to systemic corticosteroids, anti-malarial drugs, cytotoxic drugs, biologic agents, or combinations of such for significantly symptomatic disease and/or progressive decline in lung function (41-44). However, whether the use of systemic corticosteroids or other agents such as TNF-α inhibitors can prevent the development or halt the progression of pulmonary fibrosis remains debatable (45,46).

Patients who report persistent dyspnea despite therapy and have normal left ventricular function have an estimated prevalence of PH that approximates 53% (47), and patients listed for lung transplant have an even higher incidence of PH at approximately 74% (26). Although most forms of PH associated with underlying parenchymal lung disease are classified as WHO group 3 PH, SAPH is categorized as WHO group 5 due to its complex and multifactorial pathogenesis, and there can be substantial dissociation between the magnitude of physiologic measures of restriction as a surrogate marker for parenchymal disease burden and the presence and severity of SAPH. Such discordance is likely due to the multifactorial nature of circulatory impairment in SAPH, which can be due to various combinations of distal capillary bed destruction due to fibrotic parenchymal remodeling combined with areas of hypoxemic vasoconstriction, direct involvement of vessels by granulomatous inflammation, and increased vasoreactivity that may respond to vasodilators such as nitric oxide or prostacyclin, upregulation of vasoactive cytokines such as endothelin-1, or mechanical extrinsic compression of pulmonary vessels by bulky intrathoracic adenopathy (28). Because of the multifactorial nature of SAPH, some patients may show a significant response to interventions such as supplemental oxygen, treatment of obstructive sleep apnea if present, treatment of cardiac dysfunction, identification and treatment of thromboembolic disease, or immunosuppressive therapies targeting active sarcoidosis. The administration of vasoactive agents that show efficacy for WHO Group 1 PH remains controversial, but responses have been reported for pharmacologic therapies that target the endothelin pathway (endothelin receptor antagonists such as bosentan), the nitric oxide pathway (selective phosphodiesterase inhibitors), or prostacyclin pathway inhibitors such as epoprostenol (28). However, such therapies, while having potential benefit for some patients, may also cause harm by worsening ventilation-perfusion mismatching and hypoxemia, and such pharmacologic intervention should only be considered on a case-by-case basis by experienced referral center clinicians (and preferably in the setting of a randomized clinical trial) (28). Additionally, vasoactive drugs for targeted treatment of SAPH should probably be avoided for patients with mPAP values <40 mm Hg.

Evaluation and listing for lung transplantation

Progressive pulmonary fibrosis, SAPH, and recurrent/chronic respiratory infection are leading causes of respiratory failure and mortality in patients with advanced pulmonary sarcoidosis (6, 48). Independent predictors of mortality that were identified in long-term follow-up (≥8 years) after adjustment for various confounders were older age, extensive fibrosis on HRCT scanning, and the presence of PH (49). However, quantitative models that can predict clinical behavior of disease and mortality are lacking (50). Therefore, decisions concerning timing of a referral to a transplant center are generally made via case-by-case assessments of patients with advanced lung disease.

A series of consensus documents created by task forces working under the auspices of the ISHLT have provided guidance for decisions regarding referral and evaluation of patients with various forms of advanced lung disease with the most recent published in 2015 (51). While these recommendations have not necessarily been validated, they are widely followed and provide a very useful roadmap for referral, evaluation, bridging to transplant, and transplantation. All potential candidates must lack absolute contraindications to lung transplantation (Table 3), and relative contraindications must be carefully weighed on a case-by-case basis. The majority of patients with advanced pulmonary disease due to sarcoidosis fall into the category of interstitial lung disease (ILD),

Table 3. Contraindications to lung transplantation

Absolute
- Recent history of malignancy (2-year disease-free interval if low risk of recurrence; 5-year interval for higher risk; risk be remain too high for some cancers beyond 5 years)
- Severely limited functional capacity with poor rehabilitation potential
- Significant dysfunction of other major organ systems (unless multiple combined organ transplantation is feasible)
- Acute medical instability (e.g. acute myocardial infarction, sepsis, hepatic failure)
- Uncorrectable bleeding diathesis
- Significant, uncorrected atherosclerotic disease with suspected/confirmed dysfunction (or ischemia or significant coronary artery disease that cannot be revascularized)
- Chronic infection with highly virulent and/or antibiotic-resistant microbes with poor control pre-transplant
- Active infection with *Mycobacterium tuberculosis*
- Chest wall or spinal deformity that would cause severe ventilator restriction post-transplant
- Body mass index (BMI) ≥35 kg/m^2 (Class II/III obesity)
- Non-adherence to recommended medical therapies
- Psychiatric/psychologic conditions causing inability to cooperate with health care team interactions and/or adherence to complex medical therapies
- Lack of an adequate and/or reliable social support system
- Substance abuse or dependence (must demonstrate meaningful/persistent risk reduction behaviors and verified abstinence from substances of concern (e.g. tobacco, alcohol, marijuana, or other illicit substances))

Relative
- Age >65 years if other relative contraindications are present of physiologic reserve is significantly impaired
- Malnutrition if progressive or severe
- Osteoporosis if severe, symptomatic
- BMI 30.0-34.9 kg/m^2 (Class I obesity, especially if truncal/central obesity)
- Extensive prior thoracic surgery (e.g. lung resection)
- Receiving mechanical ventilation
- Receiving extracorporeal life support
- Hepatitis B and/or C infection
- Human immunodeficiency virus infection
- Colonization/infection with highly virulent and/or antibiotic-resistant bacteria or fungi
- Significant atherosclerotic disease burden
- Other significant medical conditions (e.g. diabetes mellitus, systemic hypertension, gastroesophageal reflux) that have not caused advanced organ system damage (especially if not optimally treated/controlled)

for which guidance for timing of referral and timing of placing on the transplant waitlist are provided (Table 4). However, some patients with sarcoidosis may have predominantly vascular involvement but lack extensive pulmonary fibrosis, and ISHLT recommendations provided for pulmonary vascular diseases may be more appropriate for such patients (Table 4). Because waitlist mortality is quite high among patients with pulmonary fibrosis, timely referral for transplant evaluation is essential for patients who have severe disease despite maximal therapy and wish to be considered for lung transplantation.

Evaluation of potential candidates for lung transplant should include (1) an objective determination of disease severity, (2) elucidation of the nature of disease characteristics that are causing the patient's symptoms, (3) a determination of whether the benefits (prolonged survival, improved quality of life) of undergoing lung transplantation clearly outweigh the risks associated with receiving a lung transplant (Table 5). Many patients will unfortunately not be eligible for lung transplantation due to the presence of an absolute contraindication or combinations of relative contraindications and comorbidities that make the possibility of achieving a successful transplant unlikely (e.g. severe corticosteroid-induced diabetes and obesity). On the other hand, some patients with sarcoidosis can be very symptomatic from their disease yet not have enough physiologic impairment to receive a high enough lung allocation score (LAS) value (for countries that use a LAS system to prioritize transplant candidates) to have a reasonable chance of receiving a donor lung offer if placed on the waitlist.

The LAS system (Table 6) was implemented in the US in 2005 with the goals of (1) balancing the

Table 4. Guidelines for timing of referral for potential lung transplantation

Referral to a transplant center (all patients with ILD)*
- Impaired lung function
  - FVC <80% predicted
  - DLCO <40% predicted
- Any dyspnea or functional limitation due to lung disease
- Any requirement for supplemental oxygen (even if only required during exertion)
- Failure to improve dyspnea, reduce/eliminate requirement for supplemental oxygen, and/or improve lung function with a clinically indicated trial of medical therapy if inflammatory ILD is present

Referral to a transplant center (all patients with PVD)*
- NYHA Functional Class III or IV symptoms despite escalating therapy
- Rapidly progressive disease (rule out body weight or rehabilitation concerns)
- Use of parenteral targeted vasoactive therapy regardless of symptoms or NYHA Functional Class

Suggested timing of referral to a transplant center for patients with sarcoidosis
- Dyspnea or functional limitation due to lung disease
- Significantly impaired lung function (e.g. FVC <80% predicted, DLCO <40% predicted)
- Requirement for use of supplemental oxygen
- Evidence of SAPH
- NYHA Functional Class III or IV symptoms
- Rapidly progressive disease
- Lack of response to clinically indicated pharmacologic therapies
- Life-threatening complications of suppurative bronchiectasis (e.g. episode of respiratory failure requiring non-invasive ventilation, poor clinical recovery from exacerbations and/or increasing antibiotic resistance, pneumothorax, life-threatening hemoptysis)

urgency of need for transplantation due risk of death without receiving a transplant with the likelihood of an acceptable outcome following transplantation, (2) optimally placing organs according to LAS values combined with matching characteristics of potential recipients (e.g. blood type, thoracic cage dimensions), and (3) reducing the number of candidates on transplant center waitlists who die without the opportunity to undergo a transplant (52,53). The LAS is weighted more by transplant urgency than likelihood of surviving for at least one year post-transplant, but its successful aspects have led to its adoption by a number of countries outside of the US. Because patients with ILD (especially those with IPF) tend to have higher LAS values than candidates with other disease indications, the total number of transplants for ILD (mostly IPF) in the US surpassed that for other indications (e.g. chronic obstructive pulmonary disease, cystic fibrosis, PH due to pulmonary vascular disease) in 2007, and IPF is the leading indication for lung transplantation in the US at present (54). The major indications for lung transplantation for sarcoidosis are advanced fibrotic lung disease, severe pulmonary hypertension, or a combination of both.

A key question when evaluating a patient with sarcoidosis for potential lung transplantation is whether their lung disease has been adequately treated. Our center has had candidates who were using supplemental oxygen and very incapacitated but improved markedly and were able to be weaned off supplemental oxygen and achieve acceptable quality of life when placed on adequate pharmacotherapy. Another key question is whether significant extrapulmonary sarcoidosis is present that may have an impact on post-transplant outcome, and appropriate screening should be performed to detect significant left ventricular dysfunction or sustained ventricular dysrhythmias.

Prior to placement on a transplant waitlist, comorbidities should be aggressively and optimally managed. This includes angioplasty and/or stent placement for coronary artery disease if needed, anti-resorptive therapies to reduce fracture risk if osteoporosis is present, joining a weight loss program if overweight, and medical treatment of systemic hypertension or diabetes mellitus. Because dyspnea may limit physical activity and promote deconditioning, pulmonary rehabilitation with physical training and breathing exercises should be prescribed, and pulmonary rehabilitation programs can provide educational and psychological support and optimize exercise tolerance and functional status

Table 5. Evaluation of potential lung transplant candidates with sarcoidosis

Disease-specific considerations for patients with sarcoidosis
- Is the disease adequately treated/managed?
- Does the risk of death from the disease clearly outweigh risks associated with lung transplantation?
- Is significant involvement of other organ systems present?
- Is chronic bacterial infection associated with bronchiectasis present?
- Does the patient have sarcoidosis-associated pulmonary hypertension?
- Is fungal disease an issue (especially mycetomas due to *Aspergillus* spp)?

Evaluation and testing
- Careful physical examination
  - Is diaphragmatic movement impaired?
  - Is axial skeleton and especially chest wall mobility significantly impaired?
  - Is there evidence of systemic disease with significant organ system involvement (cardiac, nervous system, liver, spleen, skin)?
- Thoracic imaging studies
  - HRCT, routine chest x-ray
  - Quantitative nuclear medicine ventilation/perfusion scan
  - Barium esophagram (? Esophageal dysfunction, significant reflux)
- Lung function assessment
  - Spirometry
  - Lung volumes
  - Single breath diffusion capacity for CO (DLCO)
  - Paranasal sinus imaging if pertinent
- 6-minute walk test
  - Walk distance
  - Oxyhemoglobin saturation at rest and with exertion
  - Quantification of supplemental oxygen requirements if significant desaturation present
- Cardiac evaluation
  - Electrocardiogram
  - Echocardiography with bubble study to detect possible intracardiac shunt
  - Left & right heart catheterization
  - Ambulatory electrocardiography (e.g. 24-72 hrs to rule out significant dysrhythmia)
- Laboratory testing (complete blood count with differential, BUN, creatinine, electrolytes, liver function testing, fasting lipid profile, viral serologies [HIV, HBsAg, HBsAb, HCV, CMV, HSV, EBV, VZV], toxoplasma and aspergillus antibodies, type and screen [blood group and Rh type], prostate-specific antigen [males >40 years of age], panel reactive antibody testing, anti-HLA antibody screening, urinalysis
- Bone densitometry
- PPD testing
- Screening for *Aspergillus* (sputum culture, serum precipitins)
- If significant/suppurative bronchiectasis is present:
  - Sputum bacterial culture and sensitivities
  - Screen for non-tuberculous mycobacteria
- Age- and gender-appropriate cancer screening
- Consultations:
  - Psychosocial evaluations
  - Nutritionist evaluation
  - Rehabilitation medicine
  - Dental evaluation
  - Others as indicated (e.g. ophthalmologic)

prior to transplantation. Because of the prolonged and variable disease course for patients with sarcoidosis, the decision as to when to proceed with transplantation is challenging, even for experienced clinicians at referral/transplant centers. Guidance for timing the placement of lung transplant candidates on the waitlist has been provided by the ISHLT (Table 7).

Surgical considerations

Previous thoracic surgical procedures are generally not a contraindication to performing a lung transplant, but higher risk of hemorrhage, increased need for chest re-exploration, and renal dysfunction can be encountered in patients who have had previous chest surgical procedures, especially if prolonged

Table 6. Values/factors* used to calculate the lung allocation score**

- Lung diagnosis code
- Age (years)
- Body mass index (BMI)
- Functional status
- Forced vital capacity (FVC) percent predicted
- Requirement for supplemental oxygen
- 6-minute walk distance (feet)
- Pulmonary artery systolic pressure (mm Hg)
- Mean pulmonary artery pressure (mPAP; mm Hg)
- Cardiac index (CI) in L/min/m^2
- Central venous pressure (mm Hg)
- Ventilation status
- pCO_2 (current, highest, lowest) mm Hg
- Presence of diabetes
- Serum creatinine (current, highest, lowest) in mg/dL
- Total bilirubin (current, highest, lowest) in mg/dL

* Some values are adjusted according to Disease Group (A-D); sarcoidosis is classified as Group A if mPAP is ≤30 mm Hg but switches to Group D if mPAP is >30 mm Hg.
** The LAS calculation incorporates three different measures (waiting list urgency, post-transplant survival, and transplant benefit) to derive a Raw Allocation Score that is then normalized on a continuous scale of 0 to 100.
For additional information see concerning LAS components and calculations see https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf#nameddest=Policy_10. Organ Procurement and Transplantation Network Policies; Policy 10: Allocation of Lungs. Date accessed, 1/26/18.

Table 7. Guidelines for timing of waitlist placement for transplant candidates

Timing of placing a patient on the lung transplant waitlist (all patients with ILD)*
- Decline in FVC ≥10% during a 6-month follow-up period (a lesser degree of decline has been associated with a poorer prognosis and may call for earlier listing)
- Decline in DLCO ≥15% during a 6-month follow-up period
- Oxyhemoglobin desaturation to <88% or 6-MWT distance <250 meters or >50 meter decline in 6-MWT distance over a 6-month period

Timing of placing a patient on the lung transplant waitlist (all patients with PVD)*
- NYHA Functional Class III or IV symptoms despite 3 months of combination vasoactive therapies (including prostanoids)
- Cardiac index <2 L/min/m^2
- Mean right atrial pressure >15 mm Hg
- 6-MWT distance <350 meters
- Significant hemoptysis, pericardial effusion, or progressive right heart failure (as evidenced by renal dysfunction, increasing serum bilirubin, increasing serum BNP, or recurrent ascites)

Suggested timing of waitlist placement for patients with sarcoidosis
- Decline in FVC ≥10% during a 6-month follow-up period (a lesser degree of decline has been associated with a poorer prognosis and may call for earlier listing)
- Decline in DLCO ≥15% during a 6-month follow-up period
- Oxyhemoglobin desaturation to <88% or 6-MWT distance <250 meters or >50 meter decline in 6-MWT distance over a 6-month period
- NYHA Functional Class III or IV symptoms despite 3 months of combination vasoactive therapies (including prostanoids)
- Cardiac index <2 L/min/m^2
- Mean right atrial pressure >15 mm Hg
- 6-MWT distance <350 meters
- Significant hemoptysis, pericardial effusion, or progressive right heart failure (as evidenced by renal dysfunction, increasing serum bilirubin, increasing serum BNP, or recurrent ascites)

cardiopulmonary bypass times are required. The decision of whether to perform a single, bilateral, or heart-lung transplant involves consideration of the nature of lung involvement and extent of physiologic impairment, whether significant extrapulmonary disease is an issue, what comorbid conditions are pre-

sent, and the likelihood of procuring a donor organ that matches a candidate's thoracic cage dimensions and ABO blood group status. Explanting lungs from patients with advanced sarcoidosis can be extremely challenging due to pleural adhesions and perihilar fibrosis, and substantial intraoperative bleeding is more likely to occur if resection of the native lung(s) proves to be difficult (55).

The presence of one or more mycetomas, especially if abutting the pleura, increases the risk of seeding the pleural spaces during explantation. The risk and degree of pleural bleeding (especially if patients require cardiopulmonary bypass) is likely to be increased, and a prolonged dissection to explant the native lungs may significantly increase donor lung cold ischemic time, thereby increasing the risk of significant reperfusion injury. One case series reported that post-transplant outcomes were significantly worse for patients with mycetomas (56), but aggressive pre-transplant antifungal therapy and prolonged post-transplant prophylaxis may successfully prevent post-transplant Aspergillus infection (57). Additionally, irrigation of the pleural space with an anti-fungal agent (e.g. amphotericin B) when donor lungs are implanted should be considered. Patients with mycetomas, even if apparently unilateral, should only be listed for bilateral lung transplant (BLT).

If suppurative bronchiectasis is present, sputum cultures should be obtained as native lungs are explanted to identify all infecting organisms and their sensitivities to antibiotics. Peri-operative and post-operative antibiotics should be administered according to culture and sensitivity results. Because a bronchiectatic native lung can serve as a reservoir of infection that places a transplanted single lung at risk for post-transplant infection, BLT is the preferred approach for patients with bronchiectasis and chronic suppurative infection.

Bilateral transplant may also be a better choice than single lung transplant (SLT) for patients with significant SAPH, although recipients can do well with SLT despite the presence of PH with mPAP values greater than 40 mm Hg (58). Indeed, a SLT may be a reasonable choice for patients in whom BLT is not required, and listing for SLT may improve chances for a donor organ offer and reduce risk of dying on the waitlist (59). A heart-lung transplant can be considered for patients with significant left ventricular dysfunction or cardiac dysrhythmias.

Post-transplant management, complications, and outcomes

Post-transplant management is multi-faceted and complicated, yet few randomized, prospective controlled trials are available to provide robust evidence for optimal recipient management. Peri-operative care in the ICU requires both ventilator and circulatory support, and early surgical and/or medical complications must be promptly identified and addressed. Protocols should be in place to facilitate prevention of infections (e.g. prophylaxis for cytomegalovirus and *Pneumocystis jiroveci*) as well as protocols to rapidly identify and treat infectious complications that may develop. Immunosuppressive regimens typically consist of pulse corticosteroid and an induction agent given at the time of surgery, and maintenance immunosuppression with a calcineurin inhibitor (tacrolimus or cyclosporine A), anti-metabolite (usually mycophenolate or azathioprine), and a corticosteroid that is gradually weaned to a low dose.

Transplant recipients are at risk for a multitude of immediate, acute, and subacute/chronic complications following successful transplantation (Table 8) (60,61). Approximately one third of lung transplant recipients will develop grade 3 primary graft dysfunction (PGD) (62), but while a number of markers have been identified that correlate with increased risk of high-grade PGD (63), interventions other than providing supportive care have been relatively ineffective in preventing or treating PGD.

Acute/subacute complications include antibody-mediated rejection, acute cellular rejection, lymphocytic bronchiolitis, and infection. Because up to 90% of recipients have pre-formed anti-HLA antibodies of which approximately one third are donor-specific antibodies (DSAs) (64), effective and carefully monitored immune suppression is essential to establish allograft immune tolerance. Monitoring recipients for evidence of lung function decline as well as monitoring for the appearance of numerous complications and co-morbidities is essential to optimize post-transplant allograft function and recipient quality of life and survival. Many centers subject recipients to surveillance bronchoscopy with BAL and transbronchial biopsies (TBBs) at protocol-determined intervals to detect occult infection and/or evidence of rejection, although other centers perform few if any protocol-driven surveillance bronchos-

Table 8. Complications of lung transplantation

Pulmonary complications

Lung allograft complications

- Primary graft dysfunction (PGD)
  - Rejection (hyperacute, acute, chronic)
  - Anastomosis dysfunction (dehiscence, malacia, stricture)
- Phrenic nerve dysfunction
- Chronic lung allograft dysfunction (CLAD)
  - Bronchiolitis obliterans syndrome (obstructive CLAD)
  - Restrictive allograft syndrome (restrictive CLAD)
- Disease recurrence (e.g. sarcoidosis)
- Complications of bronchoscopy with transbronchial biopsy

Lung allograft and/or native lung complications

- Infection (bacterial, fungal, viral)
- Pleural complications (empyema, effusion, hemothorax, fistula)
- Pulmonary embolic disease
- Malignancy (primary lung cancer, post-transplant lymphoproliferative disease [PTLD])

Native lung complications (single lung transplant recipients)

- Hyperinflation (emphysematous lung)
- Reactivated and/or refractory infection

Extrapulmonary/systemic complications (may impact lung function)

- Adverse drug reactions (e.g. immunosuppressant side effects, drug-drug interactions)
- Renal dysfunction
- Infection (e.g. wound, sepsis)
- Metabolic/endocrine
  - Hyperglycemia, diabetes, obesity
  - Electrolyte abnormalities
  - Dyslipidemia
- Cardiovascular (e.g. systemic hypertension, cardiac rhythm disturbance, infarction)
- Thromboembolism
- Hematologic (anemia, leukopenia, thrombocytopenia, thrombotic microangiopathy)
- Gastrointestinal
  - GERD (can affect lung allograft)
  - Biliary tract disease
  - Gastroparesis, other bowel disorders
- Musculoskeletal (osteopenia, osteoporosis, myopathy)
- Neurologic
  - Tremor, headache, seizure, memory loss
  - Cerebrovascular accident, blindness, coma
- Malignancy (skin, primary lung cancer, PTLD)

copies and may only perform such when clinically indicated by deterioration in lung function with the suspicion that infection or allograft rejection may be the cause. Consensus guidelines for using or not using post-transplant surveillance bronchoscopies have not yet become available.

For lung transplant recipients who survive beyond the first year post-transplant, the development of chronic lung allograft dysfunction (CLAD) is the greatest threat to long-term allograft and recipient survival (65, 66). The ISHLT/American Thoracic Society (ATS)/European Respiratory Society (ERS) clinical practice guideline systematically examined available evidence for the prevention and treatment of BOS/CLAD and provided recommendations for the diagnosis and treatment of BOS/CLAD. Identified risk factors included PGD, various forms of alloimmune rejection (acute cellular rejection, antibody-mediated rejection, lymphocytic bronchiolitis), infections (viral, bacterial, fungal), pathologic GER, autoimmunity, and persistent bronchoalveolar lavage (BAL) neutrophilia. Although evidence from randomized controlled trials (RCTs) for preventing and treating BOS/CLAD was found to be of low or very low quality, a number of conditional recommendations were made by consensus among task force members following a comprehensive review of available publications. These include ruling out other causes of delayed, persistent allograft function decline, administering azithromycin, adjustment of

immunosuppressive regimens, and the detection/treatment of significant gastrointestinal reflux that may be affecting the lung allograft (65). Evidence for other salvage therapies for CLAD, such as extracorporeal photopheresis or total lymphoid irradiation, are weak at best (65, 67, 68).

A meta-analysis of 13 different reports that included a total of 10,042 lung transplant recipients of which 98 were transplanted for sarcoidosis concluded that sarcoidosis patients had a 50% prevalence of PGD (69). Additionally, the risk of short-term mortality has been reported to be significantly increased for African-American recipients (9). Furthermore, a higher incidence of hemothorax in sarcoidosis recipients was found to be associated with longer need for ventilator support, increased length of stay in intensive care units, and more prolonged length of hospital stay following lung transplant (70). Nonetheless, despite concerns that short-term outcomes and risk of early mortality may be somewhat worse for sarcoidosis lung recipients and especially African-American recipients, long-term post-transplant survival for recipients with sarcoidosis appears to be generally similar to survival rates for patients with other forms of fibrotic ILD. Tamieh et al. (11) examined a cumulative cohort of 695 patients with sarcoidosis (out of a total of 20,896 recipients) transplanted over a 25-year time period and reported that median survival rates for sarcoidosis recipients were not significantly different from that of non-sarcoid recipients. Additionally, the incidence of BOS does not appear to be increased for patients transplanted for sarcoidosis (11, 71).

Recurrence of non-caseating granulomata in transplanted lungs despite intense chronic immune suppression is a frequent observation in sarcoid recipients (72-78). The majority of cases were detected via transbronchial biopsy, many of which were surveillance procedures, but recurrent granulomas may also be significant enough to allow detection via HRCT scanning. Ionescu et al. (75) showed via DNA analysis that recurrence of granulomas in the lung allografts appeared to be of recipient origin. Additionally, granulomas tend to appear within the first 6-12 months post-transplant, are usually detected via surveillance biopsies, and rarely seem to have a significant impact on allograft function, although disease recurrence has been occasionally reported to cause significant allograft dysfunction (78, 79). Currently available data suggest that granuloma recurrence in the transplanted lungs occurs in approximately a third of recipients, but the impact of disease recurrence on survival is minimal.

We have detected subclinical recurrence of allograft granulomas in 5 of 22 recipients with sarcoidosis at our center, and all spontaneously regressed with the passage of time. Interestingly, an additional non-sarcoid recipient (a 37-year-old Caucasian female) with severe constrictive bronchiolitis caused by an inhalation injury (whose explanted lungs showed no evidence of granulomatous inflammation) had asymptomatic granulomas appear on surveillance transbronchial biopsies at one year post-transplant. These persisted until two years post-transplant (present on multiple sequential surveillance bronchoscopies) and then regressed spontaneously over a period of approximately one year without any change in her chronic immunosuppression regimen. Although BAL lymphocyte percentages on differential cell count and CD4/CD8 lymphocyte ratios are generally very low in lung transplant recipients on surveillance biopsies and this recipient's percent lymphocytes on BAL were 3%, 3%, and 6% at 2, 6, and 24 weeks post-transplant, her BAL lymphocyte percentage had increased to 24% at 52 weeks with a CD4/CD8 ratio of 2.7 (versus 0.6±0.1 for clinically stable non-sarcoid lung recipients [N=20]) along with the appearance of typical well-formed, non-caseating granulomata on transbronchial biopsies. While repeat surveillance bronchoscopies up to 48 months post-transplant showed BAL lymphocyte percentages that ranged up to 49% with CD4/CD8 lymphocyte ratios as high as 5.7 along with persistence of well-formed non-caseating granulomata on transbronchial biopsies, serial HRCT imaging showed no changes and lung function remained completely stable. BAL culture and special stains showed no evidence of infection, and her maintenance immunosuppression and other medications were not altered. At 2.5 years post-transplant, the granulomas had regressed and were no longer detectable, the BAL lymphocytosis resolved, and the BAL lymphocyte CD4/CD8 ratio returned to a low ratio consistent with stable lung transplant recipient status. We suspect that this individual, who was of northern European ethnicity, likely developed a sarcoidosis syndrome with lung-limited infiltration of recipient immune cells into the lung allograft that gradually peaked and then eventually regressed spontaneously.

Key Points

1. A small number of patients diagnosed with sarcoidosis develop advanced lung disease.
2. Advanced pulmonary disease phenotypes include extensive pulmonary fibrosis, pulmonary hypertension, and purulent bronchiectasis.
3. Lung transplantation is an appropriate treatment for sarcoidosis patients with advanced lung disease that progresses to respiratory insufficiency despite other therapies.
4. Post-transplant survival is generally similar to that of recipients with other transplant indications such as IPF.
5. Although bilateral lung transplantation is generally a preferred procedure, single lung transplant may be an appropriate procedure for patients without complications of their lung disease such as purulent bronchiectasis, chronic fungal infection, or severe pulmonary hypertension.
6. Although recurrence of granulomas in transplanted lungs may occur, this rarely has a significant impact on lung allograft function or recipient survival.

Acknowledgment

Supported in part by the George and Julie Mosher Pulmonary Research Fund.

Financial/nonfinancial disclosures:
Within the past 3 years Dr. Meyer has been an investigator in clinical trials sponsored by Boehringer-Ingelheim, Bristol Meyers Squibb, Genentech, National Institutes of Health, Nivalis, Parion, Promedior, Roche, and Vertex. Dr. Meyer does not report any other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. No writing assistance was utilized in the production of this manuscript.

References

1. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis 1999 Sep; 16(2): 149-73.
2. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med 1997 Apr 24; 336(17): 1224-34.
3. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Müller-Quernheim J. Sarcoidosis. Lancet 2014 Mar 29; 383(9923): 1155-67.
4. Culver DA, Baughman RP. It's time to evolve from Scadding: phenotyping sarcoidosis. Eur Respir J 2018 Jan 25; 51(1).
5. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 2001 Nov 15; 164(10 Pt 1): 1885-9.
6. Patel DC, Budev M, Culver DA. Advanced ("end-stage") pulmonary sarcoidosis. In Pulmonary Sarcoidosis: A Guide for the Practicing Clinician, Respiratory Medicine 17, Ed Judson M, 2014: pp 79-110.
7. Kotloff RM, Thabut G. Lung transplantation. Am J Respir Crit Care Med 2011 Jul 15; 184(2): 159-71.
8. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant 2015 Oct; 34(10): 1264-77.
9. Shorr AF, Helman DL, Davies DB, Nathan SD. Sarcoidosis, race, and short-term outcomes following lung transplantation. Chest 2004 Mar; 125(3): 990-6.
10. Shah L. Lung transplantation in sarcoidosis. Semin Respir Crit Care Med 2007; 28: 134-140.
11. Taimeh Z, Hertz MI, Shumway S, Pritzker M. Lung transplantation for pulmonary sarcoidosis. Twenty-five years of experience in the USA. Thorax 2016 Apr; 71(4): 378-9.
12. Shorr AF, Davies DB, Nathan SD. Outcomes for patients with sarcoidosis awaiting lung transplantation. Chest 2002 Jul; 122(1): 233-8.
13. Shorr AF, Davies DB, Nathan SD. Predicting mortality in patients with sarcoidosis awaiting lung transplantation. Chest 2003 Sep; 124(3): 922-8.
14. Baughman RP, Lower EE, Tami T. Upper airway. 4: Sarcoidosis of the upper respiratory tract (SURT). Thorax 2010; 65(2): 181-6.
15. Chambellan A, Turbie P, Nunes H, Brauner M, Battesti JP, Valeyre D. Endoluminal stenosis of proximal bronchi in sarcoidosis: bronchoscopy, function, and evolution. Chest 2005 Feb; 127(2): 472-81.
16. Patil SN, Levin DL. Distribution of thoracic lymphadenopathy in sarcoidosis using computed tomography. J Thorac Imaging 1999 Apr; 14(2): 114-7.
17. Murdoch J, Müller NL. Pulmonary sarcoidosis: changes on follow-up CT examination. AJR Am J Roentgenol 1992 Sep; 159(3): 473-7.
18. McLoud TC, Epler GR, Gaensler EA, Burke GW, Carrington CB. A radiographic classification for sarcoidosis: physiologic correlation. Invest Radiol 1982 Mar-Apr; 17(2): 129-38.
19. Battesti JP, Saumon G, Valeyre D, et al. Pulmonary sarcoidosis with an alveolar radiographic pattern. Thorax 1982 Jun; 37(6): 448-52.
20. Nunes H, Brillet PY, Valeyre D, Brauner MW, Wells AU. Imaging in sarcoidosis. Semin Respir Crit Care Med 2007 Feb; 28(1): 102-20.
21. Abehsera M, Valeyre D, Grenier P, Jaillet H, Battesti JP, Brauner MW. Sarcoidosis with pulmonary fibrosis: CT patterns and correlation with pulmonary function. AJR Am J Roentgenol 2000 Jun; 174(6): 1751-7.
22. Primack SL, Hartman TE, Hansell DM, Müller NL. End-stage lung disease: CT findings in 61 patients. Radiology 1993 Dec; 189(3): 681-6.
23. Padley SP, Padhani AR, Nicholson A, Hansell DM. Pulmonary sarcoidosis mimicking cryptogenic fibrosing alveolitis on CT. Clin Radiol 1996 Nov; 51(11): 807-10.
24. Handa T, Nagai S, Miki S, Fushimi Y, Ohta K, Mishima M, Izumi T. Incidence of pulmonary hypertension and its clinical relevance in patients with sarcoidosis. Chest 2006 May; 129(5): 1246-52.
25. Nunes H, Humbert M, Capron F, Brauner M, Sitbon O, Battesti JP, Simonneau G, Valeyre D. Pulmonary hypertension associated with sarcoidosis: mechanisms, haemodynamics and prognosis. Thorax 2006 Jan; 61(1): 68-74.
26. Shorr AF, Helman DL, Davies DB, Nathan SD. Pulmonary hypertension in advanced sarcoidosis: epidemiology and clinical characteristics. Eur Respir J 2005 May; 25(5): 783-8.

27. Sulica R, Teirstein AS, Kakarla S, Nemani N, Behnegar A, Padilla ML. Distinctive clinical, radiographic, and functional characteristics of patients with sarcoidosis-related pulmonary hypertension. Chest 2005 Sep; 128(3): 1483-9.
28. Shlobin O, Nathan SD. Sarcoidosis-associated pulmonary hypertension. In Pulmonary Sarcoidosis: A Guide for the Practicing Clinician, Respiratory Medicine 17, Ed Judson M, 2014: pp 111-128.
29. Baughman RP, Engel PJ, Taylor L, Lower EE. Survival in sarcoidosis-associated pulmonary hypertension: the importance of hemodynamic evaluation. Chest 2010 Nov; 138(5): 1078-85.
30. Lewis MM, Mortelliti MP, Yeager H Jr, Tsou E. Clinical bronchiectasis complicating pulmonary sarcoidosis: case series of seven patients. Sarcoidosis Vasc Diffuse Lung Dis 2002 Jun; 19(2): 154-9.
31. Xu L, Kligerman S, Burke A. End-stage sarcoid lung disease is distinct from usual interstitial pneumonia. Am J Surg Pathol 2013 Apr; 37(4): 593-600.
32. Udwadia ZF, Pilling JR, Jenkins PF, Harrison BD. Bronchoscopic and bronchographic findings in 12 patients with sarcoidosis and severe or progressive airways obstruction. Thorax 1990 Apr; 45(4): 272-5.
33. Soskel NT, Sharma OP. Pleural involvement in sarcoidosis. Curr Opin Pulm Med 2000 Sep; 6(5): 455-68.
34. Parker JM, Torrington KG, Phillips YY. Sarcoidosis complicated by chylothorax. South Med J 1994 Aug; 87(8): 860-2.
35. Froudarakis ME, Bouros D, Voloudaki A, et al. Pneumothorax as a first manifestation of sarcoidosis. Chest 1997 Jul; 112(1): 278-80.
36. Nguyen LD, Viscogliosi E, Delhaes L. The lung mycobiome: an emerging field of the human respiratory microbiome. Front Microbiol 2015 Feb 13; 6: 89.
37. Pena TA, Soubani AO, Samavati L. Aspergillus lung disease in patients with sarcoidosis: a case series and review of the literature. Lung 2011 Apr; 189(2): 167-72.
38. Judson MA, Boan AD, Lackland DT. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. Sarcoidosis Vasc Diffuse Lung Dis 2012 Oct; 29(2): 119-27.
39. Minces LR, Bhama JK, Abdel-Massih R, et al. Successful double lung transplantation in a patient with bilateral pulmonary and sinus aspergillomas. Transpl Infect Dis 2011 Oct; 13(5): 485-8.
40. Panselinas E, Judson MA. Acute pulmonary exacerbation of sarcoidosis. In Pulmonary Sarcoidosis: A Guide for the Practicing Clinician, Respiratory Medicine 17, Ed Judson M, 2014: pp 65-78.
41. Moller DR, Ho L. Pulmonary sarcoidosis. In: Clinical Handbook of Interstitial Lung Disease. Ed. Thillai M, Moller DR, Meyer KC. CRC Press, Taylor & Francis Group, Boca Raton 2018: pp 257-270.
42. Schutt AC, Bullington WM, Judson MA. Pharmacotherapy for pulmonary sarcoidosis: a Delphi consensus study. Respir Med 2010 May; 104(5): 717-23.
43. Korsten P, Strohmayer K, Baughman RP, Sweiss NJ. Refractory pulmonary sarcoidosis - proposal of a definition and recommendations for the diagnostic and therapeutic approach. Clin Pulm Med 2016 Mar; 23(2): 67-75.
44. Baughman RP, Drent M. The treatment of pulmonary sarcoidosis. In Pulmonary Sarcoidosis: A Guide for the Practicing Clinician, Respiratory Medicine 17, Ed Judson M, 2014: pp 41-64.
45. Grutters JC, van den Bosch JM. Corticosteroid treatment in sarcoidosis. Eur Respir J 2006 Sep; 28(3): 627-36.
46. Baughman RP, Drent M, Kavuru M, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 2006 Oct 1; 174(7): 795-802.
47. Baughman RP, Engel PJ, Meyer CA, Barrett AB, Lower EE. Pulmonary hypertension in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2006 Jun; 23(2): 108-16.
48. Valeyre D, Nunes H, Bernaudin JF. Advanced pulmonary sarcoidosis. Curr Opin Pulm Med 2014 Sep; 20(5): 488-95.
49. Kirkil G, Lower EE, Baughman RP. Predictors of Mortality in Pulmonary Sarcoidosis. Chest 2018 Jan; 153(1): 105-113.
50. Judson MA. Strategies for identifying pulmonary sarcoidosis patients at risk for severe or chronic disease. Expert Rev Respir Med 2017 Feb; 11(2): 111-118.
51. Weill D, Benden C, Corris PA, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant 2015 Jan; 34(1): 1-15.
52. Eberlein M, Garrity ER, Orens JB. Lung allocation in the United States. Clin Chest Med 2011 Jun; 32(2): 213-22.
53. Egan TM, Edwards LB. Effect of the lung allocation score on lung transplantation in the United States. J Heart Lung Transplant 2016 Apr; 35(4): 433-9.
54. Lamas DJ, Lederer DJ. Lung transplantation for idiopathic pulmonary fibrosis. In; Idiopathic Pulmonary Fibrosis: A Comprehensive Clinical Guide. Eds Meyer KC, Nathan SD. Humana Press, Springer, New York 2014, pp 363-377.
55. Shlobin OA, Nathan SD. Management of end-stage sarcoidosis: pulmonary hypertension and lung transplantation.Eur Respir J 2012 Jun; 39(6): 1520-33.
56. Hadjiliadis D, Sporn TA, Perfect JR, Tapson VF, Davis RD, Palmer SM. Outcome of lung transplantation in patients with mycetomas. Chest 2002 Jan; 121(1): 128-34.
57. Minces LR, Bhama JK, Abdel-Massih R, et al. Successful double lung transplantation in a patient with bilateral pulmonary and sinus aspergillomas. Transpl Infect Dis 2011 Oct; 13(5): 485-8.
58. Julliard WA, Meyer KC, De Oliveira NC, Osaki S, Cornwell RC, Sonetti DA, Maloney JD. The presence or severity of pulmonary hypertension does not affect outcomes for single-lung transplantation. Thorax 2016 May; 71(5): 478-80.
59. Nathan SD, Shlobin OA, Ahmad S, Burton NA, Barnett SD, Edwards E. Comparison of wait times and mortality for idiopathic pulmonary fibrosis patients listed for single or bilateral lung transplantation. J Heart Lung Transplant 2010 Oct; 29(10): 1165-71.
60. Jaksch P, Koinig H, Klepetko W. Critical care management. In: Lung Transplantation. Eds Vigneswaran WT, Garrity ER Jr. Lung Biology in Health and Disease, Volume 243. Informa, London, 2010: pp 224-236.
61. Meyer KC. Lung transplantation: chronic complications and management. In: Lung Transplantation. Eds Vigneswaran WT, Garrity ER Jr. Lung Biology in Health and Disease, Volume 243. Informa, London, 2010: pp 357-374.
62. Shah RJ, Diamond JM, Cantu E, et al. Latent class analysis identifies distinct phenotypes of primary graft dysfunction after lung transplantation. Chest 2013; 144(2): 616-622.
63. Morrison MI, Pither TL, Fisher AJ. Pathophysiology and classification of primary graft dysfunction after lung transplantation. J Thorac Dis 2017 Oct; 9(10): 4084-4097.
64. Brugière O, Suberbielle C, Thabut G, et al. Lung transplantation in patients with pretransplantation donor-specifi c antibodies detected by Luminex assay. Transplantation 2013; 95(5): 761-765.
65. Meyer KC, Raghu G, Verleden GM, et al. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. Eur Respir J 2014 Dec; 44(6): 1479-503.
66. Verleden GM, Raghu G, Meyer KC, Glanville AR, Corris P. A new classification system for chronic lung allograft dysfunction. J Heart Lung Transplant 2014 Feb; 33(2): 127-33.
67. Benden C, Haughton M, Leonard S, Huber LC. Therapy options for chronic lung allograft dysfunction-bronchiolitis obliterans syndrome following first-line immunosuppressive strategies: A systematic review. J Heart Lung Transplant 2017 Sep; 36(9): 921-933.
68. Meyer KC. Diagnosis and management of bronchiolitis obliterans

syndrome following lung or hematopoietic cell transplantation. Expert Rev Respir Med 2016 Jun; 10(6): 599-602.
69. Liu Y, Liu Y, Su L, Jiang SJ. Recipient-related clinical risk factors for primary graft dysfunction after lung transplantation: a systematic review and meta-analysis. PLoS One 2014 Mar 21; 9(3): e92773.
70. Hong A, King CS, Brown AW, Ahmad S, Shlobin OA, Khandhar S, Bogar L, Rongione A, Nathan SD. Hemothorax following lung transplantation: incidence, risk factors, and effect on morbidity and mortality. Multidiscip Respir Med 2016 Nov 15; 11: 40.
71. Wille KM, Gaggar A, Hajari AS, et al. Bronchiolitis obliterans syndrome and survival following lung transplantation for patients with sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2008; 25(02): 117–124.
72. Walker S, Mikhail G, Banner N, Partridge J, Khaghani A, Burke M, Yacoub M. Medium term results of lung transplantation for end stage pulmonary sarcoidosis. Thorax 1998 Apr; 53(4): 281-4.
73. Nunley DR, Hattler B, Keenan RJ, Iacono AT, Yousem S, Ohori NP, Dauber JH. Lung transplantation for end-stage pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 1999 Mar; 16(1): 93-100.
74. Collins J, Hartman MJ, Warner TF, Müller NL, Kazerooni EA, McAdams HP, Slone RM, Parker LA. Frequency and CT findings of recurrent disease after lung transplantation. Radiology 2001 May; 219(2): 503-9.
75. Ionescu DN, Hunt JL, Lomago D, Yousem SA. Recurrent sarcoidosis in lung transplant allografts: granulomas are of recipient origin. Diagn Mol Pathol 2005 Sep; 14(3): 140-5.
76. Schultz HH, Andersen CB, Steinbruuchel D, Perch M, Carlsen J, Iversen M. Recurrence of sarcoid granulomas in lung transplant recipients is common and does not affect overall survival. Sarcoidosis Vasc Diffuse Lung Dis 2014 Jul 8; 31(2): 149-53.
77. Banga A, Sahoo D, Lane CR, Farver CF, Budev MM. Disease Recurrence and Acute Cellular Rejection Episodes During the First Year After Lung Transplantation Among Patients With Sarcoidosis. Transplantation 2015 Sep; 99(9): 1940-5.
78. Bjørtuft O, Foerster A, Boe J, Geiran O. Single lung transplantation as treatment for end-stage pulmonary sarcoidosis: recurrence of sarcoidosis in two different lung allografts in one patient. J Heart Lung Transplant 1994 Jan-Feb; 13(1 Pt 1): 24-9.
79. Yserbyt J, Wuyts WA, Verleden SE, Verleden GM, Van Raemdonck DE, Verbeken EK, Vanaudenaerde BM, Vos R. Solid Organ Transplantation in Sarcoidosis. Semin Respir Crit Care Med 2017 Aug; 38(4): 538-545.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 108-115

Multidisciplinary management of interstitial lung diseases: A real-life study

Caroline Biglia[1], Benoît Ghaye[2], Gregory Reychler[1, 2], Sandra Koenig[1], Halil Yildiz[3], Valérie Lacroix[4], Farah Tamirou[5], Delphine Hoton[6], Thierry Pieters[1], Antoine Froidure[1, 2]

[1] Pneumology department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium; [2] Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Belgium; [3] Radiology department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium; [4] General internal medicine department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium; [5] Thoracic surgery department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium; [6] Rheumatology department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium; [7] Pathology department, Cliniques universitaires Saint-Luc, Bruxelles, Belgium

Abstract. *Background:* The guidelines on idiopathic pulmonary fibrosis (IPF) diagnosis established the crucial role of multidisciplinary discussion (MDD) in the diagnosis of interstitial lung diseases (ILD). However, real-life evaluation of MDD remains scarce. Our aim was to study the impact of a well-structured MDD on etiological assessment, diagnosis, and management of ILD. *Methods:* We collected and analysed all relevant data on patients concerning diagnosis and treatment before and after MDD during the year 2017. *Results:* One hundred fifty patients were included in the analysis. MDD had a significant impact on management: 42% of diagnoses were revised and the number of unclassifiable ILD was significantly reduced. Lung biopsy was performed in 26 patients (12 cryobiopsies and 14 surgical biopsies). The most prevalent diagnoses were connective-tissue disease associated ILD (32%), idiopathic pulmonary fibrosis (23%), hypersensitivity pneumonitis (13%) and granulomatous ILD (7%). MDD led to a change or initiation of treatment in 55% of cases. Nine patients were evaluated for transplantation, 23 patients were screened for academic or sponsored clinical trials and an 8-fold increase in rehabilitation inclusion was observed. *Conclusion:* Our results confirm the benefits of MDD on ILD management and diagnosis. MDD also facilitates access to non-pharmacological therapies and clinical trials. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 108-115)*

Key words: interstitial lung diseases, multidisciplinary management

Introduction

The diagnostic and management of interstitial lung diseases (ILD) are complex, as this group of disorders encompasses a wide heterogeneity of diseases, presenting with different causes, requiring personalized management and leading to variable outcomes. In 2001, The American Thoracic Society/European Respiratory Society (ATS/ERS) already highlighted the need for a multidisciplinary and dynamic process in diagnosing idiopathic interstitial pneumonias (IIP) (1). Few years later, the ATS/ERS guidelines recommended multidisciplinary discussion (MDD) among experts to diagnose idiopathic pulmonary fibrosis (IPF) (1). This recommendation was reconducted in the last 2018 guidelines (2, 3). The emergence of anti-fibrotic drugs and the potential danger of misused immunosuppressive therapy (4) makes discrimination between IPF and non IPF-ILD critically important in clinical practice (4, 5).

Received: 14 January 2019
Accepted after revision: 11 May 2019
Correspondence: Pr Antoine Froidure, MD, PhD
Service de pneumologie
Cliniques universitaires Saint-Luc, Bruxelles, Belgium
Université catholique de Louvain, Bruxelles, Belgium
Avenue Hippocrate, 10, 1200 Bruxelles - Belgium
Tel. 0032 (0) 2/7642832
E-mail: antoine.froidure@uclouvain.be

Some studies have tackled the issue of the role of MDD in ILD diagnosis. Flaherty et al have shown that, in idiopathic interstitial pneumonia's (IIP), level of diagnostic agreement between observers and diagnostic confidence improves as more data are shared during a multidisciplinary discussion, especially for the non IPF-ILD (6). Disagreement in term of diagnosis was at the highest level in non-academic centres with no access to MDD meetings, reflecting the need for referring ILD in expert centres and for promoting the use of these MDD meetings (7). Walsh et al have demonstrated that MDD increases frequency and confidence of IPF diagnosis. They have also shown that inter-MDD agreement was good, especially in IPF. Regarding the subgroup of IPF diagnosed without requirement of a biopsy (typical clinical context and typical HRCT pattern), the level of inter-observers and inter-MDD diagnostic agreement was high and the difference between levels of inter-individual and inter MDD agreement was low (8). This is probably explained by the existence of validated guidelines that are easy to apply for clinicians with experience in ILD. Another observation that emerged from these studies was that diagnosis of chronic hypersensitivity pneumonitis and disease with a non-specific interstitial pneumonia (NSIP) HRCT pattern were still challenging despite the input of MDD. Therefore, evaluating MDD performance in real-world setting is valuable.

In the light of recent evidence, a well-structured MDD was set up in our department. The aim of the present study was to assess the impact of these MDD in our daily clinical practice. We hypothesized that MDD would significantly impact (1) ILD diagnosis and (2) ILD management. The purpose of this study was not only to observe the effects on diagnoses, but also on diagnostic processes, choices of treatment and recommendations for non-pharmacological treatment.

Methods

Study design

This is a single-centre retrospective study. All information of ILD patients discussed in MDD between January 1st and December 31st2017 were included in a database and eligible for the study. For each case, relevant clinical and demographic characteristics were collected. We reported also data about pre- and post-referral investigations, diagnosis and treatment. For every patient, we had a "pre-MDD" diagnosis (i.e. the suspected diagnosis, based on the form filled by the clinician) and a "post-MDD" diagnosis, corresponding to the conclusion.

Recommendations on rehabilitation program, transplantation valuation and academic or sponsored clinical trials were analysed. Data collection was performed between January 1st and July 1st 2018.

We included patients only once even if the case was presented again during the year. We excluded patients for which no structured form had been completed and validated after the MDD.

We applied the STROBE criteria for observational studies (http://www.equator-network.org/wp-content/uploads/2015/10/STROBE_checklist_v4_combined.pdf).

Multidisciplinary discussion meetings

MDD are organized every other week and last about 90 minutes. The panel comprises two pulmonologists with experience in ILD, at least one chest radiologist, 1 rheumatologist, 1 surgeon and 1 histopathologist. A study coordinator involved in ILD-related clinical trials also attends the meeting. Other specialties (general internal medicine, nephrologists) occasionally refer patients for an ILD workup. For each patient, information about medical history, symptoms and signs, toxic exposure (smoking status, drugs, environmental and occupational), functional respiratory test, bronchoalveolar lavage findings and autoantibody profile are collected in a structured computer form by the referral specialist (supplementary figure 1). The clinical context is exposed briefly and then images from high resolution computed tomography (HRCT) are presented by the radiologist who defined a typical CT pattern whenever possible. In case a biopsy was performed, selected images are presented by the pathologist. For each case presented, the structured form is completed with a definitive CT pattern, histopathology pattern when available, final diagnosis and recommendations for further management and follow up. Finally, a pulmonologist specialized in ILD validates this form and inserts it in the patient's medical file. Some cases are discussed twice or more: Patients who underwent a lung biopsy

and cases requiring treatment response assessment or a significant change in management.

Analysis and statistics

For every patient included, we compared suspected diagnosis at referral to final diagnosis established by the MDD. Chi-square test was used to estimate the impact of MDD impact on the number of unclassifiable ILD after MDD.

Ethics

The present study was approved by our local ethics committee (study PNEU-ILD-02, approval number 2018/15MAR/116).

Results

Study population

One hundred fifty-three patients were discussed in MDD. A mean of 9 (range 7-16) patients are discussed at every meeting. We excluded three cases for who the structured form had not been filled properly and validated, meaning that one hundred fifty patients were included in the analysis (table 1). Sex ratio was 78/72 (M/F). The mean age was 63.1 years (SD=15.3). One third of the subjects were former or current smokers of at least 20 pack-years. Most patients were referred by pulmonologists (n= 58, 38 %) and rheumatologists (n=46, 30%) working in our hospital. Others (19%) were addressed by pulmonologists from primary and secondary centres. The others were referred by various department of our centre (internal medicine 10; intensive care 3; oncology 1; haematology 1; nephrology 1; geriatric service 1). At the time of analysis, follow-up ranged from 7 months to 19 months.

Table 1. Demographic characteristics of the cohort

Subjects	
Sexfemale/male	78/72
Age mean±SD (years)	63.1±15.3
Smoking status	
Ex/currentsmokers	50
Non smokers	100
Environmental exposure	
Occupational/Environmental	37
Drugs	8
Comorbidities/pre-existing diseases	
Chronicrespiratorydisease	42
Rheumatologicdisease	61
Ethnic group	
Europeans	115
Africans	26
Asians	7
Americans	2

Diagnostic assessment

The most prevalent diagnosis was connective-tissue disease associated ILD (CTD-ILD) with 48 cases reported (32%). Other frequent diagnoses were idiopathic pulmonary fibrosis (IPF, n=35, 23%), chronic hypersensitivity pneumonitis (HP, n=20, 13%) and granulomatosis (n=11, 7%). Despite MDD, 15 cases (10%) remained unclassifiable ILD (figure 1).

Ninety-five cases of ILD (63%) were from known causes: CTD-ILD, HP, granulomatosis and ILD from rarer causes (drugs n=3; hemopathy and lymphoproliferative disease n=8). While 55 cases (37%) remained idiopathic: unclassifiable ILD, IPF, cryptogenic organizing pneumonia (n=3) and pleuroparenchymal fibroelastosis (n=2).

Sarcoidosis was the most common granulomatosis (n=9). Two cases of eosinophilic granulomatosis

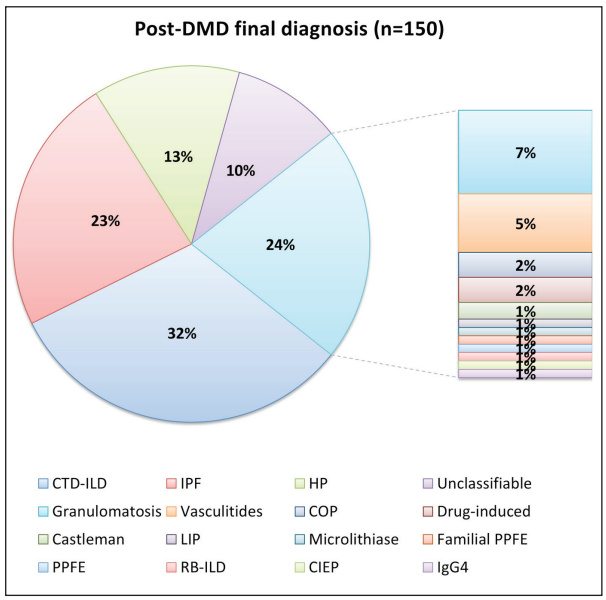


Fig. 1. Proportion of MDD final diagnoses

with polyangiitis (EGPA, formerly Churg-Strauss syndrome) and one case of granulomatosis with polyangiitis (GPA, formerly Wegener granulomatosis) were also reported.

Nine cases of familial interstitial lung diseases were detected (6 % of study cohort), including 6 IPF, 2 cases of pleuroparenchymal fibroelastosis and 1 case of chronic hypersensitivity pneumonitis. Following genetic testing, we identified a telomerase mutation in 5 cases of familial IPF (TERC n=2, TERT mutations n=2, RTEL1 mutation n=1). In 3 cases, gene sequencing was done but no known mutations were found, and in 1 case genetic testing is still ongoing.

Impact of MDD on diagnoses

Reviewing cases in MDD led to a change between suspected diagnosis (pre-MDD) and final diagnosis (post-MDD) in 63 of cases (42%) (figure 2 and figure S2). We observed a 5-fold increase in diagnosis of IPF after MDD: From 7 suspected IPF (5%) to 35 cases of confirmed IPF (23%). These changes were in most cases due to face-to-face discussion between radiologists and clinicians (n=15). In nine cases a biopsy was required by the multidisciplinary team and led to the diagnosis of IPF. In 3 cases, a pre-existing biopsy was examined by our expert pathologist and the pattern was UIP, leading to MDD diagnosis of IPF.

MDD led to a significant increase of the number of HP diagnoses (from 11 to 20 cases). Four cases required histological confirmation because of atypical presentation. The other sixteen cases were diagnosed by combination of symptoms, clinical examination, proved sensitivity towards an antigen, broncho-alveolar lavage (BAL) composition and CT pattern. MDD emphasized the need to search for an incriminated antigen by dosing serum precipitins and searching antigen at home. Despite this, we only identified a relevant antigen exposure in 9 cases of HP. At referral, 11 cases of HP were proposed, 2 became IPF after performing a biopsy. From the 11 cases newly diagnosed as HP, the majority were referred as ILD of undetermined aetiology.

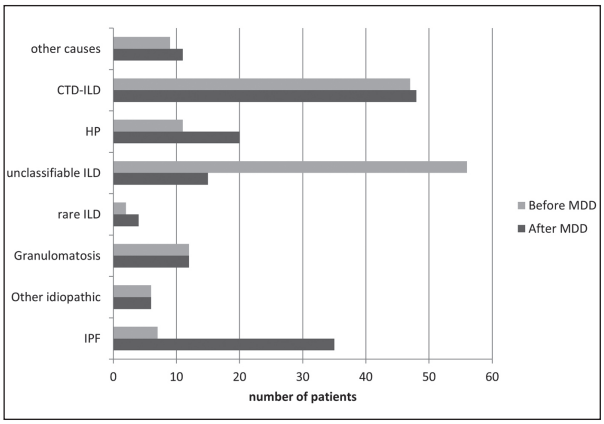


Fig. 2. Comparison between suspected diagnosis at referral (light grey bars) and final MDD diagnosis (dark grey bars)

Following MDD, the amount of unclassifiable ILD was significantly reduced (from 56 to 15, p<0.0001). The majority of unclassifiable ILD (n=23) at referral were diagnosed as IPF by the multidisciplinary team: 13 patients met the ATS-ERS criteria for probable or definite UIP pattern at the CT-scan, seven patients required histological confirmation and underwent lung biopsy. Finally, we confirmed a histological UIP pattern in three patients that had been biopsied elsewhere. (figure 3). As previously said, a part of unclassifiable ILDs were finally diagnosed as HP without requirement of a biopsy. MDD recommended performing biopsy in 19 cases of unclassifiable at referral but only 13 subjects underwent a biopsy. Among the 15 cases of unclassifiable after MDD, three patients remained unclassifiable despite a lung biopsy (2 cryobiopsies and 1 surgical biopsy). In three cases, the patient declined the procedure. Two patients had formal contraindication for either surgical biopsy or transbronchial-cryobiopsy. For five patients, biopsies were not proposed because of spontaneous clinical recovering, old age or very mild disease.

Impact of MDD on ILD management

In total, lung biopsy was proposed in 37 cases (25%) and effectively performed in 26 patients (12 cryobiopsies and 14 surgical biopsies). Input of histopathology allowed to change diagnosis in 18 cases (69% of biopsied patient) and to change treatment in 14 cases (54% of biopsied patients). The number of lung biopsies increased significantly between 2016 and 2017 with implementation of the structured MDD (from 4 to 14 surgical biopsies and from 6 to 12 cryobiopsies).

MDD led to a change or initiation of treatment in 81 cases (54%). Anti-fibrotic were prescribed for IPF but also for unclassifiable ILD with work-

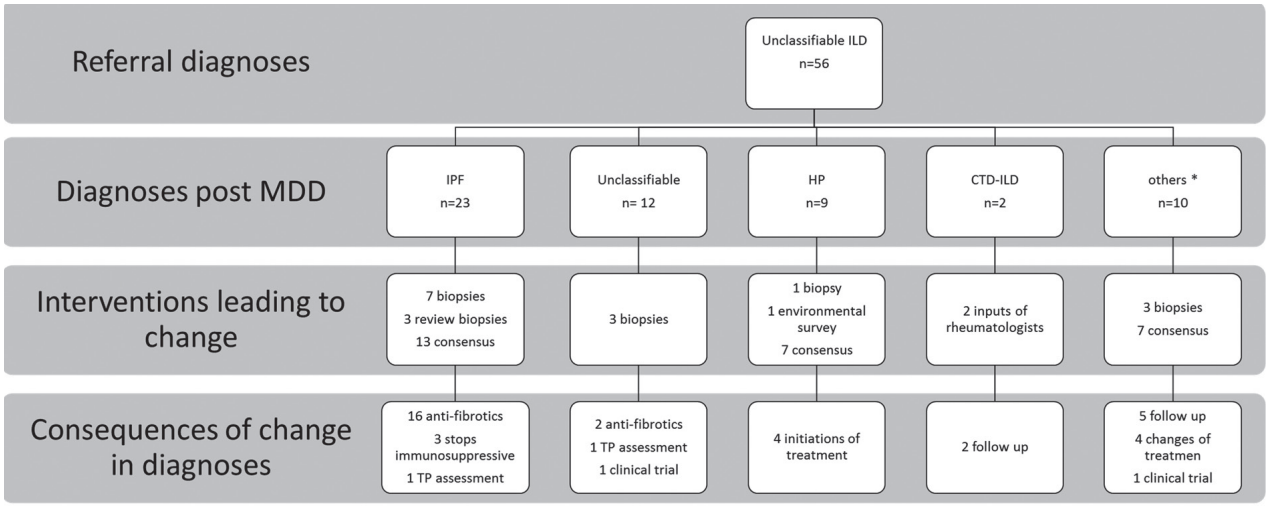


Fig. 3. Flowchart describing patients addressed for unclassifiable fibrosis, including interventions leading to a change in diagnosis and the consequences on treatment

ing diagnosis of IPF (total of 32 new antifibrotic treatments initiated). 6 patients were included in sponsored clinical trial, providing them an access to treatment. MDD strictly recommended to stop corticosteroids and immunosuppressive therapies in case of IPF or unclassifiable ILD in 6 cases. In contrast, in HP group MDD recommended to start corticosteroid in 9 cases and steroid sparing agent in 2 cases (1 mycophenolate mofetil and 1 azathioprine). Similarly, MDD recommended starting steroid sparing agent in 7 cases of sarcoidosis.

In the CTD-ILD subgroup, there was an increased recommendation for initiation of corticosteroid-sparing immunosuppressive therapies: In 14 cases, intravenous cyclophosphamide pulse therapy was advocate because of clinical, radiological and/or functional decline. Advices were given about oral medication after the pulse therapy. Thirteen patients received azathioprine or mycophenolate mofetil after recommendation of MDD. Half of patients included in rehab program following the MDD were CTD-ILD patients (n=16). Four CTD-ILD patients were assessed for lung transplantation due to an end-stage respiratory disease (figure 4).

Identifying definite diagnoses for 44 cases of unclassifiable ILD at referral led to change or initiation of treatment in 28 cases. Despite the absence

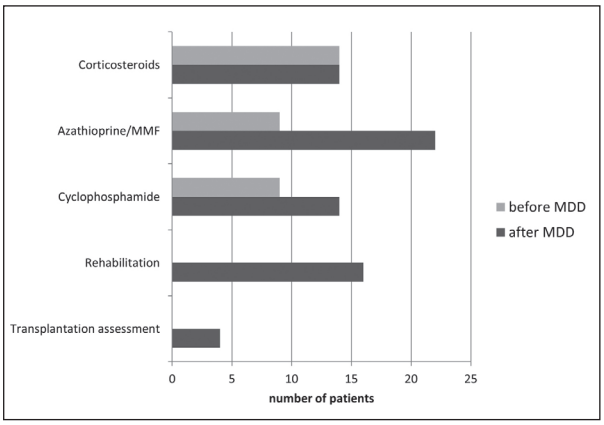


Fig. 4. Changes in pharmacological and non-pharmacological treatment in CT-ILD. Light grey bars represent treatment before MDD, dark grey bars correspond to treatment after MDD

of definite diagnosis, treatments with pirfenidone in clinical trials were proposed for three cases.

MDD strongly supported inclusion on rehabilitation program for 29 patients. Sixteen patients were really included in the outpatient pulmonary rehabilitation program of our centre, an 8-fold increase compared with inclusion in 2016. MDD led to an increasing recommendation for early transplantation evaluation (9 patients in total, including 4 CTD-ILD, 3 IPF and 2 HP). Finally, 23 patients were screened for academic or sponsored clinical trials.

Discussion

In this study, we assessed the effect of a well-structured MDD meeting on ILD management. Our local results confirm that MDD has a significant impact on final diagnosis (42% modification), pharmacological treatment as well as non-pharmacological therapies (54% change in therapy). The most prevalent diagnoses were CTD-ILD, IPF and HP. Our epidemiological data are consistent with those presented in three recent publications from the Greater Paris region (9) (Seine-Saint-Denis), United States (10) and Leuven (11). However, small differences are worth noticing: We collected a higher percentage of IPF (23%) than in Seine Saint Denis (11%) and approximately the same percentage as in USA (20%). One explication given by authors for the relative low frequency of IPF in Seine Saint-Denis is that their population is especially young and not representative of the general population. Our higher percentage of IPF is also explained by local regulations that condition access to antifibrotic drugs to MDD discussion. We found a higher proportion of HP (13%) than in Seine-Saint-Denis (3%) but less than in USA (20%). Comparatively, we have reported a larger proportion of CTD-ILD (32%) than in Leuven (7%), Seine-Saint-Denis (17%) and in USA (20%). This is explained by the facts that (1) our hospital is a tertiary referral centre for systemic sclerosis and systemic lupus erythematosus, and (2) MDD is systematically attended by at least one rheumatologist, as recommended by the recent Fleishner's Society position paper (3). The impact of MDD on CTD-ILD management in our study underlines the benefits of a proper collaboration between pulmonology and rheumatology departments: On one side, input of rheumatologists allows detecting unrecognized connective tissue disease in case of ILD associated with atypical autoimmune serological findings or clinical signs that can be difficult to integrate by respiratory physicians. On the other side, input of pulmonologists enables to standardize monitoring of functional test, to detect cases requiring treatment and to optimize the non-pharmacological management (rehabilitation, transplantation assessment, oxygen therapy). Finally, the presence of a rheumatologist is required by national regulatory rules (Belgian rules for the reimbursement of antifibrotic drugs, www.inami.fgov.be). Of note, several studies have placed great value on this close collaboration: Jo et al have shown in their survey that when a rheumatologist is attending the meeting, he always or frequently contributes to discussion (12). Walsh et al have highlighted in a case cohort-study the importance of rheumatologists input to distinguish IIP from CT-ILD. They suggested that rheumatological consultation might be part of the diagnostic process in selected patients, whereas one study from Castelino et al. advocated systematic rheumatological assessment for all ILD-patients (8, 13). We reported a relatively low proportion of granulomatous diseases (i.e. sarcoidosis). This low rate is explained by the facts of those diseases are not systematically discussed in MDD because of time limitations and relatively simple diagnosis of lung sarcoidosis compared to other forms of ILD.

Analysis of our data confirms that MDD meetings have a real impact on several aspect of daily clinical management of ILD. First, as shown in previous studies, MDD increases degree of diagnostic certainty and leads to more definite diagnosis in challenging cases by advising complementary investigations. In this study, diagnoses were changed in 42% of cases thanks to MDD. Similarly to what was described in previous study from Ryerson et al, 10% of our cases remain unclassifiable ILD after MDD (14), mostly because there was contraindication or patient refusal for lung biopsies. In these cases, a working diagnosis was proposed and treatment, non-pharmacological management and follow-up were recommended.

We observed almost a 2-fold increases in HP diagnosis. This highlights the importance of occupational and environmental interrogation, BAL findings and searching for serum precipitins. HP diagnosis requires integration of clinical history, environmental and occupational exposure assessment, biological and broncho-alveolar lavage findings and radiological features. The clinical and radiological presentation varies over time and can mimic other ILD. MDD emphasized the need to scrutinize for a culprit antigen by interviewing patients, dosing serum precipitins and searching antigen at home. In cases of occult exposure, atypical presentation and pejorative evolution despite adequate treatment MDD argued for confirming the diagnosis by histopathologic findings. In line with the results of previous studies,we reported an increase in IPF diagnoses after MDD (7, 15, 16). Lung biopsies were recommended by MDD when clinical context and radio-

logic pattern were discordant. The number of lung biopsies increased with implementation of regular and standardized MDD, reflecting the obsession of the MDD to approach more accurately the diagnosis and adapt management.

Secondly, changing diagnosis led to changes in treatment in 54% cases. With the increase of IPF diagnoses we observed a significant increase in anti-fibrotic therapies prescription and recommendation against immunosuppressive therapies and corticosteroids was made. In contrast, combination of corticosteroids and immunosuppressive therapies were shown to increase risk of death and hospitalization (4). MDD helps to optimize immunosuppressive therapy in CTD-ILD.

Finally, MDD brought non-pharmacological measures that improved global management of ILD: Based on two controlled trials (17, 18), ATS/ERS guidelines for IPF management promoted inclusion in pulmonary rehabilitation program. These two studies have shown improvement in walked distance and symptoms or quality of life (18-20). Despite this, recommendations for pulmonary rehabilitation remain weak in guidelines (21). Over the last few years, many studies were published and have strengthened the conviction that ILD patients benefits from exercise training (17, 22). After implementation of structured MDD, recommendations for pulmonary rehabilitation attendance and effective participation increases. Efforts still need to be made to propose more systematically rehabilitation programs and to convince patients to participate.

Clinical trials are crucial in ILD to improve therapies and outcomes in this area where our therapeutic action remains limited in certain cases. MDD allowed screening more patients for inclusion in clinical trial.

This study comprises several limitations: It is a retrospective study, so we could not compare MDD and absence of MDD face-to-face. In line with our inclusion criteria (files discussed between January and December 2017), we lack long-term follow up data that may provide hints regarding morbidity and mortality outcomes. Furthermore, our study was not designed for longitudinal evaluation of patients. Finally, the implementation of a MDD *per se* is likely to have improved ILD management locally.

In conclusion, we report our experience on one-year use of a well-structured MDD. Our results emphasize the multiple benefits related to MDD in ILD management. Furthermore, MDD management fosters collaboration between different departments of the hospital, favouring integrated medicine and holistic care of patients.

References

1. 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(6): 788-824.
2. Raghu G, Remy-Jardin M, Myers JL, 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-e68.
3. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. Lancet Respir Med 2017.
4. Idiopathic Pulmonary Fibrosis Clinical Research N, Raghu G, Anstrom KJ, et al. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med 2012; 366(21): 1968-77.
5. Raghu G, Rochwerg B, Zhang Y, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med 2015; 192(2): e3-19.
6. Flaherty KR, King TE, Jr., Raghu G, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med 2004; 170(8): 904-10.
7. Flaherty KR, Andrei AC, King TE, Jr., et al. Idiopathic interstitial pneumonia: do community and academic physicians agree on diagnosis? Am J Respir Crit Care Med 2007; 175(10): 1054-60.
8. Walsh SL, Wells AU, Desai SR, et al. Multicentre evaluation of multidisciplinary team meeting agreement on diagnosis in diffuse parenchymal lung disease: a case-cohort study. Lancet Respir Med 2016; 4(7): 557-65.
9. Duchemann B, Annesi-Maesano I, Jacobe de Naurois C, et al. Prevalence and incidence of interstitial lung diseases in a multi-ethnic county of Greater Paris. Eur Respir J 2017; 50(2).
10. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. N Engl J Med 2018; 378(19): 1811-23.
11. De Sadeleer LJ, Meert C, Yserbyt J, et al. Diagnostic Ability of a Dynamic Multidisciplinary Discussion in Interstitial Lung Diseases: A Retrospective Observational Study of 938 Cases. Chest 2018; 153(6): 1416-23.
12. Jo HE, Corte TJ, Moodley Y, et al. Evaluating the interstitial lung disease multidisciplinary meeting: a survey of expert centres. BMC Pulm Med 2016; 16: 22.
13. Castelino FV, Goldberg H, Dellaripa PF. The impact of rheumatological evaluation in the management of patients with interstitial lung disease. Rheumatology (Oxford) 2011; 50(3): 489-93.
14. Ryerson CJ, Urbania TH, Richeldi L, et al. Prevalence and prognosis of unclassifiable interstitial lung disease. Eur Respir J 2013; 42(3): 750-7.
15. Jo HE, Glaspole IN, Levin KC, et al. Clinical impact of the interstitial lung disease multidisciplinary service. Respirology 2016; 21(8): 1438-44.
16. Chaudhuri N, Spencer L, Greaves M, et al. A Review of the Multidisciplinary Diagnosis of Interstitial Lung Diseases: A Retrospective Analysis in a Single UK Specialist Centre. J Clin Med 2016; 5(8).
17. Ryerson CJ, Cayou C, Topp F, et al. Pulmonary rehabilitation im-

proves long-term outcomes in interstitial lung disease: a prospective cohort study. Respir Med 2014; 108(1): 203-10.
18. Dowman LM, McDonald CF, Hill CJ, et al. The evidence of benefits of exercise training in interstitial lung disease: a randomised controlled trial. Thorax 2017.
19. Nishiyama O, Kondoh Y, Kimura T, et al. Effects of pulmonary rehabilitation in patients with idiopathic pulmonary fibrosis. Respirology 2008; 13(3): 394-9.
20. Holland AE, Hill CJ, Conron M, et al. Short term improvement in exercise capacity and symptoms following exercise training in interstitial lung disease. Thorax 2008; 63(6): 549-54.
21. Spruit MA. Pulmonary rehabilitation. Eur Respir Rev 2014; 23(131): 55-63.
22. Huppmann P, Sczepanski B, Boensch M, et al. Effects of inpatient pulmonary rehabilitation in patients with interstitial lung disease. Eur Respir J 2013; 42(2): 444-53.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 116-123

Systemic glucocorticoids plus cyclophosphamide for acute exacerbation of idiopathic pulmonary fibrosis: a retrospective nationwide study

Shotaro Aso[1], Hiroki Matsui[1], Kiyohide Fushimi[2], Hideo Yasunaga[1]

[1] Department of Clinical Epidemiology and Health Economics, School of Public Health, The University of Tokyo, Tokyo, Japan; [2] Department of Health Policy and Informatics, Tokyo Medical and Dental University Graduate School of Medicine, Tokyo, Japan

Abstract. *Purpose:* Mortality of acute exacerbation of idiopathic pulmonary fibrosis is high, and it remains unknown whether cyclophosphamide is an effective treatment for this condition. *Objectives:* This study compared the effects of cyclophosphamide combined with systemic glucocorticoids with those of systemic glucocorticoids alone. *Methods:* Using the Diagnosis Procedure Combination database in Japan, adult patients with idiopathic pulmonary fibrosis who had received high-dose methylprednisolone and mechanical ventilation at admission from July 1, 2010, to March 31, 2014, were identified. Instrumental variable analyses based on a hospital preference for cyclophosphamide were performed to compare in-hospital outcomes. *Results:* Eligible patients (n=1847) were divided into the methylprednisolone plus cyclophosphamide group (n=104) and the methylprednisolone alone group (n=1743). The results of an instrumental variable analysis detected no significant differences between the groups with respect to in-hospital mortality (odds ratio, 1.11; 95% confidence interval, 0.19-6.43), ventilator-free days (difference, 2.2; 95% confidence interval, –2.6 to 7.0). *Conclusions:* In a Japanese inpatient database study analyzing outcomes from patients with acute exacerbation idiopathic pulmonary fibrosis receiving systemic glucocorticoids, the addition of cyclophosphamide was not associated with improved in-hospital mortality and ventilator-free days. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 116-123)*

Key words: idiopathic pulmonary fibrosis, mortality, cyclophosphamide, glucocorticoids

Introduction

Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive fibrotic interstitial pneumonia of unknown cause. Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) is defined as respiratory deterioration in less than 1 month (1). Respiratory failure caused by AE-IPF is associated with high in-hospital mortality (55.6%-80%) (2-5). In particular, studies have shown that the mortality of patients with AE-IPF requiring mechanical ventilation is 81.8%-94% (6, 7).

Treatment of AE-IPF has not been established, and only anecdotal treatment reports exist. International evidenced-based guidelines weakly recommend a standard therapy for AE-IPF of administrating systemic glucocorticoids, including methylprednisolone at a dosage of 1 g per day intravenously for 3 days (8). The international evidenced-base guidelines do not comment on the use of other immunosuppressant agents combined with glucocorticoids owing to a lack of conclusive results for the combina-

Received: 14 March 2018
Accepted after revision: 24 February 2019
Correspondence: Shotaro Aso, MD, MPH,
Department of Clinical Epidemiology and Health Economics,
School of Public Health, The University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan
Tel. +81-3-5841-1887
Fax +81-3-5841-1888
E-mail: asou-sin@umin.ac.jp

tion treatment (8). It remains controversial whether cyclophosphamide combined with methylprednisolone is effective for patients with IPF (9-11). Previous studies have reported that cyclophosphamide combined with high-dose methylprednisolone is potentially effective for patients with AE-IPF (12, 13). However, the patients in those studies had connective tissue diseases. To date, there has been no study comparing high-dose methylprednisolone plus cyclophosphamide with high-dose methylprednisolone alone as therapy for patients with AE-IPF, and thus it remains unknown whether cyclophosphamide would have an additive effect with high-dose methylprednisolone in these patients.

Therefore, the aim of the present study was to use data from a national inpatient database in Japan to compare the effectiveness of the administration of cyclophosphamide combined with systemic glucocorticoids to that of systemic glucocorticoids alone for reducing the mortality of AE-IPF.

Methods

Data source

Inpatient data were extracted from the Japanese Diagnosis Procedure Combination database. More than 1,000 hospitals voluntarily contribute to the database, which includes data from approximately 7 million inpatients, representing approximately 50% of all discharges from acute care hospitals in Japan. The data used in the present study included the hospital identification number; patient sex and age; body weight and height; consciousness level on admission; dates of hospitalization and discharge; main diagnoses, pre-existing comorbidities on admission, and complications that occurred during hospitalization, which were coded with the International Classification of Diseases, tenth revision (ICD-10) codes and text in Japanese; surgical and nonsurgical procedures and dates of procedures performed; dates and doses of drugs or blood products administered during the hospitalization; and discharge status.

Consciousness level on admission was evaluated using Japan Coma Scale scores (14, 15), which is widely used in Japan, and its assessment is well correlated with the Glasgow Coma Scale assessment (16).

The Institutional Review Board of The University of Tokyo approved this study. Informed consent was waived because of the anonymous nature of the data.

Patient selection

This study used data from July 1, 2010, to March 31, 2014. The inclusion criteria were patients aged ≥15 years who were diagnosed as having idiopathic pulmonary fibrosis (ICD-10 codes: J84.1, J84.8, and J84.9) and who received mechanical ventilation within 1 day after admission. The patients were divided into two groups: (1) those who received cyclophosphamide 500 to 1,000 mg per day intravenously for 1 day and methylprednisolone 1 gram per day intravenously for 3 days within 5 days after admission (termed the methylprednisolone plus cyclophosphamide group); (2) those who received methylprednisolone 500 to 1,000 mg per day intravenously for 3 days within 4 days after admission (methylprednisolone alone group).

Baseline characteristics

Baseline characteristics included the following: age; sex; Hugh-Jones classification on admission (17); consciousness level on admission; Charlson comorbidity index (CCI); smoking index (packs per year); past history of diabetes mellitus, chronic kidney disease, lung cancer, chronic obstructive pulmonary disease, and congestive heart failure; and use of cotrimoxazole, azithromycin (18), continuous renal replacement therapy, and noradrenaline within 1 day after admission. Patients were categorized into five age groups: 15-40, 41-60, 61-70, 71-80, and ≥81 years old. The CCI was classified into five groups: 0, 1, 2, 3-5, and ≥6 points. The smoking index was categorized into five groups: 0, 1-20, 21-40, 41-60, and ≥61 packs per year.

Outcomes

The primary outcome was in-hospital mortality. The secondary outcome was ventilator-free days (VFDs) (19), incidence of sepsis (ICD-10 codes: A32.7, A39.4, A40.x, and A41.x), and incidence of mycosis (ICD-10 codes: B37.1, B37.5-B37.8, B44.0,B44.1, B45.0, B45.1, B45.7, B45.9, B48.7, B49, and J17.2).

Statistical analysis

Because some values were missing for the Hugh-Jones classification, smoking index, and CCI, a multiple imputation procedure was performed to replace each missing value with a set of submitted plausible values by creating 20 filled-in complete datasets using a Markov chain Monte Carlo algorithm known as chained equations imputation (20). The multiple imputation method assumes that data are missing at random and that any systemic differences between the missing and observed values can be explained by differences in the observed data (21, 22).

An instrumental variable (IV) analysis was also performed. Unmeasured confounders can lead to incorrect inferences regarding the effectiveness of different treatments. The IV analysis can theoretically balance both measured and unmeasured confounders between two groups (23, 24). A hospital preference for cyclophosphamide was selected as an IV, because use of cyclophosphamide depended on physician preference. When hospitals are strongly consistent in whether or not they use cyclophosphamide to treat AE-IPF, it is assumed that the decision to administer the drug may be made independently of an individual patient's background. In such a situation, a hospital preference for cyclophosphamide may have acted as an IV, thereby setting the stage for a "natural experiment" that allowed an unbiased estimate of the risk of AE-IPF, even if unmeasured confounders existed (25, 26). An IV analysis assumes that patient hospital choice is made independently of the hospital's choice of a specific drug, and the hospital's use of the drug is independent of the outcomes. The number of patients with AE-IPF who received cyclophosphamide in each hospital was counted, and then the average number of patients with AE-IPF who received cyclophosphamide among all the hospitals was calculated. Hospitals with more than the average number of cyclophosphamide users were defined as hospitals with a preference for cyclophosphamide. Hospitals with less than the average number of cyclophosphamide users were defined as hospitals without a preference for cyclophosphamide. To assess the validity of hospital preference as an IV, we confirmed that hospital preference was highly correlated to the receipt of cyclophosphamide (F statistic >10) (25). We examined hospital preference was not associated with outcomes.

A two-stage residual inclusion estimation framework of the IV analysis was used (27, 28). The residual inclusion approach has been shown to generate more consistent and less biased estimates for a variety of nonlinear models. In the first stage model, the association between receipt of cyclophosphamide and hospital preference for cyclophosphamide was measured, with adjustment for patient level covariates. From this model, the raw residual for each patient was determined by calculating the difference between the model-predicted probability of receiving cyclophosphamide and the actual treatment received. The residuals were then included as an additional covariate in the second-stage model. In the second-stage model, the association between treatment and outcomes was estimated, adjusting for covariates. All IV analyses were performed using robust standard errors.

A sensitivity analysis was performed to confirm the correctness of the inclusion criteria for AE-IPF (1). First, patients who had not received a computed tomography (CT) scan within 1 day after admission were excluded. Second, patients who had not received a CT scan within 1 day after admission and with the use of furosemide within 1 day after admission were excluded.

Continuous variables are presented as an average along with the standard deviation or the median with the interquartile range. Categorical variables are presented as the number with a proportion. In the unadjusted comparisons, averages of continuous variables were compared using *t*-tests, and proportions of categorical variables using χ^2 tests.

A P value <0.05 was considered to indicate statistical significance. All statistical analyses were performed using STATA/MP version 14.0 software (STATA Corp, College Station, TX, USA).

Results

During the analyzed period, we identified 12,992 patients who received methylprednisolone at a dose of 500 to 1,000 mg per day for 3 days within 4 days after admission (Figure 1). Among them, 1,847 patients were eligible for the present study, including 104 patients administered cyclophosphamide and 1,743 patients without cyclophosphamide administration.

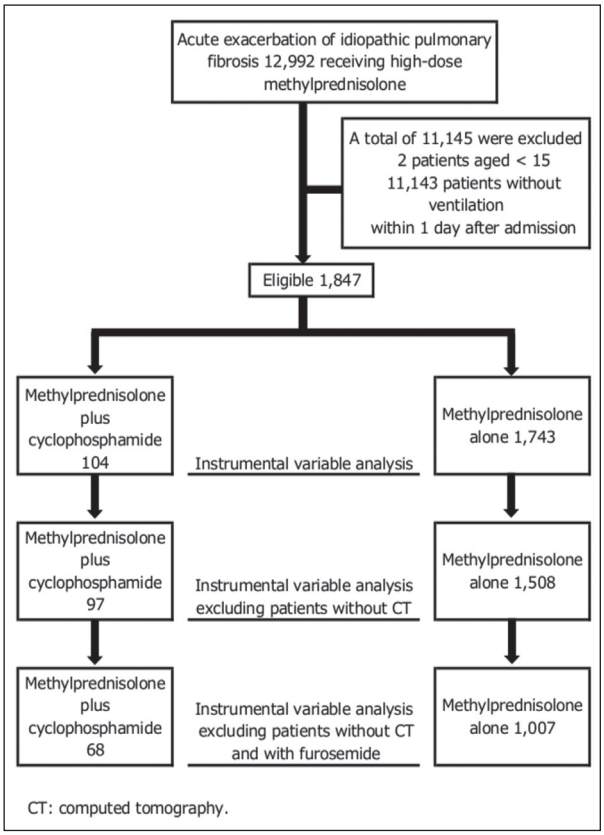


Fig. 1. Patient selection

Values were missing for smoking status, Hugh-Jones classification, and CCI (15.4%, 23.6%, and 30.0%, respectively; Table 1). Patient backgrounds in the methylprednisolone plus cyclophosphamide group were significantly different from those in the methylprednisolone alone group with respect to Hugh-Jones classification. Patients in the methylprednisolone plus cyclophosphamide group received more cotrimoxazole within 1 day after admission than those in the methylprednisolone alone group (31.7% vs. 18.0%, *P*=0.0005).

The overall in-hospital mortality was 48.6% (897/1847). Unadjusted in-hospital mortality was significantly higher in the methylprednisolone plus cyclophosphamide group than in the methylprednisolone alone group (64.4% vs 47.6%, *P*=0.0009). In the unadjusted comparison, VFDs in the methylprednisolone plus cyclophosphamide group were significantly lower than those in the methylprednisolone alone group (6.7 days vs. 10.4 days, *P*=0.0008). In the unadjusted comparison, there were no significant differences between the groups in incidence of sepsis and mycosis (6.7% vs, 3.5%, *P*=0.09; 3.9% vs. 2.1%, *P*=0.24, respectively).

The average number of patients with AE-IPF was 1.7 per year. The hospital preference for cyclophosphamide was highly associated with actual receipt of cyclophosphamide (F statistic=73.2), whereas the hospital preference for cyclophosphamide was not significantly associated with death (coefficient, −0.07; 95% confidence interval [CI], −0.32 to 0.18), VFDs (0.14; 95% CI, −0.51 to 0.80), incidence of sepsis (0.20; 95% CI, −0.33 to 0.74), or incidence of mycosis (0.21; 95% CI, −0.50 to 0.92).

In the IV analysis, no significant difference was detected between the methylprednisolone plus cyclophosphamide group and the methylprednisolone alone group with respect to in-hospital mortality (odds ratio [OR], 1.11; 95% CI, 0.19-6.43; Table 2). There were also no significant differences between the groups with respect to VFDs (difference, 2.2; 95% CI, −2.6 to 7.0), incidence of sepsis (OR, 6.68; 95% CI, 0.12-379), or incidence of mycosis (OR, 5.93; 95% CI, 0.05-665; Tables 3, 4).

The numbers of patients having a CT scan within 1 day after admission in the methylprednisolone plus cyclophosphamide and methylprednisolone alone groups were 97 and 1,508, respectively. The F statistic was 67.3, and the hospital preference for cyclophosphamide treatment was not significantly associated with death (coefficient, −0.05; 95% CI, −0.32 to 0.22), VFDs (0.32; 95% CI, −0.41 to 1.06), incidence of sepsis (0.23; 95% CI, −0.31 to 0.78) or incidence of mycosis (0.24; 95% CI, −0.47 to 0.95). There were no significant differences between the groups for in-hospital mortality (OR, 1.44; 95% CI, 0.21-9.97), VFDs (difference, 3.8; 95% CI, −1.1 to 8.7), incidence of sepsis (OR, 6.02; 95% CI, 0.12-309), or incidence of mycosis (OR, 4.82; 95% CI, 0.05-488). The numbers of patients having CT scans within 1 day after admission and without furosemide within 1 day after admission in the groups were 68 and 1,007, respectively. The F statistic was 20.3, and the hospital preference for cyclophosphamide was not significantly associated with death (coefficient, −0.25; 95% CI, −0.75 to 0.25), VFDs (0.57; 95% CI, −0.69 to 1.84), incidence of sepsis (−0.02; 95% CI, −1.20 to 1.15), or incidence of mycosis (0.52; 95% CI, −0.78 to 1.81). No significant differences between the groups were

Table 1. Baseline characteristics at admission

Variable	Methylprednisolone alone (n=1,743)		Methylprednisolone plus cyclophosphamide (n=104)		*P* value
Sex, n (%)					
male	1,182	(67.8)	72	(69.2)	0.77
Age, years, n (%)					
15-40	9	(0.5)	0	0.0	0.30
41-60	112	(6.4)	9	(8.7)	
61-70	384	(22.0)	29	(27.9)	
71-80	751	(43.1)	45	(43.3)	
≥81	487	(27.9)	21	(20.2)	
Smoking index (packs per year), n (%)					
0	779	(44.7)	50	(48.1)	0.72
1-20	143	(8.2)	9	(8.7)	
21-40	237	(13.6)	12	(11.5)	
41-60	179	(10.3)	14	(13.5)	
≥61	134	(7.7)	5	(4.8)	
missing	271	(15.5)	14	(13.5)	
Hugh-Jones classification, n (%)					
1	54	(3.1)	3	(2.9)	0.04
2	82	(4.7)	5	(4.8)	
3	107	(6.1)	2	(1.9)	
4	198	(11.4)	6	(5.8)	
5	903	(51.8)	52	(50.0)	
missing	399	(22.9)	36	(34.6)	
Charlson comorbidity index, n (%)					
0	316	(18.1)	20	(19.2)	0.38
1	321	(18.4)	17	(16.3)	
2	384	(22.0)	17	(16.3)	
3-5	119	(6.8)	7	(6.7)	
≥6	88	(5.0)	3	(2.9)	
missing	515	(29.5)	40	(38.5)	
Japan coma scale, n (%)					
0 (alert)	1,273	(73.0)	85	(81.7)	0.17
1-digit (dizziness)	283	(16.2)	14	(13.5)	
2-digit (somnolence)	87	(5.0)	3	(2.9)	
3-digit (coma)	100	(5.7)	2	(1.9)	
Lung cancer, n (%)	72	(4.1)	5	(4.8)	0.73
Chronic obstructive pulmonary disease, n (%)	140	(8.0)	7	(6.7)	0.63
Congestive heart failure, n (%)	142	(8.1)	3	(2.9)	0.06
Diabetes mellitus, n (%)	425	(24.4)	17	(16.3)	0.06
Chronic kidney disease, n (%)	46	(2.6)	3	(2.9)	0.85
Noradrenaline, n (%)	162	(9.3)	10	(9.6)	0.92
Azithromycin, n (%)	272	(15.6)	9	(8.7)	0.06
Cotrimoxazole, n (%)	313	(18.0)	33	(31.7)	0.0005
Continuous renal replacement therapy, n (%)	24	(1.4)	3	(2.9)	0.22

Table 2. Comparison of in-hospital mortality between the methylprednisolone plus cyclophosphamide and methylprednisolone alone groups

	OR*	95% CI†	*P* value
Unadjusted	1.99	1.32 - 3.01	0.0009
Instrumental variable analysis	1.11	0.19 - 6.43	0.91
Instrumental variable analysis, patients with CT‡	1.44	0.21 - 9.97	0.71
Instrumental variable analysis, patients with CT, without furosemide	0.95	0.04 - 23.97	0.97

*OR, odds ratio; †CI, confidence interval; ‡CT, computed tomography

Table 3. Comparison of ventilator-free days between the methylprednisolone plus cyclophosphamide and methylprednisolone alone groups

	Difference	95% CI*	*P* value
Unadjusted	−3.7	−5.9 - −1.6	0.0008
Instrumental variable analysis	2.2	−2.6 - 7.0	0.37
Instrumental variable analysis, patients with CT†	3.8	−1.1 - 8.7	0.13
Instrumental variable analysis, patients with CT, without furosemide	5.8	−5.2 - 16.8	0.30

*CI, confidence interval; †CT, computed tomography

Table 4. Comparison of incidence of sepsis or mycosis between the methylprednisolone plus cyclophosphamide and methylprednisolone alone groups

	OR*	95% CI†	*P* value
Sepsis			
Unadjusted	1.99	0.89 - 4.47	0.10
Instrumental variable analysis	6.68	0.12 - 379	0.36
Instrumental variable analysis, patients with CT‡	6.02	0.12 - 309	0.37
Instrumental variable analysis, patients with CT, without furosemide	1.90	0.00 - 1060	0.84
Mycosis			
Unadjusted	1.84	0.64 - 5.28	0.25
Instrumental variable analysis	5.93	0.05 - 665	0.46
Instrumental variable analysis, patients with CT	4.82	0.05 - 488	0.50
Instrumental variable analysis, patients with CT, without furosemide	1.70	0.01 - 532	0.86

*OR, odds ratio; †CI, confidence interval; ‡CT, computed tomography

detected for in-hospital mortality (OR, 0.95; 95% CI, 0.04-23.97), VFDs (difference, 5.8; 95% CI, −5.2 to 16.8), incidence of sepsis (OR, 1.90; 95% CI, 0.00-1060), or incidence of mycosis (OR, 1.70; 95% CI, 0.01-532).

Discussion

This study used data obtained from a Japanese national inpatient database to compare the effectiveness of high-dose methylprednisolone plus cyclophosphamide with high-dose methylprednisolone alone for treating patients with AE-IPF. Our IV analysis showed no significant difference between the two treatment groups for in-hospital mortality, VFDs, incidence of sepsis, or incidence of mycosis.

Two previous studies showed that cyclophosphamide was potentially effective for treating patients with AE-IPF (12, 13). However, those studies were limited by having no control group and small sample sizes (n=11, 17, respectively). Despite the high mortality associated with AE-IPF, treatment of the condition remains uncertain. International evidence-based guidelines weakly recommend administration of systemic corticosteroids and do not judge whether other medications are effective for AE-IPF because of a lack of evidence regarding the combined treatment (8). Furthermore, a recent review examining AE-IPF reported that studies investigating treatment of AE-IPF were mostly small and uncontrolled and could not adjust for confounders (1).

The advantage of the present study was that we performed an IV analysis, and this analysis generated pseudo-randomization adjusting for unmeasured and measured confounders. We found no significant difference for in-hospital mortality or VFDs between the methylprednisolone plus cyclophosphamide group and methylprednisolone alone group. One potential reason for this may be that cyclophosphamide had no effect on AE-IPF. Previous studies have reported that cyclophosphamide has no effect on patients with IPF (9, 10). Another potential reason for the lack of differences is that no currently known medication, including corticosteroids and other immunosuppressant agents, may be effective against AE-IPF because AE-IPF is a severe condition with a rapid progression. Although cyclophosphamide suppresses the immune system with depressing bone marrow, the present study showed that there were no significant differences in incidence of sepsis or mycosis between the cyclophosphamide users and non-users. One potential reason for this may be that patients receiving cyclophosphamide died before cy-

clophosphamide had time to suppress their immune systems.

This study has several limitations. First, the database did not include detailed data on patients' physical conditions, laboratory examinations, and other tests, such as respiratory rates, partial pressure of arterial oxygen/fraction of inspired oxygen ratios, lactate dehydrogenase levels, serum KL-6 levels, and CT imaging results (29). We could therefore not obtain information on the severity of IPF. Moreover, it is possible that patients receiving cyclophosphamide were treated more aggressively. We therefore used instrumental variable analysis to account for these unmeasured confounders. Furthermore, previous studies have shown that the prognosis of suspected AE-IPF is similar to that of AE-IPF (4, 5). The second limitation is that it cannot be proven that our IV analysis fully addressed unmeasured confounders (30). However, we conducted sensitivity analyses based on revised diagnostic criteria for AE-IPF, and the results of the sensitivity analysis were similar to those in the primary analysis. Furthermore, the Japanese Diagnosis Procedure Combination database has been well validated and can serve as a relatively accurate substitute for clinical data although any administrative data have some limitations to the recorded data (31). Third, it is unknown whether our results can be applied to patients with AE-IPF who are not using mechanical ventilation.

Conclusions

Despite these limitations, our IV analysis using a Japanese inpatient database showed that the administration of cyclophosphamide to patients with AE-IPF who were also receiving systemic corticosteroids was not associated with improved in-hospital mortality or VFDs. Further prospective studies will be required to confirm the effect of cyclophosphamide in the treatment of AE-IPF.

Authorship: KF contributed to the study design and data acquisition. SA, HM, and HY performed the statistical analyses and produced the first draft of the manuscript. All authors commented on the manuscript and approved the final version.

Funding: This work was supported by grants for Research on Policy Planning and Evaluation from the Ministry of Health, Labour and Welfare, Japan (grant numbers H29-Policy-Designated-009, H27-Policy-Strategy-011); Ministry of Education, Culture, Sports, Science and Technology, Japan (grant numbers 17H04141); and the Japan Agency for Medical Research and Development (AMED). The funders had no role in the execution of this study or interpretation of the results.

Disclosure Statement: HY and KF received grant support from the Japanese government. The funders had no role in the execution of this study or interpretation of the results. All authors have disclosed that they do not have any potential conflicts of interest.

References

1. Collard HR, Ryerson CJ, Corte TJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis an international working group report. Am J Respir Crit Care Med 2016;1 94(3): 265-75.
2. Song JW, Hong S-B, Lim C-M, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J 2011; 37(2): 356-63.
3. Usui Y, Kaga A, Sakai F, et al. A cohort study of mortality predictors in patients with acute exacerbation of chronic fibrosing interstitial pneumonia. BMJ Open 2013; 3: e002971.
4. Huie TJ, Olson AL, Cosgrove GP, et al. A detailed evaluation of acute respiratory decline in patients with fibrotic lung disease: Aetiology and outcomes. Respirology 2010; 15(6): 909-17.
5. Collard HR, Yow E, Richeldi L, Anstrom KJ, Glazer C. Suspected acute exacerbation of idiopathic pulmonary fibrosis as an outcome measure in clinical trials. Respir Res 2013; 14: 73.
6. Mallick S. Outcome of patients with idiopathic pulmonary fibrosis (IPF) ventilated in intensive care unit. Respir Med 2008; 102(10): 1355-9.
7. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic pulmonary fibrosis: Frequency and clinical features. Eur Respir J 2006; 27: 143-50.
8. 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.
9. Collard HR, Ryu JH, Douglas WW, et al. Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. Chest 2004; 125(6): 2169-74.
10. Johnson MA, Kwan S, Snell NJ, Nunn AJ, Darbyshire JH, Turner-Warwick M. Randomised controlled trial comparing prednisolone alone with cyclophosphamide and low dose prednisolone in combination in cryptogenic fibrosing alveolitis. Thorax 1989; 44(January): 280-8.
11. Pereira CAC, Malheiros T, Coletta EM, et al. Survival in idiopathic pulmonary fibrosis - Cytotoxic agents compared to corticosteroids. Respir Med 2006; 100(2): 340-7.
12. Novelli L, Ruggiero R, Giacomi F De, et al. Corticosteroid and cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis : a single center experience and literature review. Sarcoidosis Vasc Diffus Lung Dis 2016; 33: 385-91.
13. Ota M, Iwasaki Y, Harada H, Sasaki O, Nagafuchi Y, Nakachi S, et al. Efficacy of intensive immunosuppression in exacerbated rheumatoid arthritis-associated interstitial lung disease. Mod Rheumatol 2016; 7595(May): 1-7.
14. Ohta T, Waga S, Handa H, et al. New grading of level of disordered consciousness (author's translation). No shinkei geka 1974; 2: 623-7.
15. Shigematsu K, Nakano H, Watanabe Y. The eye response test alone is sufficient to predict stroke outcome—reintroduction of Japan Coma Scale: a cohort study. BMJ Open 2013; 3:e 002736.
16. Ono K, Wada K, Takahara T, Shirotani T. Indications for Computed

Tomography in Patients With Mild Head Injury. Neurol Med Chir 2007; 47: 291-8.
17. Fletcher CM. The clinical diagnosis of pulmonary emphysema; an experimental study. Proc R Soc Med 1952; 45(9): 577-84.
18. Oda K, Yatera K, Fujino Y, et al. Efficacy of concurrent treatments in idiopathic pulmonary fibrosis patients with a rapid progression of respiratory failure: an analysis of a national administrative database in Japan. BMC Pulm Med 2016; 16(1): 91.
19. Schoenfeld D a, Bernard GR. Statistical evaluation of ventilator-free days as an efficacy measure in clinical trials of treatments for acute respiratory distress syndrome. Crit Care Med 2002; 30(8): 1772-7.
20. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat Med 1991; 10(4): 585-98.
21. Sterne JAC, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009; 338: b2393.
22. Li P, Stuart EA, Allison DB. Multiple Imputation: A Flexible Tool for Handling Missing Data. JAMA 2015; 314(18): 1966-7.
23. Newhouse JP, McClellan M. Econometrics in outcomes research: the use of instrumental variables. Annu Rev Public Health 1998; 19(1): 17-34.
24. Iwashyna TJ, Kennedy EH. Instrumental variable analyses: Exploiting natural randomness to understand causal mechanisms. Ann Am Thorac Soc 2013; 10(3): 255-60.
25. Staiger D, Stock J. Instrumental Variables Regression with Weak Instruments. Econometrica 1997; 65(3): 557-86.
26. Stukel TA, Fisher ES, Wennberg DE, Alter DA, Vermeulen MJ. Analysis of Observational Studies in the Presence of Treatment Selection Bias. Jama 2007; 297(3): 278-85.
27. Terza J V, Basu A, Rathouz PJ. Two-stage residual inclusion estimation: Addressing endogeneity in health econometric modeling. J Health Econ 2008; 27(3): 531-43.
28. Terza J V., Bradford WD, Dismuke CE. The use of linear instrumental variables methods in health services research and health economics: A cautionary note. Health Serv Res 2008; 43(3): 1102-20.
29. Kishaba T, Tamaki H, Shimaoka Y, Fukuyama H, Yamashiro S. Staging of acute exacerbation in patients with idiopathic pulmonary fibrosis. Lung 2014; 192(1): 141-9.
30. Harris KM, Remler DK. Who is the marginal patient? Understanding instrumental variables estimates of treatment effects. Health Serv Res 1998; 33(5 Pt 1): 1337-60.
31. Yamana H, Moriwaki M, Horiguchi H, Kodan M, Fushimi K, Yasunaga H. Validity of diagnoses, procedures, and laboratory data in Japanese administrative data. J Epidemiol 2017; 27(10): 476-82.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 124-129

A new side of sarcoidosis: medication and hospitalization use in a privately insured patient population

Derek Low[1], Kit N. Simpson[2], Richard Rissmiller[2], Ennis James[2]
[1] University of Colorado, Denver CO; [2] Medical University of South Carolina, Charleston, SC

Abstract. *Objective:* This study describes patterns of medication prescriptions for sarcoidosis patients in a large commercially insured U.S. population, with specific focus on prescribing practices across medical specialties and their associated hospitalization risk. *Methods:* Using the Marketscan Database we selected adult patients with a diagnosis of sarcoidosis by ICD-9 code during the 2012 calendar year. Differences in prescribing practices were evaluated between provider types. A multivariate model controlling for age, sex, and region assessed hospitalization risk associated with provider type, prednisone dose, and use of non-steroid sarcoidosis medications. *Results:* Using the described criteria, 11,042 total patients were identified. A majority were female, mean age 49.3 years. Of these, 1,792 (16.2%) had one or more hospital admissions (mean 1.6, SD 1.3) with a mean length of stay of 8.1 days (SD 14.5). 25.5% of patients were prescribed prednisone with a 1 year mean cumulative dose of 250mg. Pulmonary/Rheumatology providers prescribed the highest cumulative prednisone dose (961 mg) and were more likely to prescribe methotrexate and monoclonal antibody medications. Sarcoidosis patients receiving a cumulative prednisone dose >500 mg had an increased risk for hospitalization (OR 2.512, 2.210-2.855), while those prescribed methotrexate and azathioprine had decreased risk (OR 0.633, 0.481-0.833 and 0.460, 0.315-0.671). Monoclonal antibody use was associated with increased OR for hospitalization at 1.359. *Conclusion:* Sarcoidosis patients treated by subspecialists were more likely to receive higher doses of prednisone and non-steroid sarcoidosis medications. Higher doses of prednisone and monoclonal antibody use were associated with higher hospitalization risk while methotrexate and azathioprine were associated with lower hospitalization risk. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 124-129)*

Key words: sarcoidosis, epidemiology, hospitalization, corticosteroids

Background

Sarcoidosis is a multisystem inflammatory disease characterized by the presence of noncaseating granulomas that affects 35.5 per 100,000 African Americans and 10.9 per 100,000 Caucasians in the United States (U.S.) (1, 2). Most sarcoidosis patients experience spontaneous resolution of their disease (3) and do not require treatment, but a third of patients will have chronic disease requiring prolonged treatment (3, 4). Corticosteroids are considered first line treatment for most forms of sarcoidosis, but can result in significant side effects and increased healthcare use (4). Patients with refractory disease may require treatment with second- or third-line medications, including methotrexate (MTX), azathioprine (AZA), and monoclonal antibody therapy (5).

Previous studies evaluating sarcoidosis treatment options have primarily addressed sarcoidosis patients from large referral centers which have reported treatment rates of 55-65% (4, 6). This contrasts starkly

Received: 30 March 2018
Accepted after revision: 11 March 2019
Correspondence: Derek Matthew Low
University of Colorado, Denver CO
E-mail: derek.low@ucdenver.edu

with a recent study which included a large, community population of patients with sarcoidosis in which only 22.8% of continuing cases received treatment (7). Scant data exists regarding prescribing patterns and outcomes in sarcoidosis patients from community-based practices. Large datasets from private insurers can provide insight into prescribing practices and associated outcomes across medical specialties from non-referral centers. The objectives of this study were to describe patterns of medication prescriptions for sarcoidosis patients in a large commercially insured U.S. population, with a specific focus on differences in prescribing practices across medical specialties and their associated hospitalization risk.

Methods

This retrospective analysis was conducted using the Truven Health MarketScan® Research Databases. These databases provide outcome measures including resource utilization and healthcare costs for inpatient and outpatient healthcare encounters of patients under employer-sponsored health insurance and Medicare-eligible individuals which covers approximately 143 million individuals. All database records were de-identified in compliance with the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

Study Variables

Variables included in the analysis were demographic data (age, sex), date and duration of hospitalizations, geographic location, and International Classification of Diseases, Clinical Modification (ICD-9-CM) code details (specific code and date of code entry into record). The Marketscan database does not record race. Details of outpatient prescriptions included the specialty of the prescribing practitioner, medication name, daily dose and cumulative dose prescribed over the 12-month study period. Specific anti-sarcoidosis medications were selected for the analysis, including prednisone, MTX, AZA, hydroxychloroquine, and monoclonal antibody (MAB) which included infliximab and adalimumab. The prescriptions of other medications used in sarcoidosis, including Leflunomide and Mycophenolate, were too infrequent to include in the analysis.

Study Definitions

Patients selected as having a diagnosis of sarcoidosis were those with an ICD-9-CM code of 135.xx on two separate outpatient encounters at least 7 days apart. Hospitalizations were defined as any inpatient admission at an acute care facility during the study period. The acute care facility designation is based on a UB04 claim indicating an acute care hospital admission or an emergency department claim record with a length of stay greater than 0 days. Provider categories, as defined by billing label in the Marketscan Database, were divided into primary care providers (PCP), Pulmonology/Rheumatology (P/R), Dermatology/Immunology (D/I), multiple subspecialty providers (Multi), and "Other" subspecialty (Other) which includes pediatric, surgical and other medical specialties. To ensure PCPs were representative of non-specialist prescribing practices, sarcoidosis patients receiving prescriptions in the PCP category were required to have no encounters with a specialty provider, while those seen by a specialist could also be seen by a PCP.

Statistical Analysis

For characteristics of the sarcoidosis patients in the Marketscan database we used descritptive statistics reporting mean and SD for continuous variables. Medication prescriptions are described by mean daily and cumulative dose. Differences between groups of providers where tested with univariate analysis using gamma distributed log link models to account for skewed distribution of data. Multivariate logistic regression controlling for age, sex, and region was used to assess hospital risk by provider, prednisone use/cumulative dose, and use of non-steroid sarcoidosis medications. Regions were defined in the MarketScan database as Northeast, North Central, South, West, and Unknown.

Results

Patient Characteristics

From January 1, 2012 to December 31, 2012, 11,042 patients met inclusion criteria (table 1). The cohort was predominately female (59.1%) with a mean age of 49.3 years.

Table 1. Patient characteristics

Total N	11,042
Sarcoidosis Prevalence by Age Group: (per 100,000)	
Age 18-34	7.0
Age 35-44	26.6
Age 45-54	42.4
Age 55-64	47.9
Mean Age (SD)	49.3 (9.8)
Female Sex (%)	6,520 (59.1)
Hospital Admission (%)	1,792 (16.2)
Number of Annual Hospital Admissions for Subjects with Admissions, Mean (SD) [IQR]	1.6 (1.3) [IRQ 1-2]
Prednisone Use (%)	2,817 (25.5)

IRQ=Interquartile range.

Medications

Review of the Marketscan database revealed that 2,817 sarcoidosis patients (25.5%) received a prescription for prednisone use during the study period. The mean annual prescribed cumulative dose across providers was 250 mg mg (SD 692). While there was significant variation in the cumulative dose between provider groups, the mean daily prednisone dose did not differ significantly between providers (Table 2).

Steroid sparing medications were not commonly prescribed (Table 3). Hydroxychloroquine was the most commonly prescribed non-steroidal anti-sarcoidosis medication after prednisone (4.0%), followed by MTX (2.7%) and AZA (1.2%). Hydroxychloroquine and MTX were also the most commonly prescribed medications in combination with prednisone (2.6% and 1.9%, respectively). Subspecialty providers provided at least 2/3 of all prescriptions for MTX, hydroxychloroquine, and monoclonal antibodies. No significant difference existed across provider groups in the number of prescriptions for AZA. Less than 1% of patients were on a combination of 2 non-steroidal medications, which was overwhelmingly prescribed (78.3%) by P/R specialties.

Table 2. Prednisone prescribing practices

	PCP	P/R	D/I	Multi	Other	P-value
Mean daily prednisone dose, mg (SD) [IRQ]	14.9 (8.1) [10-20]	14.9 (8.0) [10-20]	15.4 (8.6) [10-20]	15.3 (8.3) 10-20]	14.9 (7.2) [10-20]	.7457
Mean cumulative prednisone dose, mg (SD) [IRQ]	866 (961) [150-1200]	961 (1138) [200-1500]	755 (939) [130-1040]	930 (954) [185-1290]	961 (1065) [180-1260]	<.0001

PCP=Primary Care Physician. P/R=Pulmonology/Rheumatology. D/I=Dermatology/Immunology. Multi=Multispecialty

Table 3. Number of patients receiving steroid sparing therapy

	Total Population	PCP	P/R	D/I	Multi	Other	P-value
Methotrexate, n (%)	298 (2.7)	24	219	26	17	12	<.0001
Azathioprine, n (%)	132 (1.2)	25	72	18	9	8	.0910
Hydroxychloroquine n (%)	441 (4.0)	56	298	52	16	19	<.0001
Any Mab, n (%)	64 (0.6)	6	44	6	5	3	.0017
Pred + MTX, n (%)	210 (1.9)	11	163	19	10	7	<.0001
Pred + AZA, n (%)	92 (0.8)	15	52	14	6	5	.2052
Pred + Any MAB, n (%)	36 (0.3)	2	26	3	3	2	.0102
Pred + Hydroxychl, n (%)	261 (2.6)	28	187	29	10	7	<.0001
No pred, ≥2 steroid sparing drugs, n (%)	92 (0.8)	6	72	7	5	2	<.0001

PCP=Primary Care Physician. P/R=Pulmonology/Rheumatology. D/I=Dermatology/Immunology. Multi=Multispecialty. Pred=prednisone

Hospitalization risk

Patients seen in P/R clinics had an increased risk of hospitalization compared to those seen only by a PCP, D/I, or Other provider (Table 4). Medication use was also correlated with hospitalization risk (Table 4). OR for prednisone use was grouped into three levels; no prednisone use, low-dose (1-500 mg cumulative yearly prednisone), and high-dose (>500 mg cumulative yearly prednisone). Low-dose prednisone correlated with a slightly higher risk for hospitalization compared to those who did not receive prednisone, while patients receiving high-dose prednisone had a substantial increase in hospitalization risk. MTX and AZA showed statistically significant decrease in hospitalization risk, while MAB showed an increased rate of hospitalization.

Table 4. Odds ratios for hospitalization

	OR (CI)
Provider (ref: Pulmology/Rheumatology)	
Primary Care Physician	.604 (.521-.699)
Dermatology/Immunology	.678 (.591-.777)
Multi-Specialty	.901 (.704-1.152)
Other	.776 (.638-.944)
Medication	
Pred: None vs 1-500	1.217 (1.035-1.431)
Pred: None vs >500	2.512 (2.210-2.855)
Any Monoclonal Antibody	1.359 (1.035-1.431)
Methotrexate	.633 (.481-.833)
Azathioprine	.460 (.315-.671)

*multivariable model controlling for effects of age, sex and region

Discussion

The goal of this study was to describe a group of privately insured sarcoidosis patients with respect to medication prescribing practices across provider groups and associated hospitalization risk. As previous studies have mainly described patient cohorts at large referral centers, this study evaluates a subset of patients who have not been well described historically. The recent study by Baughman, et al, evaluated a similar group using the Optum database, however their study centered around demographic differences and health care costs (7). Our study further evaluated hospitalization risk in reference to medication use and prescribing provider. Using the Marketscan dataset we demonstrated differences in prescribing practices across provider groups, with second- and third-line prescriptions being more common in subspecialty practices. Sarcoidosis referral center studies have often cited prednisone use in 55-65% of sarcoidosis patients, however this study found that only 25.5% of sarcoidosis patients in the Marketscan database were prescribed prednisone during the study period (4, 6). It should be noted, however, in the study by Judson, et al, 2012, their evaluation for prednisone use was described as 51% "at some point during the study period" which spanned 11 years of retrospective evaluation. This study does corroborate the findings of the Sarcoidosis in America study which found only 22.8% of patients in the Optum Database receiving therapy, of which 56% were prescribed prednisone (7). These disparities can partially be attributed to the increased severity of disease in patients seen in referral centers. In addition to prednisone, the rates of second line therapy use were more in line with those found in community studies as opposed to those observed at large referral centers.

We found the mean daily dose of prednisone per patient to be similar across provider groups, but the cumulative yearly dose of prednisone was significantly higher for P/R than PCP or D/I. This is consistent with the expectation that sarcoidosis patients seen by pulmonologists and rheumatologists are more likely to have refractory disease requiring a longer duration of steroids. The hospitalization rate in the overall cohort was similar to that reported in other studies (8), and there was an increased risk of hospitalization in patients followed by P/R. While this could be related to greater disease severity, sarcoidosis patients followed by P/R also had higher cumulative doses of prednisone which has been previously been associated with increased healthcare use in sarcoidosis patients (9). We similarly found significant correlation between high cumulative doses of prednisone and increased risk of hospitalization. While this supports the concept that higher doses of prednisone are indicative of patients with more severe disease who have a higher hospitalization risk, an potential alternative explanation is that increased hospitalizations are a result of the adverse effects related to higher steroid exposure. This would agree with previous data published by Broos et. al. and others that found strong associations between increased cumulative prednisone dose and a higher prevalence of comorbidities and

healthcare use (9, 10). We believe this correlation deserves further evaluation in prospective studies.

Interestingly, this is the first study to show reduced hospitalization rates associated with MTX and AZA use. This was unexpected since patients on MTX and AZA are typically considered to have refractory disease and one would expect higher hospitalization rates. In addition, it is difficult to reconcile this with the finding that the majority of MTX and AZA prescriptions (67.7%) were in P/R patients, who had increased hospitalization risk compared to PCP-only patients in which only 11.4% were prescribed MTX or AZA. Potential explanations for the lower hospitalization risk include improved disease control with these agents and/or their use as "steroid-sparing agents" resulted in lower cumulative prednisone use which in turn could have led to decreased hospitalizations. Unfortunately, due to the retrospective nature of this study we are unable to make a causative distinction with our current analysis. While these findings could suggest that earlier initiation of steroid sparing therapies could lower hospitalization risk, prospective studies are needed to evaluate this further.

Finally, MAB use was correlated with increased OR for hospitalization. MAB are considered third-line agents in sarcoidosis (11, 12), which supports the suggestion that patients who were started on MAB are more likely to have advanced disease which could explain the increased OR for hospitalization. Additionally, certain MAB therapy has been correlated both increased morbidity in the setting of increased infection risk (13, 14) and congestive heart failue (15) which could lead to higher hospitalization risk in this cohort.

Limitations

This study evaluated a large group of commercially insured patients with a documented diagnosis of sarcoidosis. Uninsured status is associated with younger age, poverty, and in racial minorities (16, 17). Limiting our cohort to insured patients likely excludes some patients with greater disease severity, and limits the generalization of our results. It should be noted also that the age groups in the Marketscan database stop at 64, which also limits generalization of our findings to all sarcoidosis patients. Additional limitations of our study are those commonly found in retrospective database studies. The documentation of diagnosis in this study was evaluated by 2 separate billing code for sarcoidosis at least 7 days apart. While this is a commonly used method of patient identification in database studies, there exists an inherent risk that some patients in our cohort may have been incorrectly identified as having sarcoidosis. Additionally, we used billing codes for medication prescriptions however this does not represent actual medication compliance. As it specifically relates to prednisone use, the analysis was limited by the one-year duration of the study. Many patients on prednisone were likely started on their regimens prior to the evaluated calendar year while others continued their courses into the following calendar year. This would likely affect the cumulative dose analysis. We did not attempt to exclude patients with other diseases that may be treated with immunosuppressing medications, which raises the possibility that some patients may have received prednisone and other medications included in our analysis for reasons other than sarcoidosis treatment.

Conclusions

In this study we utilized a large database of privately insured patients to identify medication use and hospitalization risk for patients with sarcoidosis. We found that patients treated by subspecialists were more likely to receive higher cumulative doses of prednisone and additionally more likely to non-steroid sarcoidosis medications. Higher doses of prednisone and MAB use were associated with higher hospitalization risk while MTX and AZA were associated with lower hospitalization risk.

References

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999; 160(2): 736-55.
2. Rybicki BA, et al. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. Am J Epidemiol 1997; 145(3): 234-41.
3. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med 1997; 336(17): 1224-34.

4. Judson MA, Boan AD, Lackland DT. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. Sarcoidosis Vasc Diffuse Lung Dis 2012; 29(2): 119-27.
5. Schutt AC, Bullington WM, Judson MA. Pharmacotherapy for pulmonary sarcoidosis: a Delphi consensus study. Respir Med 2010; 104(5): 717-23.
6. Baughman RP, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 2001; 164(10 Pt 1): 1885-9.
7. Baughman RP, et al. Sarcoidosis in America. Analysis Based on Health Care Use. Ann Am Thorac Soc 2016; 13(8): 1244-52.
8. Gerke AK, et al. Increased hospitalizations among sarcoidosis patients from 1998 to 2008: a population-based cohort study. BMC Pulm Med 2012; 12: 19.
9. Ligon CB, Judson MA. Impact of systemic corticosteroids on healthcare utilization in patients with sarcoidosis. Am J Med Sci 2011; 341(3): 196-201.
10. Broos CE, et al. No evidence found for an association between prednisone dose and FVC change in newly-treated pulmonary sarcoidosis. Respir Med 2018; 138s: S31-s37.
11. Baughman RP, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 2006; 174(7): 795-802.
12. Judson MA, et al. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. European Respiratory Journal 2008; 31(6): 1189-1196.
13. Koo S, Marty FM, Baden LR. Infectious complications associated with immunomodulating biologic agents. Infect Dis Clin North Am 2010; 24(2): 285-306.
14. Singh JA, et al. Adverse effects of biologics: a network meta-analysis and Cochrane overview. Cochrane Database Syst Rev 2011(2): Cd008794.
15. Chung ES, et al. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003; 107(25): 3133-40.
16. DeNavas-Walt CP, Smith BD, Income J. Poverty, and Health Insurance Coverage in the United States: 2012. US Census Bureau Current Population Reports, 2013: 60-245.
17. Grant SR, et al. Variation in insurance status by patient demographics and tumor site among nonelderly adult patients with cancer. Cancer 2015; 121(12): 2020-8.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 130-140

Ultrasonographic evaluation of lung parenchyma involvement in sarcoidosis

Coşkun Doğan[1], Nesrin Kıral[1], Elif Torun Parmaksız[1], Benan Çağlayan[2], Seda Beyhan Sağmen[1], Banu Salepçi[1], Ali Fidan[1], Sevda Şener Cömert[1]

[1] Department of Chest Diseases, Dr. Lütfi Kırdar Kartal Training and Research Hospital, Istanbul, Turkey; [2] Department Of Chest Diseases, Koç University, Istanbul, Turkey

Abstract. *Purpose:* To use ultrasonography (USG) for the evaluation of lung parenchyma in patients with sarcoidosis, andto compare the USG findings with the results of a high-resolution computerized tomography (HRCT) and pulmonary function test-carbon monoxide diffusion test (PFT-DLCO), which are commonly used methods in the evaluation of parenchymal involvement in sarcoidosis. *Material and Methods:* Patients with sarcoidosis and healthy controls were enrolled in the study between January 2015 and December 2017. The clinical findings, HRCT and PFT-DLCO results of all subjects were recorded, and USG findings and comet tail artifact (CTA) measurements were recorded by another pulmonologist. The USG, HRCT and SFT-DLCO findings were compared between the two groups. Based on the findings of theclinical-radiologic investigations and PFT-DLCO, as the current gold standard in diagnosis, the sensitivity and specificity of USG in demonstrating lung parenchyma involvement in sarcoidosis patients were estimated. *Findings:* The sarcoidosis group consisted of 79 patients and the control group included 34 subjects. The mean number of CTAs in the sarcoidosis and control groups was 33.4 and 25, respectively (p=0.001). In the sarcoidosis group, the number of CTAs in patients with DLCO% <80 and ≥80% was 37.4 and 29.7, respectively (p=0.011), and a negative correlation was identified between the number of CTAs and DLCO% (p=0.019 r=-0.267). The mean number of CTAs in patients with and without parenchymal involvement in HRCT was 36 and 25.5, respectively (p=0.001). The number of CTAs in the patients with sarcoidosis with a normal DLCO% value (≥80%) was higher than in the control group (p=0.014). The diagnostic sensitivity and specificity of thoracic USG were found to be 76% and 53%, respectively. *Conclusion:* The number of CTAs in patients with sarcoidosis was higher than that of the healthy controls. The number of CTAs in patients with sarcoidosis with parenchymal involvement in HRCT and/or a low DLCO (<80%) was also elevated. Thoracic USG has a high sensitivity (76%) in demonstrating parenchymal involvement in patients with sarcoidosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 130-140)*

Key words: B-lines, lung ultrasonography, sarcoidosis

Introduction

Sarcoidosis is a systemic granulomatous disease with an unclear etiology, which is frequently associated with pulmonary involvement and results in non-caseating granulomatous infiltration. The majority of cases are asymptomatic at the time of diagnosis, and many cases resolve spontaneously without treatment. Approximately 25% of cases are associated with progressive pulmonary disease (1-3), and the pulmonary parenchymal involvement of sarcoidosis is crucial regarding disease staging and the initiation of treatment. Sarcoidosis is classified into five stages based on a lung X-ray findings (4). The disease is considered

Received: 30 April 2018
Accepted after revision: 16 January 2019
Correspondence: Coşkun Doğan, MD
Neslişah sok. Teknik yapı Up city sitesi,
B2 blok D 40 Uğurmumcu mah, Kartal Istanbul-Turkey
Tel. +90 555 827 64 63
E-mail: coskund24@hotmail.com

to be at an advanced stage in the presence of a parenchymal reticular appearance and traction bronchiectasis without hilar lymph node involvement, together with honeycomb appearance and reticular opacities. Progressive parenchymal radiologic changes, such as parenchymal cavitary appearance, lung honeycombing and fibrotic changes, also represent indications for treatment (5).

Ultrasonography (USG) transmits high-oscillating sound waves to tissues, and an image is presented on a monitor based on the reflection or refraction of these sound waves as they travel back to the USG probe. Multiple reflections of a sound wave between the tissue and the probe, or between two tissues, is referred to as reverberation artifact (6). Thoracic USG is commonly used for several diagnostic procedures in pulmonology practice, although its use for the assessment of lung parenchyma/interstitium is relatively limited (7). Diseases that involve the interstitium present with interstitial inflammation, fibrosis, thickened interstitial surface, and thickened interlobular septa (8). Healthy lungs are filled with air in the absence of a pathologic condition, as such they are not well-visualized in sonography. Changes due to the involvement of interstitial zones and the thickening of the interlobular septa (ILST) in the presence of interstitial lung diseases (ILD) result in comet tail artifacts (CTAs), as a type of reverberation artifact detected in USG. CTAs develops when a sound beam hits a reflective surface. A dense tail appearance with a gradually decreasing echogenicity appears on the monitor between the subsequent echoes transmitted to the transducer (9) (Figure 1).

When we looked at the literature, we have seen that there is only one study using USG for the assessment of lung parenchymal involvement in sarcoidosis (10). In the present study, the intention was to use USG for the evaluation of lung parenchyma in patients with sarcoidosis, and thento compare the USG findings (CTA) with the results of high-resolution computerized tomography (HRCT) and pulmonary function test-carbon monoxide diffusion tests (PFT-DLCO). We aimed to investigate the use of thoracic USG in the evaluation of lung parenchymal involvement in sarcoidosis, as a simple, easily-accessible, and reproducible imaging method that eliminates radiation exposure.

Material and Methods

Patient population

This prospective, controlled, cross-sectional study was conducted between January 2015 and December 2017 in accordance with the principles of the Declaration of Helsinki and was approved by the local ethics committee. The study population com-

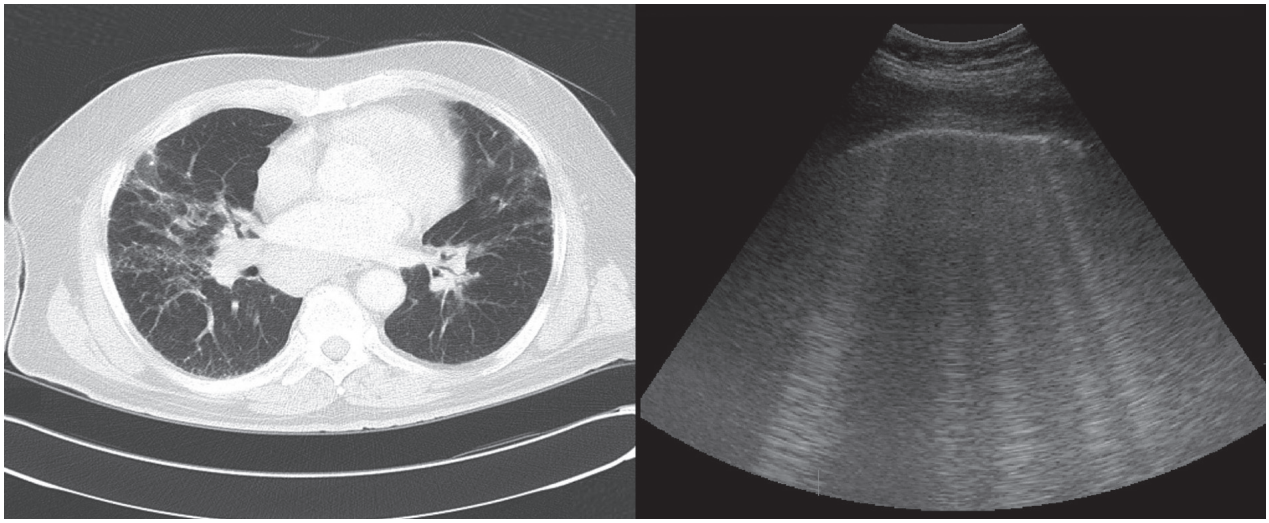


Fig. 1. Bilateral ground-glass densities in HRCT and CTAs detected using thoracic USG (CTAs marked by red arrow on the left) in patients with sarcoidosis. On the monitor, CTAs start with a narrow baseat the visceral pleural region and extend towards the peripheral along the monitor.

prised two groups: patients who had been diagnosed as having sarcoidosis based on clinical, radiologic and histopathologic findings in our pulmonology clinics (sarcoidosis group), and healthy subjects with no clinical or radiologic signs or symptoms suggestive of ILD, and who had a normal HRCT scan (control group).

Patients with other previously diagnosed interstitial lung diseases and patients with congestive heart failure (CHF) were excluded from the study. Clinical inquiry and physical examination (PE) findings, HRCT findings, and pulmonary function test (PFT) and DLCO results of all subjects were collected and recorded by a pulmonologist. Then, a thoracic USG of the subjects was performed by another pulmonologist who had no information on the diagnosis and was blinded to the HRCT and PFT-DLCO findings. The number of CTAs detected at the pre-specified anatomic lines was recorded during the thoracic USG.

Pulmonary function test and carbon monoxide diffusion test

The PFT-DLCO tests of the participants were conducted according to the guidelinesof the American Thoracic Society (ATS) and European Respiration Society (ERS) for the standardization of pulmonary function tests (11-12). The SFT and DLCO measurement tests were performedon a Sensor Medics Vi-Max 22, CareFusion, (San Diego, California) device using the single breath technique. For each lung volume, values of between 80 and 120% of the predicted value were considered normal, and forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FEV1/FVC parameters were recorded in liters (Lt) and percentages. The DLCO (mL/minute/mmHg/Lt) and DLCO/alveolar ventilation ratios (VA) (DLCO/L, %) were considered normal when values were between 80 and 120% of the predicted value for each lung, and values were recorded in liters and percentages.

High-resolution computerized tomography

HRCTs were obtained after deep inspiration using a high-resolution technique from the axial plane, starting with the apex towards the end of the diaphragm, with 15-mm table movement, at 120 kV, 200 mA, with section thickness of 2 mm, at a 512 × 512 matrix and bone algorithm using a Siemens Medical Solutions-2010 (Forchheim, Germany) device without the use of contrasting agent. Images were obtained with a window width of 1200 Hounsfield units (HU), at window level of 700 HU.

Thoracic ultrasonography

The thoracic USG was performed by a pulmonologist experienced in USG, using a General Electric (GE) Logic 7 device and a 3.5 MHz convex probe in the abdominal mode. The sonographic scanning of the thorax was performed on a total of 12 predefined bilateral anatomic lines, the first line being the linea mid-clavicular, as the vertical line passing through the mid-section of the clavicula at the anterior thorax. The other lines were as follows: linea axillaris anterior, the vertical line passing anterior to the plica axillaris anterior to the lateral thorax; linea axillaris media, the vertical line starting from the axilla apex; and linea axillaris posterior, the line starting from posterior of the linea axillaris. The final lines were the linea scapularis, the vertical line that transverses the angulus inferior scapula at the posterior thorax, and the linea paravertebralis, which progresses parallel to the vertebral column (Figure 2).

Comet tail artifact definition

CTAsare defined as hyperechogenic, adjacent bundle structures that start from the visceral pleura with a narrow base and broadening while running peripherally along the monitor and are observed when the USGprobe is located on an intercostal space (6). Along each of the pre-specified anatomic lines, the regions with the highest number of CTAs were detected while the probe was moved longitudinally along the intercostal spaces while the patient was in the sitting position. The numbers of CTAs in these regions were recorded.

Statistical analysis

The statistical analysis was performed using SPSS 17.0 (IBM Inc. Released 2008. SPSS Statistic for Windows Chicago, US) software. Descriptive statistics are presented as mean±standard deviations for continuous variables, and as percentages for cat-

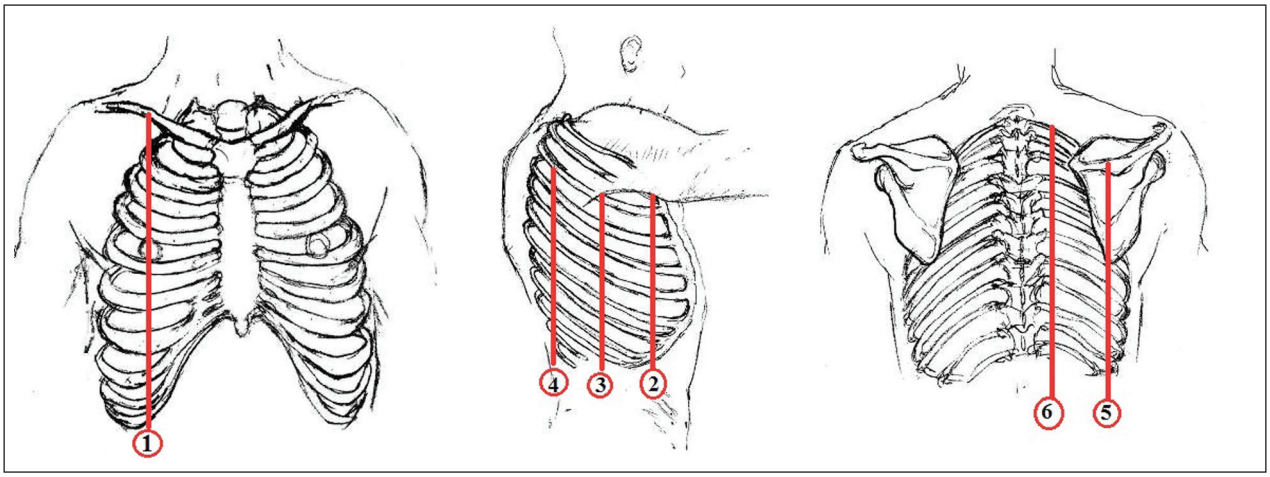


Fig. 2. Pre-specified anatomic lines (1-Linea mid-clavicularis, 2-Linea axillaris anterior, 3-Linea axillaris media, 4-Linea axillaris posterior 5-Linea scapularis 6-Linea para vertebralis).

egorical variables. The Kolmogorov-Smirnov test was used to check for normal distribution of the variables. Chi-square, t-test, Mann-Whitney U, and receiver operating curve (ROC) analysis tests were performed as necessary to compare the data between the two groups. A correlation analysis was performed to assess the relationship between the SFT-DLCO parameters and the CTA numbers of the participants. The correlation coefficient is presented with "r" values, and *p*-values lower than 0.05 were considered statistically significant.

Findings

A total of 113 patients were included in the study, with 79 (67.2%) in the sarcoidosis group and 34 (32.8%) in the control group. After being identified with concomitant CHF, two patients in the sarcoidosis group were excluded from the study. Of the remaining 77 patients with sarcoidosis, 60 (77.9%) were women, and 17 (22.1%) were men, and the mean age of the patient group was 48.1±14.4 years. Of the 34 patients in the control group, 13 (38.2%) were women, and 21 (61.8%) were men, and mean age was 39.7±12.7 years. In the sarcoidosis group, 18 (23.3%) patients had a history of smoking;the remaining 59 (76.7%) patients had no history of smoking. The mean smoking history was 8.2±4.6 pack-years. The mean age of the patient group and the proportion of females were significantly higher than in the control group (p<0.004, p<0.001, respectively) (Table 1).

Among the symptoms of the patients with sarcoidosis, the most frequently reported was exhaustion, reported by 49 (63.6%). The laboratory findings of the patients showed that four (5.1%) had hypercalcemia and four (5.1%) had hypercalciuria. In total, 14 (18.1%) patients had elevated serum ACE levels, and regarding the stage of sarcoidosis, 20 (26%), 30 (39%), 22 (28.6%), and five (6.5%) patients were at stages 0, 1, 2, and 3 of the disease, respectively. No patients were at stage 4.

The HRCT findings showed that 19 patients had normal lung parenchymaand 58 had a lung parenchyma pathology. Twenty-seven (35.1%) patients had bilateral ground-glass opacities, 10 (13%) had bilateral ILST, and 9 (11.7%) had a bilateral reticular appearance (Table 2). The HRCT findings of all patients in the control group were normal. The PFT-DLCO test results of the patients with sarcoidosis showed that the FEV1 Lt, FEV1%, FVC Lt, FVC %, DLCO (mL/dakika/mmHg) and DLCO% values

Table 1. Demographic characteristics of patients with sarcoidosis and the control group

	Sarcoidosis group (n=77)	Control group (n=34)	p value
Age (years ±sd)	48.1±14.4	39.7±12.7	p=0.004
Sex (f/m)	60/17	13/21	p<0.001
Smoking history (yes/no)	18/58	10/24	p=0.643
Smoking (pack-years±sd)	8.2±4.6	10.1±7.2	p=0.475

Table 2. HRCT findings in patients withsarcoidosis

HRCT findings	
Parenchymal abnormal findings (Yes/No)	58/19
Right lung nodule larger than 1 cm	30(39%)
Left lung nodule larger than 1 cm	25(32.5%)
Bilateral ground-glass appearance	27(35.1%)
Left lung sequela band appearance	22(28.6%)
Right lung sequela band appearance	19(24.7%)
Bilateral reticulo-nodular appearance	16(20.8%)
Bilateral peri-lymphatic, broncho-vascular, 1-2 mm nodules located along ILS	10 (13%)
Bilateral ILST	10 (13%)
Bilateral traction bronchiectasis / honeycombing appearance	8 (10.4%)
Bilateral mosaic perfusion appearance	5 (6.5%)
Other	12(15.6%)

ILST: Inter-lobular septal thickening

were significantly lower than those of the controls (p<0.05) (Table 3).

The CTA numbers of patients with sarcoidosis were not significantly different to the those of the controls in the right and left linea axillaris anterior, right linea mid-clavicularis, and left linea axillaris media regions (p>0.05). The CTA numbers in all other scanned regions were significantly higher in patients with sarcoidosis than in the controls (p<0.05). The mean number of CTAs in the patient and control groups was 33.4±13.1 and 25±6.5, respectively (p=0.001) (Table 4).

Analyses of the correlations between the PFT-DLCO parameters and the number of CTAs in the sarcoidosis group showed that DLCO% values were ≤80% and >80% in 37 (48%) and 40 (52%) of the patients, respectively. The mean number of CTAs in patients with DLCO% ≤80% was 37.4±15.8, whereas this figure was 29.7±8.7 in patients with DLCO% >80%, which represents a statistically significant difference (p=0.011) (Table 5). There was no significant relationship between the number of CTAs and FEV1% or FVC% in patients with sarcoidosis (p>0.05) (Table 5). The total number of CTAs was negatively correlated with the DLCO% of patients with sarcoidosis (p=0.019 r=-0.267) (Figure 3). No significant correlations were identified between FEV1 and the number of CTAs, FEV1% and the number of CTAs, FVC, and the number of CTAs, or FVC% and the number of CTAs (p>0.05).

The HRCT findings of the patients in the sarcoidosis group indicated that 58 patients had parenchymal involvement, and the number of CTAs in patients with parenchymal involvement in HRCT was 36±13.5, which was significantly higher than in patients without parenchymal involvement (25.5±7.9) (p=0.001). The mean number of CTAs in 19 (24.6%) patients with sarcoidosis who had a normal HRCT (no parenchymal pathology seen) was 25.5±7.9,

Table 3. PFT-DLCO findings in patients withsarcoidosis and the control group.

PFT-DLCO parameters	Sarcoidosis group (n=77)	Control group (n=34)	p value
FEV1/FVC(Mean±sd)	76.8±7.4	78.4±8.6	p=0.309
FEV1 (Mean±sd)(Lt)	2.53±0.78	3.80±0.8	p<0.001
%FEV1 (Mean±sd)	91.2±17.8	99.6±12.4	p=0.014
FVC (Mean±sd)(Lt)	3.15±0.98	4.35±1	p<0.001
%FVC (Mean±sd)	98.7±17.8	106.2±12.5	p=0.028
DLCO (Mean±sd) (mL/minute/mmHg)	6.61±2.40	9.91±2	p<0.001
%DLCO (Mean±sd)	83.4±18.3	99.7±14.5	p<0.001
DLCO/VA(mL/minute/mmHg /Lt) (Mean/sd)	1.99±1.05	1.76±0.29	p=0.082
%DLCO/VA	101.43±16.9	109.18±16.9	p=0.029

DLCO:Carbonmonoxide diffusion capacity. FVC: Forced vital capacity. FEV1: Forced expiratory volume in one second. VA: Alveolar volume. Mean: Mean SD: Standard deviation

Table 4. Number of CTAs detected on the anatomic lines in patients withsarcoidosis and the control group

Anatomic line	Sarcoidosis group (n=77)	Control group (n=34)	p value
Right linea para-vertebralis (Mean±sd)	2.4±1.4	1.3±0.8	p<0.001
Right linea scapularis (Mean±sd)	3.2±1.9	2±0.8	p<0.001
Right linea axillaris posterior (Mean±sd)	3.78±1.7	2.9±1.1	p=0.011
Right linea axillaris media (Mean±sd)	3.4±1.6	2.9±1	p=0.061
Right linea axillaris anterior (Mean±sd)	2.9±1.9	2.3±1.1	p=0.041
Right linea mid-clavicularis (Mean±sd)	2.3±1.4	1.8±1	p=0.052
Left linea para-vertebralis (Mean±sd)	1.8±1.3	1.3±0.3	p=0.039
Left linea scapularis (Mean±sd)	2.9±1.7	1.9±0.9	p=0.001
Left linea axillaris posterior (Mean±sd)	3.2±1.7	2.5±1.1	p=0.006
Left linea axillaris media (Mean±sd)	2.7±1.5	2.4±1	p=0.228
Left linea axillaris anterior (Mean±sd)	2.3±1.4	1.9±0.9	p=0.095
Left linea mid-clavicularis (Mean±sd)	2.1±1.3	1.5±0.8	p=0.014
Mean number of CTAs (Mean±sd)	33.4±13.1	25±6.5	p=0.001
Number of CTAs per ICS (Mean±sd)	2.7±1	2±0.5	p=0.001

CTA: Comet tail artefact. ICS: Intercostal space Mean: Mean SD: Standard deviation

Table 5. The relation between PFT-DLCO values and number of CTAs in patients with sarcoidosis

PFT-DLCO parameters		Number of patients (n/%)	Number of CTAs (Mean/SD)
	%FEV1<80%	21 (27.2%)	34.6±15.7
%FEV1	%FEV1>80%	56 (72.8%)	32.9±12.2
	p value	-	p=0.868
	%FVC <80%	13 (16.8%)	33.5±14.4
%FVC	%FVC >80%	64 (83.2%)	33.4±13
	p value	-	p=0.962
	%DLCO <80%	37 (48%)	37.4±15.8
%DLCO	%DLCO >80%	40 (52%)	29.7±8.7
	p value	-	**p=0.011**

DLCO:Carbonmonoxide diffusion capacity. FVC: Forced vital capacity. FEV1: Forced expiratory volume in one second. VA: Alveolar volume. Mean: Mean SD: Standard deviation

whereas the mean number of CTAs in the control group was 25±6.5. The difference between the two groups was not statistically significant (p=0.801).

When the relationship between disease stage and the number of CTAs in patients with sarcoidosis was investigated [number of CTAs in patients with stage 0-1-2 and 3 disease; 26.5±10.9, 32.5±10.9, 36.7±11.2, and 51.6±18.5, respectively (Figure 4)], both a one-way ANOVA test and a Bonferroni posthoc test indicated that a significant relationship existed between the disease stage groups and the number of CTAs (p<0.05) (Table 6). No significant

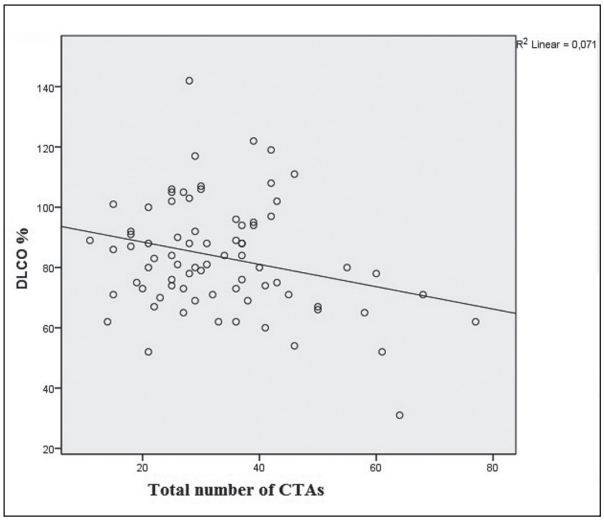


Fig. 3. The line shows the negative correlation between DLCO% value and total number of CTAs in patients with sarcoidosis

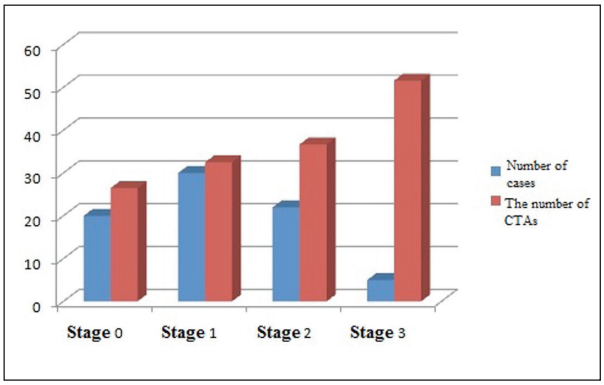


Fig. 4. Graph showing the relationship between disease stage and the number of CTAs in patients with sarcoidosis (Stage 0 number of CTAs 26.5, Stage 1 number of CTAs 32.5, Stage 2 number of CTAs 36.7, Stage 3 number of CTAs 51.6)

relationship was found between disease duration and the number of CTAs (p>0.05).

A total of 40 (51.9%) patients in the sarcoidosis group had a DLCO% of 80 or higher, and the mean number of CTAs in these patients was 29.7±8.7, whereas the mean number of CTAs in the control group was 25±6.5. The mean number of CTAs in patients with sarcoidosis with normal DLCO% values (≥80%) was significantly higher than in the controls (p=0.014).

When HRCT is considered the gold standard, the ROC analysis showed that the cut-off value for the detection of the optimal number of CTAs using USG was 25.5 (AUC:0.725). When the number of CTAs was higher than 25.5, the sensitivity and specificity of USG were estimated as 76% and 53%, respectively (Figure 5).

Table 6. The relation between sarcoidosis stage and number of CTAs in patients with sarcoidosis

Sarcoidosis stage		p value
Stage 0	Stage 1	0.516
Stage 0	Stage 2	0.042
Stage 0	Stage 3	p<0.001
Stage 1	Stage 2	p=0.999
Stage 1	Stage 3	p=0.009
Stage 2	Stage 3	p=0.085

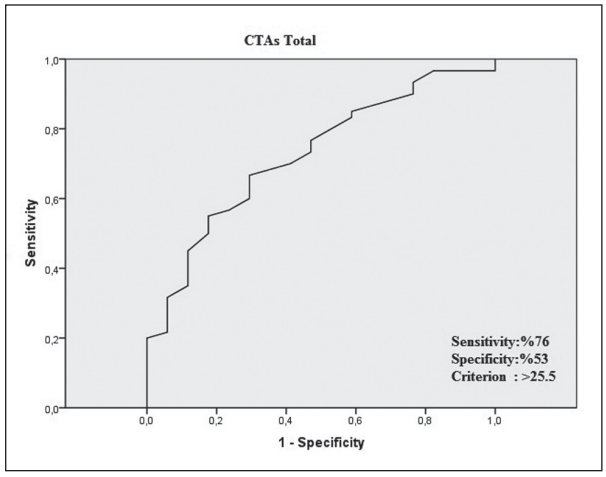


Fig. 5. CTAs evaluation according to high resolution computed tomography involvement

Discussion

The results of the present study show that the number of CTAs in patients with sarcoidosis were significantly elevated when compared to the healthy controls. The number of CTAs detected in a thoracic USG was also elevated in sarcoidosis patients with lung parenchymal involvement in HRCT and decreased DLCO (<80%) levels. The primary, and the most important, outcome of this study is the increased number of CTAs identified in the sarcoido-

sis patients when compared to the healthy controls, while of secondary importance is the correlation found between the thoracic USG findings and the HRCT and DLCO results, as markers of lung parenchyma involvement, in patients with sarcoidosis. The third most important outcome is the significant increase noted in the number of CTAs detected by USG in sarcoidosis patients with normal DLCO% values (DLCO>80%) when compared to the control group. When HRCT findings were considered as the gold standart, the sensitivity and specificity of thoracic USG for demonstrating parenchymal involvement were found to be 76% and 53%, respectively.

Sarcoidosis is a systemic granulomatous disease that most frequently affects the lungs. The rate of disease-related morbidity and mortality depends on the presence of lung involvement (14). Although lung X-ray is the first and the most commonly used method in the identification of lung parenchyma, the most effective radiologic method for this purpose in the present day is HRCT. Large-scale cohort studies have established the superiority of HRCT over other methods in demonstrating lung parenchyma involvement in sarcoidosis (15-17). In addition to such conventional methods as lung radiography, CT and HRCT, previous studies have also drawn attention to magnetic resonance imaging (MR) and radionucleotide methods (Gallium scintigraphy, PET-CT) as potential imaging modalities for sarcoidosis (18). In contrast, there are very few studies that investigated the use of USG as an imaging method in sarcoidosis (10), which remains under-researched. The transmission of sound waves through normal lung parenchyma is weak given that the lungs are filled with air, and this prevents them from being visualized as clearly as other, more solid, organs (19). However, USG may be a good diagnostic tool in the presence of conditions associated with lung edema or in diseases with diffuse involvement of the lung parenchyma, such as ILD. In 1997, Lichtenstein et al. (20) were the first researchers to demonstrate the increased number of CTAs through the use of thoracic USG in patients who had developed diffuse interstitial fibrosis. In the presence of diseases that cause lung fibrosis, mainly in connective tissue disorders, previous studies that compared thoracic USG with HRCT reported that evaluating the number of CTAs in thoracic USG was a valuable method, and several studies confirmed that there were significant increases in the number of CTAs when HRCT showed findings consistent with ILD (21-23). In the present study, the total number of CTAs detected in patients withsarcoidosis (33.4) was significantly higher than in the healthy controls (25) (p=0.001).

Parenchymal abnormalities that can be observed in the presence of sarcoidosis include ground-glass opacities, reticular opacities, interlobular septal thickening, micronodules (1-4 mm), macronodules (>5mm), patched or diffuse consolidations, fibrotic lesions, honeycombing and traction bronchiectasis (9,15,25). In the present study, USG findings were significantly different between subjects with HRCT findings that suggested lung involvement of sarcoidosis and subjects with a normal HRCT. The total number of CTAs in patients with sarcoidosis with parenchymal involvement in HRCT was 36, compared with 25.5 in patients without parenchymal involvement (p=0.001). A prospective, controlled study that investigatedthe diagnostic value of transthoracic USG in diffuse parenchymal lung diseases used thoracic USG to evaluate 53 patients with various ILDs and reported significant differences in the number of CTAs when compared with healthy controls (p<0.001). The authors of the study classified patients based on the number of CTAs as low (≤6/scan) and multiple (>6/scan), with seven patients with sarcoidosis in their ILD group (27);six (85.7%) of these patients had multiple (>6/scan) CTAs. Reissig et al. (26) is one of the few studies that reportedan increased number of CTAs in patients with sarcoidosis when compared with healthy individuals, supporting the findings of the present study. In Reissig et al's study (26) and in similar research (22, 27), the increased number of CTAs was associated with findings of ILD, including interlobular septal thickening and ground-glass densities. Sarcoidosis is a member of the diffuse parenchymal lung diseases family (28), and in the present study, we determinedthat the number of CTAs detected using thoracic USG was significantly elevated in patients with sarcoidosis who had pulmonary involvement, as demonstrated using HRCT.

DLCO is a more valid approach to demonstrating the lung parenchyma involvement of sarcoidosis than simple spirometric tests. In a recent study by Mañá et al. (29) on a very large sarcoidosis case series (640 patients), 30.3% of patients had abnormal DLCOs, although the ratio of patients with abnormal FVC values was only 16.2%. In the presence of

diffuse parenchymal lung diseases, DLCO decreases as a result of disease involvement in the alveolar-capillary membrane. Young et al. (30) demonstrated previously that DLCO was reduced in patients with sarcoidosis with lung parenchyma involvement, and Carrington et al. (31) developed an index they referred to as the "mean interstitial cell index (MICI)," which demonstrates lung involvement in sarcoidosis, and reported a correlation between MICI and DLCO. Interestingly, they also high lighted the additional importance of DLCO by providing evidence that it could decrease by almost 10 to 30 mL/min/mmHg during exercise in patients with sarcoidosis with near-normal MICI values. In another study by Edis et al. (32), who evaluated the effectiveness of thoracic USG in patients with systemic sclerosis (SS), a total of 48 patients were evaluated using thoracic USG and a negative correlation was found between the DLCO values and the number of CTAs of the patient group. In a prospective study, Hassan et al. (33) compared the number of CTAs detected using thoracic USG in patients with ILD and HRCT and PFT findings and found a negative correlation between DLCO values and CTA frequency, concluding that thoracic USG might be a beneficial tool for the evaluation of ILDs. In a study with 33 patients who were diagnosed with systemic sclerosis Gargani et al. (34) classified the patients based on the number of CTAs detected using thoracic USG as patients with >10 and <10 CTAs and found that DLCO% values in patients with above and below 10 CTAs were 66 and 83, respectively ($p<0.05$). In the present study, we identified no significant relation between CTA and FVC ($p=0.718$ $r=-0.042$), whereas the number of CTAs was found to be negatively correlated with DLCO values($p=0.019$ $r=-0.267$). Moreover, the number of CTAs in patients with sarcoidosis with DLCO% <80 was significantly higher (37.4) than inpatients with DLCO% ≥80 (29.7) ($p=0.011$). To our knowledge, no previous studies have evaluated the relationship between CTA and DLCO in patients with sarcoidosis, although there have been several studies related to diseases that involved the lung parenchyma, which reported a negative correlation between CTA and DLCO values (32-36). Our findings are consistent with the literature because sarcoidosis is a component of ILDs (32-36).

A literature review shows that almost all of the studies on assessment of pulmonary interstitium by USG focused on the diagnostic usability of USG (37). We found two case reports investigating the usability of CTAs detectable by thoracic USG in monitoring disease acitivity or evaluating the response to treatment. A study by Buda N et al. (38) demonstrated a reduction in the number of CTAs in a patient with scleroderma following the treatment. The other study by Laria A et al. (39) showed that CTAs disappeared after the therapy in a patient diagnosed with rheumatoid arthritis associated ILD. Considering the significance of pulmonary parenchymal involvement and improvement after treatment, it should be kept in mind that USG can be used in conjunction with HRCT in monitoring the disease activity and evaluating the response to treatment in sarcoidosis. This can be further elucidated by randomized controlled studies demonstrating that number of CTAs detected by thoracic USG are reducable by treatment.

We found that thoracic USG had a sensitivity of 76% and specificity of 53% in demonstrating the parenchymal involvement in patients with sarcoidosis, which showed that we had a lower sensitivity and specificity for thoracic USG in our study. Relatively lower sensitivity may be associated with high number of false negatives. In cases where USG fails to detect any involvement whereas HRCT shows it, false negatives rate may be associated with the type of the parenchymal disease. For example, the number of CTAs may be different in areas of ground glass opacity compared to the micronodular pattern. Lower specificity may be associated with high number of cases with false positives. Although no parenchymal involvement was demonstrated in HRCT, which is considered to be the golden standard, an increased number of CTAs detectable by USG may increase the number of cases with false positives. It can also be explained by higher sensitivity of USG in parenchymal involvements that are not on a macroscopic level to be detected by HRCT. Further studies are required to evaluate whether HRCT is a golden standard in assessment of parenchymal involvement, and the correlation between the type of parenchymal disease and number of CTAs in sarcoidosis.

This study, which represents a starting point for the use of thoracic USG for the evaluation of lung parenchyma in patients with sarcoidosis, has some limitations. Intra-observer and inter-observer variabilities were not calculated for the assessments of

thoracic USG and identification of CTAs, as well as for HRCT findings, which represents the most important limitation of this study. Therefore, while interpreting the study results one should consider that thoracic USG is a highly user-dependent imaging technique. Another limitation is that this study was performed on a relatively low number of patients and reflected the experiences of a single center. Accordingly, the findings of this study cannot be generalized.

In conclusion, this study has shown that statistically significant correlations exist between thoracic USG findings, which represent a novel method for the assessment of lung parenchyma involvement in patients with sarcoidosis, and the findings of HRCT, which is currently considered to be the most sensitive imaging method for the demonstration of lung parenchymal involvement of sarcoidosis, as well as DLCO, currently known to be the most effective test for showing the diffusion of gases through the alveolar-capillary membrane, and therefore, the potential involvement of the alveolar-capillary membrane. We believe that although it may not be the most preferred method for the evaluation of parenchymal involvement in patients with sarcoidosis, thoracic USG may still have an area of use in the regular monitoring and assessment of treatment response in such patients. Further studies are required to investigate methods of early detection of changes in lung parenchyma. We evaluated the use of USG for assessment of sarcoidosis. We believe that, as much as the healthy lung parenchyma cannot be evaluated using USG, it may be effective for the evaluation of lung parenchyma in the presence of such diseases as ILD, which extensively affects the lung parenchyma.

Acknowledgement

We thank MD İlker Akin for his contribution with his illustrations.

References

1. Wijsenbeek MS, Culver DA. Treatment of Sarcoidosis. Clin Chest Med 2015; 36: 751.
2. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med 2007; 357: 2153.
3. Baughman RP, Grutters JC. New treatment strategies for pulmonary sarcoidosis: antimetabolites, biological drugs, and other treatment approaches. Lancet Respir Med 2015; 3: 813.
4. Bradley B, Branley HM, Egan JJ, et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax 2008; 63: v1-58.
5. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med 2011; 183: 573.
6. Mallamaci F, Benedetto FA, Tripepi R, et al. Detection of pulmonary congestion by chest ultrasound in dialysis patients. JACC Cardiovasc Imaging 2010; 3: 586-94.
7. Koenig SJ, Narasimhan M, Mayo PH. Thoracic ultrasonography forthe pulmonary specialist. Chest 2011; 140: 1332-1341.
8. Soldati G, Inchingolo R, Smargiassi A, et al. Ex vivo lung sonography: morphologic-ultrasound relationship. Ultrasound Med Biol 2012; 38: 1169-79.
9. Volpicelli G, Elbarbary M, Blaivas M, et al. International evidence-basedrecommendations for point-of-care lung ultrasound. Intensive Care Med 2012; 38: 577-91.
10. Targhetta R, Chavagneux R, Balmes P, et al. Sonographic lung surface evaluation in pulmonary sarcoidosis: preliminary results. J Ultrasound Med. 1994; 13: 381-8.
11. Miller MR, Hankinson J, Brusasco V, et al. ATS/ERS task force standardisation of lung function testing. Edited by V. Brusasco, R. Crapo and G. Viegi Number 2 in this Series Standardisation of spirometry. EurRespir J 2005; 26: 319-338.
12. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995; 152: 1107-1136.
13. Barskova T, Gargani L, Guiducci S, et al. Lung ultrasound for the screening of interstitial lung disease in very early systemic sclerosis. Ann Rheum Dis 2013; 72: 390-5.
14. Criado E, Sánchez M, Ramírez J, et al. Pulmonary sarcoidosis: typical and atypical manifestations at high-resolution CT with pathologic correlation. Radiographics 2010; 30: 1567-86.
15. Dhagat PK, Singh S, Jain M, Singh SN, Sharma RK. Thoracic Sarcoidosis: Imaging with High Resolution Computed Tomography. J ClinDiagn Res. 2017; 11: 15-18.
16. Spagnolo P, Sverzellati N, Wells AU, Hansell DM. Imaging aspects of the diagnosis of sarcoidosis. EurRadiol 2014; 24: 807-16.
17. Walsh SL, Wells AU, Sverzellati N, et al. An integrated clinicoradiological staging system for pulmonary sarcoidosis: a case-cohort study. Lancet Respir Med 2014; 2: 123-30.
18. Silva M, Nunes H, Valeyre D, SverzellatiN. Imaging of Sarcoidosis. Clin Rev Allergy Immunol 2015; 49: 45-53.
19. Smargiassi A, Inchingolo R, Soldati G, et al. The role of chest ultrasonography in the management of respiratory diseases: Document 2. MultidiscipRespir Med 2013; 8: 55.
20. Lichtenstein D, Mézière G, Biderman P, Gepner A, Barré O. The comet-tail artifact. An ultrasoundsign of alveolar-interstitial syndrome. Am J RespirCrit Care Med 1997; 156: 1640–6.
21. Doveri M, Frassi F, Consensi A, et al. Ultrasound lung comets: New echographic sign of lung interstitial fibrosis in systemic sclerosis. Reumatismo 2008; 60: 180–4.
22. Lim JH, Lee KS, Kim TS, Chung MP. Ring-down artifacts posterior to the right hemidiaphragm on abdominal sonography: sign of pulmonary parenchymal abnormalities. J Ultrasound Med 1999; 18: 403-10.
23. Sperandeo M, Varriale A, Sperandeo G, et al. Transthoracic ultrasound inthe evaluation of pulmonary fibrosis: our experience. Ultrasound Med Biol 2009; 35: 723-9.
24. Austin JH, Müller NL, Friedman PJ, et al. Glossary of terms for CT of the lungs: recommendations of the Nomenclature Committee of the Fleischner Society. Radiology 1996; 200: 327-31.
25. Al Jahdali H, Rajiah P, Koteyar SS. Atypical radiological manifestations of thoracic sarcoidosis: A review and pictorial essay. Annals of Thoracic Medicine. 2013; 8: 186–96.
26. Reissig A, Kroegel C. Transthoracic sonography of diffuse parenchy-

mal lung disease: the role of comet tail artifacts. J Ultrasound Med. 2003; 22: 173-80.
27. Gryminski J, Krakowka P, Lypacewicz G. The diagnosis of pleural effusion by ultrasonic and radiological techniques. Chest 1976; 70: 33-37.
28. American Thoracic Society, European Respiratory Society. International multidisciplinary consensus classification of the idiopathic interstitial pneumonias. Am J RespirCrit Care Med 2002; 165: 277-304.
29. Mañá J, Rubio-Rivas M, Villalba N, et al. Multidisciplinary approach and long-term follow-up in a series of 640 consecutive patients with sarcoidosis: Cohort study of a 40-year clinical experience at a tertiary referral center in Barcelona, Spain. Medicine (Baltimore) 2017; 96: e7595.
30. Young RL, Loudon RE, Krumholz RA, Harkleroad LE, Branam GE, Weg, JG. Pulmonary sarcoidosis: I. Pathophysiologic considerations. Am Rev Respir Dis 1968; 97: 997-1008.
31. Carrington CB. Structure and function in sarcoidosis. Ann N Y Acad Sci 1976; 278: 265-83.
32. Edis EÇ, Hatipoğlu ON, Pamuk ÖM, Eraslan RM, Aktöz M, Tuncel SA. Effectiveness of thoracic ultrasonography in the evaluation of the severity of pulmonary involvement in patients with systemic sclerosis. Arch Rheumatol 2016; 31: 364-370
33. Hasan AA, Makhlouf HA. B-lines: Transthoracic chest ultrasound signs useful in assessment of interstitial lung diseases. Ann Thorac Med 2014; 9: 99-103.
34. Gargani L, Doveri M, D'Errico L, et al. Ultrasound lung comets in systemic sclerosis: a chest sonography hallmark of pulmonary interstitial fibrosis. Rheumatology (Oxford) 2009; 48: 1382-7.
35. Ostojić P, Damjanov N. Different clinical features in patients with limited and diffuse cutaneous systemic sclerosis. Clin Rheumatol 2006; 25: 453-7.
36. Gigante A, Rossi Fanelli F, Lucci S, et al. Lung ultrasound in systemic sclerosis: correlation with high-resolution computed tomography, pulmonary function tests and clinical variables of disease. Intern Emerg Med 2016; 11: 213-7.
37. Wang Y, Gargani L, Barskova T, et al. Usefulness of lung ultrasound B-lines in connective tissue disease-associated interstitial lung disease: a literature review. Arthritis Res Ther 2017; 19(1): 206.
38. Buda N, Kosiak W, Smoleńska Z, et al. Transthoracic lung ultrasound in the monitoring of interstitial lung disease: a case of scleroderma. Pol Arch Med Wewn 2013;123(12):721-2.
39. Laria A, Lurati A, Scarpellini M. Ultrasound in rheumatologic interstitial lung disease: a case report of nonspecific interstitial pneumonia in rheumatoid arthritis. Case Rep Rheumatol 2015;2015:107275. doi: 10.1155/2015/107275.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 141-147

Cathepsin S, a new serum biomarker of sarcoidosis discovered by transcriptome analysis of alveolar macrophages

Hiroyuki Tanaka[1], Etsuro Yamaguchi[1], Nobuhiro Asai[2], Toyoharu Yokoi[3], Masaki Nishimura[1], Haruhisa Nakao[4], Masashi Yoneda[4], Yoshinori Ohtsuka[5], Satoshi Konno[6], Noritaka Yamada[7]

[1] Division of Respiratory Medicine and Allergology, Department of Internal Medicine, School of Medicine, Aichi Medical University, Nagakute, Aichi, Japan; [2] Department of Infection Control and Prevention, School of Medicine, Aichi Medical University, Nagakute, Aichi, Japan; [3] Department of Pathology, Nagoya Ekisaikai Hospital, Nagoya, Aichi, Japan; [4] Division of Hepatology and Pancreatology, Department of Internal Medicine, School of Medicine, Aichi Medical University, Nagakute, Aichi, Japan; [5] Department of Medicine, Hokkaido Chuo Rosai Hospita, Iwamizawa, Japan; [6] First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan; [7] Department of Respiratory Medicine, National Hospital Organization, Higashinagoya Hospital, Nagoya, Aichi, Japan

Abstract. *Background:* Development of reliable new biomarkers remains crucial to improve diagnosis and assessing disease activity in sarcoidosis. The objective of this study was to seek such markers from the gene expression signature of alveolar macrophages by transcriptome analysis. *Methods:* Pooled RNA extracted from alveolar macrophages from patients with active sarcoidosis and control patients was subjected to transcriptome analysis using microarrays. Expressed gene intensity in sarcoidosis relative to that in control was calculated. We measured serum cathepsin S (CTSS) concentrations in 89 healthy volunteers, 107 patients with sarcoidosis, 26 with interstitial pneumonia, 150 with pneumoconiosis, and 76 with pulmonary mycobacteriosis by the enzyme-linked immunosorbent assay. *Results:* Among 12 genes with ratios higher than that of a housekeeping gene, we selected CTSS for scrutinizing protein expression in serum because of the feasibility of the protein assay. CTSS concentrations were significantly increased in sarcoidosis compared with not only controls but also all the other lung diseases. Receiver operating characteristics curve for sarcoidosis and parenchymal lung diseases revealed an area under the curve of 0.800 (95% confidence interval, 0.751-0.850; $p=1.4 \times 10^{-18}$) with 70% sensitivity and 78% specificity at a CTSS concentration of 15.5 ng/ml. A significant trend was identified between CTSS concentrations and the number of affected organs. Serum CTSS concentrations varied in parallel with clinical courses both spontaneously and in response to corticosteroid therapy. Epithelioid cells in granulomas were positive for immunohistochemical staining with CTSS. *Conclusions:* CTSS has the potential to be a useful biomarker in sarcoidosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 141-147)*

Key words: biomarkers, cathepsins, macrophages, sarcoidosis, transcriptome

Introduction

Sarcoidosis is a widespread multisystem disease that preferentially involves the lungs, intra-thoracic lymph nodes, eyes, and skin. The gold standard of diagnosis includes pathological findings compatible with sarcoidosis and exclusion of other infectious and non-infectious granulomatous diseases along with the presence of compatible clinical features (1). The presence of multiple typical clinical manifestations

Received: 11 August 2018
Accepted after revision: 20 August 2018
Correspondence: Etsuro Yamaguchi, MD, PhD,
Division of Respiratory Medicine and Allergology,
Department of Internal Medicine, School of Medicine,
Aichi Medical University, Karimata 1-1, Yazako,
Nagakute, Aichi, Japan, 480-1195
E-mail: etsuro@aichi-med-u.ac.jp

alone such as bilateral hilar lymph node enlargement, uveitis, and skin rash strongly suggests sarcoidosis but insufficient for confident diagnosis. In such cases, positive results for serum biomarkers with high sensitivity and specificity are a strongly supportive of the diagnosis. Since the discovery of angiotensin converting enzyme (ACE) by Lieberman (2), several but not many markers have been added to the list of candidate markers, such as soluble IL-2 receptor (3), Krebs von den Lungen 6 (4), tryptase (5), and amyloid A (6). Recently, omics studies such as of the transcriptome of blood (7,8) and the proteomes of alveolar macrophages (9) and serum (10,11) have been conducted. However, translation of the results from those omics studies to the development of biomarkers useful in clinical practice has been unsuccessful mainly because test specimens are hard to obtain or assays require complicated laboratory works (7-9).

Since no biomarkers have been validated as gold surrogates for diagnosis and the monitoring of disease course of sarcoidosis, such new markers are sorely needed. The current study attempted to seek such markers by conducting comparative transcriptome analysis between patients with sarcoidosis and controls. As specimens for screening by transcriptome analysis, we selected alveolar macrophages as cells that are easily obtained by bronchoalveolar lavage (BAL) and are believed to reflect immune responses at the site of disease.

Materials and Methods

Study Subjects

This study included not only healthy volunteers and patients with sarcoidosis but also a wide variety of disease controls in order to fairly assess the specificity of potential markers (Table 1). All subjects were Japanese.

Healthy volunteers without any prior major illness or any symptoms at the time of blood sampling served as controls. They all were never-smokers and were not included if they reported regular medication use.

Patients with sarcoidosis had evidence of noncaseating epithelioid cell granulomas in at least one organ and compatible clinical features in at least two organs such as bilateral hilar and/or mediastinal lymph node enlargement with or without lung parenchymal infiltrates, eye lesions, and skin lesions, and without evidence of mycobacterial, fungal, or parasitic infection as described in the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Other Granulomatous Disorders publications (1). None had a history of exposure to organic or inorganic materials known to cause lung diseases. No patients were taking systemic corticosteroids when serum was first tested for CTSS concentration. The presence or absence of active lesions in each organ was assessed by inspection of skin visually and palpation, auscultation of lungs and heart, chest roentgenography, spirometry, electrocardiography, echocardiography, routine laboratory tests including leukocyte count, hemoglobin concentration, hepatic enzyme activity, calcium ion concentration, and ACE activity, and ophthalmic examination by specialists. Since apparent absence of active lesions assessed by these noninvasive routine clinical examinations does not necessarily mean truly inactive state, the current study included eight patients who had been previously diagnosed but had no clinical signs of affected organs at the time of serum collection. The number of patients in Scadding stage

Table 1. Demographic data of study subjects*

	n	sex (male/female)	age (year)	VC (%)	FEV1/FVC (%)	$D_{L_{CO}}$/VA (%)	BAL lymphocyte (%)	BAL CD4/CD8
Control	89	47/42	40 [20-78]	-	-	-	-	-
sarcoidosis	107	31/76	59 [23-85]	104 [77-147]	77 [59-97]	103 [46-143]	43 [1-89]	7.9 [0.8-74.9]
Interstitial pneumonia	26	16/10	76 [58-88]	79 [24-134]	82 [60-99]	78 [27-121]	-	-
Pneumoconiosis	150	150/0	76 [54-93]	95 [31-149]	69 [32-97]	-	-	-
Pulmonary mycobacteriosis	76	40/36	68 [21-86]	-	-	-	-	-

* Data are median [range]. Definitions of abbreviations: FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; $D_{L_{CO}}$, diffusing capacity of lung for carbon monoxide; VA, volume of alveolar gas; BAL, bronchoalveolar lavage

0, stage I, stage II, and stage III were 16 (15%), 56 (52%), 31 (29%), and 4 (4%), respectively. Clinical courses of sarcoidosis were judged by changes in the number of affected organs or apparent changes in the severity of lesions clinically assessed as described above.

Patients with idiopathic interstitial pneumonia had either a usual interstitial pneumonia pattern (n=14) or a nonspecific interstitial pneumonia pattern (n=12) on high-resolution computed tomography according to the ATS/ERS consensus classification (12) or a published document (13) with no evidence of underlying diseases judging from occupational history, physical examination, and serological tests for collagen vascular diseases or other vasculitides. No patients were taking systemic corticosteroids when sera were collected.

Pneumoconiosis was diagnosed based on long-standing occupational exposure to silica or coal, and discrete nodular shadows of variable sizes in predominantly upper lung areas on chest roentgenography or computed tomography. According to the management classification by the Ministry of Health, Labor and Welfare of Japan, 50 patients were classified into type 2 (a number of opacities smaller than 1 cm in diameter [ILO classification: p, q, r, s, t, u]), 50 into type 3 (numerous opacities smaller than 1 cm in diameter), and 50 into type 4 (nodular shadows larger than 1 cm in diameter).

Diagnosis of pulmonary mycobacteriosis was made based on compatible radiological findings and demonstration of mycobacteria in sputum and/or bronchoalveolar lavage fluid. Forty-six patients had tuberculosis and 30 had nontuberculous mycobacteriosis.

Microarray

BAL was conducted in three nonsmoking patients with active sarcoidosis with biopsy evidence and four nonsmoking controls who were suspected to have lung cancer. BAL was done in the middle lobe or lingular segments for patients with sarcoidosis, or in segments unaffected by pulmonary lesions for patients suspected of lung cancer. All were women. Since most lymphocytes of BAL cells are cluster of differentiation (CD) 2+ T cells (14) and the proportion of granulocytes was less than 3% in all the subjects studied, a cellular fraction of BAL cells depleted of CD2+ cells by microbeads coated with anti-CD2 monoclonal antibody (MicroBeads, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) was used as alveolar macrophages. Total RNA from this cell fraction was extracted using BioRobot EZ1 (Qiagen GmbH, Hilden, Germany) and EZ1 RNA Cell Mini Kit (Qiagen). Equal amount of total RNA from each subject was pooled for the patient group or the control group, and 0.4 μg of RNA from each group was subjected to microarray analysis using CodeLink Human Whole Genome Bioarray (Applied Microarrays, Tempe, AZ) and CodeLink Expression Bioarray System (Applied Microarrays). Array slides were hybridized with biotin-labelled cRNA, stained with streptoavidin-Cy5, washed according to the manufacturer's protocol, and scanned by arrayWoRx (Applied Precision, Issaquah, WA).

Measurement of CTSS Protein and Immunostaining

Sera were obtained at the time of annual health check-up in healthy volunteers, at the time of initial visit or at the oldest time point in patients with sarcoidosis, and at variable time points in patients with other diseases. Concentrations of CTSS in sera diluted 100-fold with assay diluent were measured using a Human Total Cathepsin S DuoSet (R&D Systems, Minneapolis, MN). Specific immunostaining for human CTSS was performed on formalin-fixed paraffin-embedded slides prepared from a mediastinal lymph node in a patient with sarcoidosis using goat anti-CTSS antibody (M-19, sc-6505; Santa Cruz Biotechnology, Santa Cruz, CA), and peroxidase-labelled anti-goat immunoglobulin (Histofine Simple Stain MAX-PO [G]; Nichirei Bioscience, Tokyo, Japan).

Statistical Analysis

Difference between two study groups was assessed by the Mann-Whitney test followed by Bonferroni's correction to counteract multiple comparisons. Receiver operating characteristics curve was used to determine sensitivity and specificity for discriminating sarcoidosis from control subjects or other lung diseases. The Youden index was used to determine the cut-off point. The Jonckheere-Terpstra test was used to assess trend in CTSS concentrations across patient groups ranked by the number of

affected organs. The Wilcoxon signed-rank test was used to compare paired concentrations of CTSS during the clinical course of sarcoidosis. These statistical analyses were performed using SPSS Statistics 23.0 (SPSS, Chicago, IL). P values less than 0.05 were considered statistically significant. Sample size estimation was calculated by the PS program (Power and Sample Size Calculation version 3.1.2, http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).

The ethics committee of the Department of Medicine at Aichi Medical University approved all study protocols (No 260, No 2009-18, No 13-141), and all study subjects provided informed consent prior to participation.

Results

Among approximately 34,000 array probes including expressed sequence tags, those expressing good quality signals were selected. Ratios of normalized signal intensity for individual genes in the sarcoidosis sample to that in the control sample were calculated. Twelve genes showed ratios higher than 6.7 which was the ratio for glyceraldehyde-3-phosphate dehydrogenase, a housekeeping gene used as the reference gene (Table 2). The standard deviation of ratio of all genes on microarray was approximately 2.0 assuming the ratio as continuous variable. If the difference of ratio means between sarcoidosis and controls was set as more than 5.7 (control=1.0 and sarcoidosis >6.7, Table 2), we need only 3 experimental subjects and 3 control subjects to be able to reject the null hypothesis that the means of the experimental and control groups are equal with power 0.8 and the type I error probability 0.05. Thus, this power calculation justified the actual sample size (3 controls and 4 patients) in the present study. Since enzyme-linked immunosorbent assay (ELISA) kits for the target proteins in serum were not available for the first- and second-ranked genes (*LRAP* and *ZNF101*, respectively, in Table 2), we selected the third-ranked gene, *CTSS*, for which there was a commercially available ELISA kit.

We measured serum concentrations of CTSS in not only sarcoidosis patients and healthy controls, but also in patients with several pulmonary diseases. They were significantly increased not only in sarcoidosis, but also in other diseases except for pulmonary mycobacteriosis compared with those in healthy controls (Figure 1). Although ages differed significantly between controls and other diseases ($p<0.001$), no significant correlation was identified between CTSS concentrations and ages in control subjects ($\rho=-0.71$, $p=0.507$).

Table 2. Ratios of probe intensity for individual genes in a pooled sarcoidosis sample to that in a pooled control sample

Ratio	Description (NCBI/UniGene database)
31.2	leukocyte-derived arginine aminopeptidase (LRAP)
24.3	cDNA DKFZp570I0164 (ZNF101)
21.7	cathepsin S (CTSS)
11.9	28206323prime NIH MGC 7 cDNA clone
10.8	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase
9.1	cathepsin D (CTSD)
7.5	cDNA DKFZp686P21116
7.2	ty83h04x1 NCI CGAP Kid11 cDNA clone
7.1	secretoglobin, family 3A, member 1 (SCGB3A1)
7.0	hepatitis C virus core-binding protein 6 (HCBP6)
6.9	602533729F1 NIH MGC 15 cDNA clone
6.9	insulin-like growth factor 1 (somatomedin C) (IGF1)
6.7	glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

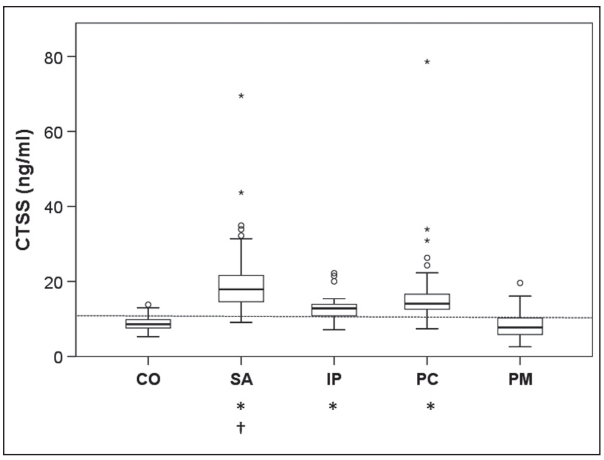


Fig. 1. Serum CTSS concentrations.
Data are presented in box-and-whisker plots with ends of the whiskers representing the lowest datum still within the 1.5 interquartile range (IQR) under the lower quartile and the highest datum still within 1.5 IQR above the upper quartile. Circles represent outliers between 1.5 and 3.0 IQR under the lower quartile or above the upper quartile. Asterisks represent extreme values below 3.0 IQR under the lower quartile or above 3.0 IQR over the upper quartile. The horizontal dotted line indicates the 95th percentile value of controls.
* Significantly higher than controls. P values for SA, IP, and PC were $1.3X10^{-30}$, $8.6X10^{-8}$, and $4.0X10^{-31}$, respectively. † Significantly higher than the other pulmonary diseases. P values for IP, PC, and PM were $6.7X10^{-6}$, $7.8X10^{-8}$, and $2.1X10^{-9}$, respectively.
Definitions for abbreviations: CO, control; SA, sarcoidosis; IP, interstitial pneumonia; PC, pneumoconiosis; PM, pulmonary mycobacteriosis.

The median value in sarcoidosis was the highest (17.9 ng/ml) and was significantly increased compared with all other diseases (Figure 1). Ninety-nine of the 107 (93%) patients with sarcoidosis exceeded the upper 95th percentile value (11.7 ng/ml) of controls. In receiver operating characteristics curve analysis for control and sarcoidosis, area under the curve was 0.985 (95% CI, 0.972-0.997; $p=1.8 \times 10^{-31}$). Controls and patients with sarcoidosis were most effectively discriminated at a CTSS concentration of 11.9 ng/ml with 93% sensitivity and 97% specificity. When interstitial pneumonia, pneumoconiosis, and pulmonary mycobacteriosis were combined into a group of parenchymal lung diseases which is usually subject to differentiation from sarcoidosis, sarcoidosis and the combined group were most effectively discriminated at a CTSS concentration of 15.5 ng/ml with 70% sensitivity and 78% specificity (Figure 2).

An increasing trend in CTSS concentrations was seen among patients with sarcoidosis as a greater number of organs are affected, and this trend was significant by the Jonckheere-Terpstra test (p=0.008). CTSS concentrations were measured at a given reference date, mostly at the initial visit, and revaluated at a later date in a proportion of patients with sarcoidosis (n=40). No significant change was seen in nine patients with unchanged activity (median [range] number of affected organs at the reference date and date of revaluation: 1 [1-4] and 1 [1-4], respectively) (Figure 3A). Meanwhile, significant decreases were seen in 19 patients with spontaneous improvement (2 [1-5] and 1 [0-4], respectively) (Figure 3B) and in seven patients following the administration of systemic corticosteroids (3 [2-6] and 2 [1-3], respectively) (Figure 3C). Opposite changes were observed in five patients with disease progression (1 [1-4] and 1 [1-5], respectively) (Figure 3D).

Immunohistochemical staining with anti-CTSS antibody for lymph nodes obtained from nine patients with sarcoidosis was positive for epithelioid cells in sarcoid granulomas (Figure 4).

Discussion

We have conducted a transcriptome study of alveolar macrophages for the screening of serum biomarkers that are potentially adoptable for clini-

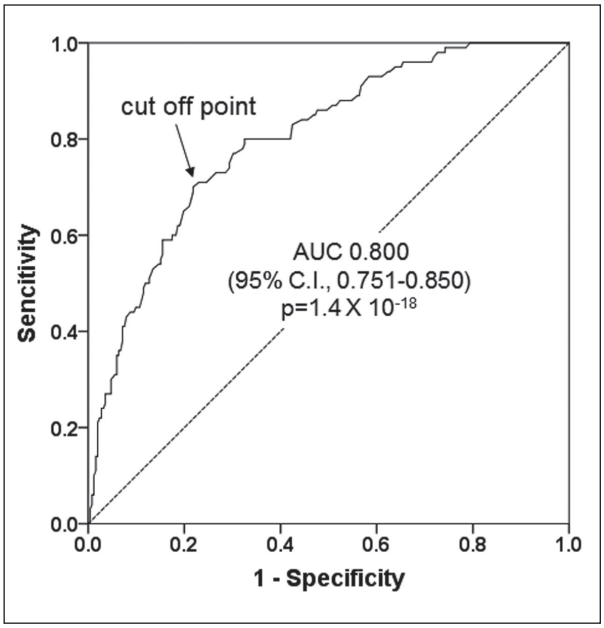


Fig. 2. Receiver operating characteristics analysis for differentiation between sarcoidosis and some of parenchymal lung diseases.
Receiver operating characteristics curve was generated as the actual state and higher values of CTSS as positive.
Definitions of abbreviations: AUC, area under the curve; CI, confidence interval

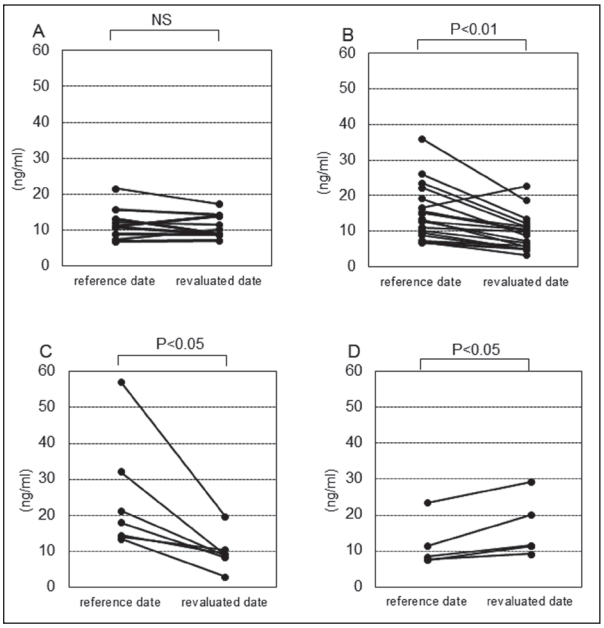


Fig. 3. Changes in CTSS concentrations and clinical course of sarcoidosis.
CTSS concentrations in serum were measured twice and compared in patients with unchanged clinical activity (A), spontaneous improvement (B), improvement by systemic corticosteroids (C), and disease progression (D)

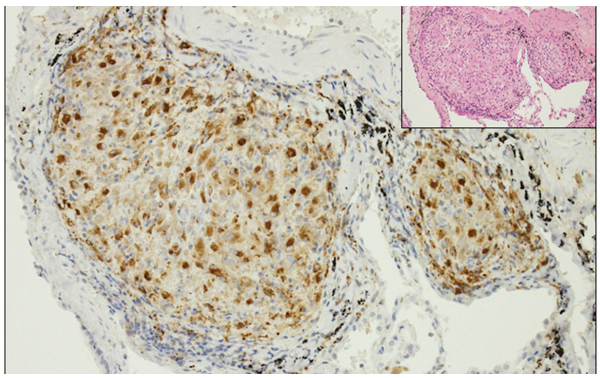


Fig. 4. Representative immunohistochemical staining with anti-CTSS antibody for a lymph node from a patient with sarcoidosis. Positive cells for CTSS are stained brown. A hematoxylin-eosin stained picture of the same area is shown in the upper right corner.

cal use in sarcoidosis. We found that CTSS had the potential to be such a biomarker, which has not been reported in previous proteomic or transcriptomic studies (7-9,11). We used samples of pooled RNA that were expressed by alveolar macrophages in order to save the number of arrays required and evaluated the relative expression of each RNA by calculating the ratio of the signal intensity in sarcoidosis patients to that in the control subjects. Although this method is not frequently used (15), it proved to be effective in finding a new serum biomarker in sarcoidosis.

The sensitivity for sarcoidosis was higher for the CTSS concentrations than those for the other markers reported to date (6,10,16,17). The source of CTSS in serum was thought to be alveolar macrophages and epithelioid cells in the granulomas, since CTSS was expressed by these cells and the serum concentrations tended to rise in parallel with the number of affected organs, which roughly reflected the total granuloma load. Similar to ACE as a classic marker of sarcoidosis, CTSS could also be a marker of disease activity, because its concentrations varied in parallel with both the natural clinical course and in response to corticosteroid treatment.

The specificity of CTSS concentrations in terms of discriminating other lung diseases was modest, as these were elevated in a proportion of patients with other parenchymal lung diseases. This fact suggested that CTSS partially reflected inflammation in general, like the other serum markers of sarcoidosis (3,18,19,20). Nevertheless, the CTSS concentrations in sarcoidosis were significantly higher than those in all the other lung diseases examined. This fact enabled establishment of an appropriate cut-off concentration of CTSS, which is potentially useful for differentiating sarcoidosis from other parenchymal lung diseases that occasionally exhibit lung shadows that are similar to those in sarcoidosis.

CTSS is expressed in B cells, macrophages, and dendritic cells and is required for invariant chain (Ii) degradation and antigen processing (21,22). Ii is a peptide within antigen-presenting cells and covers the peptide-binding groove of the major histocompatibility complex (MHC) II molecules until it encounters a foreign peptide in the lysosomal compartment of the cells (7). In the presence of interferon-γ, CTSS becomes the main enzyme that can cleave Ii, leaving class II-associated Ii peptides, which are then displaced by HLA-DM in the presence of antigens. This allows antigen peptides to bind to class II molecules and to be transported to the cell membrane for presentation (22,23). CTSS is supposedly involved in the pathogenic mechanism of sarcoidosis through these processes.

Acknowledgments

We are grateful to Ms. Kamiya and Ms. Takagi for their excellent technical assistance.This study was supported in part by a grant of the Strategic Research Foundation Grant-aided Project for Private Universities from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (MEXT), 2011-2015 (S1101027) and by a grant for Rare Lung Diseases (pulmonary alveolar proteinosis, congenital interstitial lung disease) from the Ministry of Health Labor and Welfare, Japan.

References

1. Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis 1999; 16: 149-73.
2. Lieberman J. Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis. Am J Med 1975; 59: 365-72.
3. Tsutsumi T, Nagai S, Imai K, Setoyama Y, Uchiyama T, Izumi T. Soluble interleukin-2 receptor in blood from patients with sarcoidosis and idiopathic pulmonary fibrosis. Sarcoidosis 1994; 11: 102-9.
4. Miyoshi S, Hamada H, Kadowaki T, et al. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest 2010; 137: 1391-7.
5. Bargagli E, Mazzi A, Mezzasalma F, et al. The analysis of tryptase in serum of sarcoidosis patients. Inflammation 2009; 32: 310-4.
6. Eroglu H, Genc NS. Conventional markers in determination of activity of sarcoidosis. Int Immunopharmacol 2015; 25: 174-9.
7. Bloom CI, Graham CM, Berry MP, et al. Transcriptional blood signa-

tures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS One 2013; 8: e70630.
8. Su R, Li MM, Bhakta NR, et al. Longitudinal analysis of sarcoidosis blood transcriptomic signatures and disease outcomes. Eur Respir J 2014; 44: 985-93.
9. Silva E, Souchelnytsukyi S, Kasuga K, Eklund A, Grunewald J, Wheelock A. Quantitative intact proteomics investigations of alveolar macrophages in sarcoidosis. Eur Respir J 2013; 41: 1331-9.
10. Bons JA, Drent M, Bouwman FG, Mariman EC, van Dieijen-Visser MP, Wodzig WK. Potential biomarkers for diagnosis of sarcoidosis using proteomics in serum. Respir Med 2007; 101: 1687-95.
11. Zhang Y, Chen X, Hu Y, et al. Preliminary characterizations of a serum biomarker for sarcoidosis by comparative proteomic approach with tandem-mass spectrometry in ethnic Han Chinese patients. Respir Res 2013; 14: 18.
12. 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.
13. 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: 733-48.
14. Yamaguchi E, Okazaki N, Itoh A, Furuya K, Abe S, Kawakami Y. Enhanced expression of CD2 antigen on lung T cells. Am Rev Respir Dis 1991; 143(4 Pt 1): 829-33.
15. Rego N, Bianchi S, Moreno P, et al. Search for an aetiological virus candidate in chronic lymphocytic leukaemia by extensive transcriptome analysis. Br J Haematol 2012; 157: 709-17.
16. Furuya K, Yamaguchi E, Itoh A, et al. Deletion polymorphism in the angiotensin I converting enzyme (ACE) gene as a genetic risk factor for sarcoidosis. Thorax 1996; 51: 777-80.
17. Grutters JC, Fellrath JM, Mulder L, Janssen R, van den Bosch JM, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. Chest 2003; 124: 186-95.
18. Witkowska AM. On the role of sIL-2R measurements in rheumatoid arthritis and cancers. Mediators Inflamm 2005; 2005: 121-30.
19. Ishii H, Iwata A, Oka H, Sakamoto N, Ishimatsu Y, Kadota J. Elevated serum levels of lysozyme in desquamative interstitial pneumonia. Intern Medicine 2010; 49: 847-51.
20. Sipeki N, Antal-Szalmas P, Lakatos PL, Papp M. Immune dysfunction in cirrhosis. World J Gastroenterol 2014; 20: 2564-77.
21. Nakagawa TY, Rudensky AY. The role of lysosomal proteinases in MHC class II-mediated antigen processing and presentation. Immunol Rev 1999; 172: 121-9.
22. Honey K, Rudensky Y. Lysosomal cysteine proteases regulate antigen presentation. Nat Rev Immunol 2003; 3: 472-82.
23. Rocha N, Neefjes J. MHC class II molecules on the move for successful antigen presentation. EMBO J 2008; 27:1-5.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 148-156

The role of video-assisted thoracoscopic surgery in the diagnosis of interstitial lung disease

Keishi Sugino[1, 5], Hajime Otsuka[2], Yusuke Matsumoto[1], Yasuhiko Nakamura[1], Keiko Matsumoto[3], Yoko Azuma[2], Takashi Makino[2], Akira Iyoda[2], Kazutoshi Shibuya[4], Sakae Homma[1]

[1]Department of Respiratory Medicine, [2]Department of Chest Surgery, [3]Department of Diagnostic Radiology, [4]Department of Pathology, Toho University Omori Medical Center, Tokyo, Japan; [5]Department of Respiratory Medicine, Jizankai Medical Fundation Tsuboi Cancer Center Hospital, Asakamachi, Koriyama City, Fukushima, Japan

Abstract. *Background:* When a clinical context is indeterminate for idiopathic pulmonary fibrosis (IPF), or a chest high-resolution computed tomography (HRCT) pattern is not indicative of typical or probable usual interstitial pneumonia (UIP) in patients with interstitial lung disease (ILD), surgical lung biopsy should be considered to make a confident diagnosis on the basis of multidisciplinary diagnosis (MDD). *Aim:* The aim of this study was to evaluate the role and safety of video-assisted thoracoscopic surgery (VATS) in patients with ILD. *Methods:* A total of 143 patients with ILD underwent VATS at Toho University Medical Center Omori Hospital between March 2004 and April 2017. We conducted a retrospective study on the usefulness and safety of VATS in the diagnosis of ILD under MDD. *Results:* The 30-day mortality was 0%. The postoperative complication rate was 12.6%, which included 5 cases of pneumothorax after discharge (3.5%), 4 cases of prolonged air leakage (2.8%), and 2 cases of acute exacerbation (1.4%). Three of 9 cases (33.3%) complicated by pneumothorax after discharge or prolonged air leakage were resected specimens of pleuroparenchymal fibroelastosis (PPFE). Two patients had acute exacerbation, who were ultimately diagnosed as having idiopathic unclassifiable IP and had histologically significant irregular dense fibrosis and numerous fibroblastic foci. The comparison between chest HRCT and histopathological findings revealed 55 cases of possible UIP [UIP (45%), NSIP (25%), and unclassifiable IP (29%)] and 21 cases of inconsistent with UIP [UIP (10%), NSIP (33%), organizing pneumonia (10%), unclassifiable IP (24%), and PPFE (24%)]. *Conclusion:* VATS can be safely performed to obtain a confident diagnosis for appropriate treatment strategies in patients with ILD. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 148-156)*

Key words: interstitial lung disease, video-assisted thoracoscopic surgery, complication, survival, multidisciplinary discussion diagnosise

Abbreviations List:
SLB: surgical lung biopsy
VATS: video-assisted thoracoscopic surgery
OLB: open lung biopsy
HRCT: high resolution computed tomography
ILD: interstitial lung disease
MDD: multidisciplinary discussion diagnosis
IIPs: idiopathic interstitial pneumonias
IPF: idiopathic pulmonary fibrosis
NSIP: nonspecific interstitial pneumonia
UIP: usual interstitial pneumonia
CHP: chronic hypersensitivity pneumonia
PPFE: pleuroparenchymal fibroelastosis
AE: acute exacerbation
KL-6: Krebs von den Lungen-6
SP-D: surfactant protein-D
FVC: forced vital capacity
DLco: diffusing capacity for carbon monoxide

Received: 3 November 2018
Accepted after revision: 28 April 2019
Correspondence: Keishi Sugino, MD. PhD
Department of Respiratory Medicine,
Toho University School of Medicine,
6-11-1, Omori-nishi, Ota-ku, Tokyo 143-8541, Japan
Tel: +81-3-3762-4151 - Fax: +81-3-3766-3551
E-mail: keishi.sugino@med.toho-u.ac.jp

Introduction

In recent years, the necessity of surgical lung biopsy (SLB) for the purpose of diagnosis of interstitial lung disease (ILD) has been questioned not only because of the development of the chest high-resolution computed tomography (HRCT) but also because of the high morbidity and mortality associated with the procedure. More recently, Lynch, et al. (1) emphasized in a Fleischner Society White Paper that a confident diagnosis of idiopathic pulmonary fibrosis (IPF) can be made in a correct clinical context without SLB when CT imaging shows a pattern of typical or probable usual interstitial pneumonia (UIP). However, it is very important and challenging work-up for pulmonologist to make a correct diagnosis from over 100 different ILDs (2). When a clinical context is indeterminate for IPF, or a chest HRCT pattern is not indicative of typical or probable UIP in patients with ILD, surgical lung biopsy should be considered to make a confident diagnosis on the basis of multidisciplinary discussion diagnosis (MDD). Indeed, the chest HRCT findings do not always represent typical features of patients with ILD. Sverzellati et al. (3) reported that 34 out of 55 patients diagnosed as IPF on biopsy had received a diagnosis of NSIP, CHP, or sarcoidosis on SUGINO, The role VATS in ILD chest CT. In addition, Morris et al. (4) described that only 54% of patients who received a consensus diagnosis of UIP after video-assisted thoracoscopic surgery (VATS) lung biopsy, had received a diagnosis of probable UIP on chest HRCT. Therefore, VATS can be considered as one of necessary tool for the accurate diagnosis of ILD.

It has been reported that in general, risk factors of SLB are male sex, increasing age, increasing comorbidity, unstable condition such as rapidly progressive ILD requiring mechanical ventilation, severely impaired pulmonary function, coexisting of pulmonary hypertension in patients with ILD, undergoing open lung biopsy (OLB), and a provisional diagnosis of IPF or connective tissue disease–related ILD (5-8). VATS is generally considered as a safe procedure to provide adequate lung tissue samples for definitive histological diagnosis. However, postoperative complications may outweigh the potential benefits in patients with ILD because postoperative acute exacerbation (AE) or prolonged air leakage is one of the particularly critical and significant complications. According to a comprehensive literature review by Nguyen and Meyer (9), the overall 30-day mortality for OLB was 4.3% versus 2.1% for VATS biopsy, and non-lethal complications appeared to occur more frequently with OLB (18.1%) vs. VATS (9.6%) procedures. Given that VATS reduces risks of morbidity and mortality in this study, we aimed to assess the usefulness and postoperative complications of VATS in patients with ILD at our institution.

Methods

Patients

This study cohort included a total of 143 consecutive patients underwent VATS for suspected ILD at Toho University Omori Medical Center between March 2004 and April 2017. We conducted a retrospective review of the usefulness and postoperative complications of VATS in the diagnosis of ILD under MDD. All patients underwent a preoperative work-up including spirometry, blood gas analysis, electrocardiogram, and ultrasonic cardiogram. The data collected include: baseline patient characteristics, pulmonary function test findings, chest HRCT images, surgical parameters such as biopsy site, location, operation and anesthesia time, duration of chest drainage, postoperative complications, and 30-, 60-, and 90-day mortality. All scans were reviewed by consensus by 2 clinicians (S. H., K. Su.) and 1 radiologist (K. M.) with vast experience in ILD, who were blinded to histopathology results and clinical information. All patients were reviewed and interpreted by 2 expert pulmonary pathologists (K. S., T. U.). The postoperative diagnosis of ILD was determined at MDD. Patients excluded for VATS had a definite UIP pattern on chest HRCT, which was determined in accordance with the 2011 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association (ATS/ERS/JRS/ALAT) consensus statement (10). In addition, patients with diffusion capacity <30% predicted, AE-IPF and mechanical ventilation were excluded from VATS in the present study.

Informed consent for VATS was obtained from all patients. The study protocol was approved by the institutional ethics committee of Toho University Omori Medical Center (IRB No. M16266).

Surgical lung biopsy

A number of 2 or 3 biopsy sites, which were mild or moderate lesions adjacent to normal lungs avoiding severely affected areas, were determined through discussion with respiratory physicians, surgeons, and radiologists. Basically, VATS was performed with 3-port access under general anesthesia using a double-lumen endotracheal tube for single-lung ventilation as a standard procedure. In a case of severe pleural adhesions, conversion to a minithoracotomy was performed. As a result, triangle-shaped specimens with 3 to 5 cm in each margin were obtained. All biopsy specimens were inflated with formalin using a syringe and a needle after removing staples. Tissue samples for microscopic analyses were embedded in paraffin after being fixed with 10% formaldehyde. Sections with a thickness of 4 μm were routinely stained with hematoxylin and eosin and elastic van Gieson stains.

Definitions of postoperative complications

All complication occurred within 30 days after VATS. Postoperative AE was diagnosed by modified criteria for AE of IPF proposed by Collard et al. (11) and all of the following 3 conditions had to be fulfilled: i) worsening or development of dyspnea within 30 days after undergoing VATS; ii) chest HRCT scan with new bilateral ground-glass opacities and/or consolidation superimposed on a background ILD; iii) no evidence of pulmonary infection, heart failure, pulmonary embolism, and alternative causes for acute lung injury. Prolonged air leak was defined as a status requiring chest tube placement for 5 days or more. In addition, pneumothorax after discharge indicated that there was no evidence of air leak during hospitalization.

Measurement of the levels of the serum markers

The serum level of Krebs von den Lungen (KL)-6 and surfactant protein (SP)-D were measured using a KL-6 enzyme-linked immunosorbent assay (ELISA) kit (Eisai Co. Ltd., Tokyo, Japan) and a SP-D ELISA kit (Yamasa, Tokyo, Japan), respectively. These were both commercially available kits. The cut-off levels of serum KL-6 and SP-D were <500 U/ml and <110 ng/ml, respectively.

Chest CT scan

A helical CT scanner (Aquilion 16, Toshiba, Tokyo, Japan) was applied. Thin-section CT scans were obtained at full inspiration and CT images were reconstructed by 1-2-mm collimation sections with a high spatial frequency algorithm and photographed at window settings appropriate for viewing the lung parenchyma (window level from -600 Hounsfield Units (HU); width from 1600 HU).

Statistical analysis

Data were expressed as median value and range for continuous variables, and as number and percentage for categorical variables. Data analyses were performed using statistical software (JMP, version 10.0.0, SAS Institute, Cary, NC, USA).

Results

Baseline patient characteristics

Baseline patient characteristics of this study are summarized in Table 1.

The study population consisted of 143 patients. There were 69 male (48%) and 74 female (52%) patients with a median age of 64 years (range 33-81 years). In 90 patients (62.9%), arterial blood gas analysis showed a ≥PaO_2 of 80 Torr. The median serum KL-6 and SP-D were 743 U/mL and 127 ng/mL, respectively. The pulmonary function test revealed that a ≥ forced vital capacity (FVC) of predicted of 60% and a ≥ diffusion capacity for carbon monoxide (DLco) of predicted of 50% were seen in 128 patients (89.5%) and 122 patients (89.1%), respectively (Table S1).

Surgical parameters in VATS

Characteristics of VATS lung biopsy procedure are summarized in Table S2.

While 3-port approach was carried out in 139 patients (97.2%), 4 patients were converted to minithoracotomy because of extensive pleural adhesion. The number of biopsy sites was 2 in 109 patients (76.2%). One biopsy was taken in 13 (9.0%) patients, and 3 biopsies were taken in 21 (14.7%) patients.

Table 1. Baseline demographic characteristics of 143 patients with ILD

Variable	Median or number	Range
Age, yrs	64	33-81
Sex, male/female	69/74	
Smoking history, Never/Former/Current	52 (36.4%)/76 (53.1%)/15 (10.5%)	
PaO_2, Torr	81.5	54.9-112
$PaO_2 \geq 80$	90 (62.9%)	
$70 \leq PaO_2 < 80$	36 (25.2%)	
$60 \leq PaO_2 < 70$	15 (10.5%)	
$PaO_2 < 60$	2 (1.4%)	
KL-6, U/ml	743	147-7580
SP-D, ng/ml	127	29.5-1530

ILD; interstitial lung disease, PaO_2; partial pressure of arterial oxygen, KL-6: Kreb von den Lungen-6, SP-D: surfactant protein D

The most common locations of biopsy sites were right lower lobe (32.3%) and left lower lobe (33.0%). Mean operation time was 96 min, and mean anesthesia time was 169 min. Intraoperative blood loss was extremely low (median; 0 mL, under 40 mL). The median duration of chest drainage and hospital stay was 1 and 6 days, respectively.

Complications of VATS

Total of 18 patients (12.6%) experienced postoperative complications such as pneumothorax after discharge in 5 (3.5%), prolonged air leakage in 4 (2.8%), AE of ILD in 2 (1.4%), and other complications in 7 patients. Three patients with pneu-

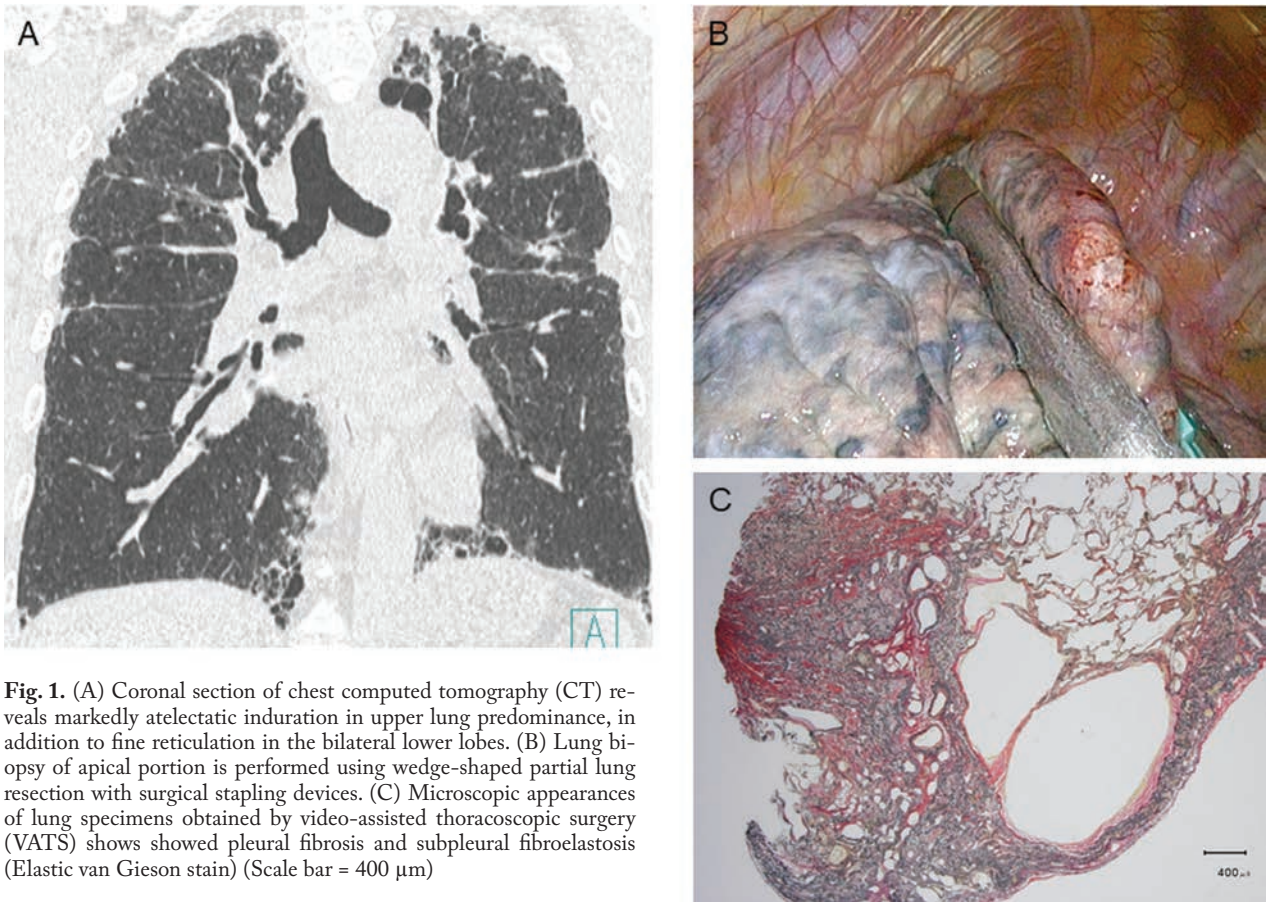


Fig. 1. (A) Coronal section of chest computed tomography (CT) reveals markedly atelectatic induration in upper lung predominance, in addition to fine reticulation in the bilateral lower lobes. (B) Lung biopsy of apical portion is performed using wedge-shaped partial lung resection with surgical stapling devices. (C) Microscopic appearances of lung specimens obtained by video-assisted thoracoscopic surgery (VATS) shows showed pleural fibrosis and subpleural fibroelastosis (Elastic van Gieson stain) (Scale bar = 400 μm)

mothorax after discharge required re-drainage and the remaining 2 patients had to undergo operation to close pulmonary fistulas. Prolonged air leakage in 2 patients resolved with continuous drainage, and the leakage in 2 other patients resolved with re-drainage. Three of 9 patients, who had a complication associated with pneumothorax after discharge along with a prolonged air leakage, underwent a biopsy for pleuroparenchymal fibroelastosis (PPFE) lesions in upper lobes (Figure 1, 2). The clinical characteristics of 2 patients who developed postoperative AE are summarized in Table 2. AE were observed on the second and fifth day after VATS. Additionally, locations of GGO accompanied by AE were primarily seen in the non-operated lungs of the present 2 cases. These histopathological findings were consistent with unclassifiable IP, including significant irregular dense fibrosis and numerous fibroblastic foci (Figure 3). Two patients complicated by AE were treated with 3-day intravenous administration of methylprednisolone (1000 mg/day). Thereafter, the dose was tapered based on their respiratory condition. Furthermore, synthetic neutrophil elastase inhibitor and cyclosporine A were added to the treatment. The both patients were consequently discharged at least once (Figure 4). No patients died 30 days after VATS. Although 1 patient died as a result of the second onset of AE within 90 days, this patient was classified as non-procedure related mortality (Table 3).

MDD of ILD

All 143 patients of ILD received a definite diagnosis after VATS under MDD. The most common diagnoses were IIPs (52.4%), including IPF (21.7%), NSIP (15.4%), and unclassifiable IP (10.5%), in addition to IP related collagen tissue disease (CTD) and chronic hypersensitivity pneumonitis (CHP) (Table 4). The comparison between chest HRCT and histopathological findings revealed 55 cases of possible UIP [UIP (45%), NSIP (25%), and unclassifiable IP (29%)] and 21 cases of inconsistent with UIP [UIP (10%), NSIP (33%), organizing pneumonia (10%), unclassifiable IP (24%), and PPFE (24%)] (Table 5).

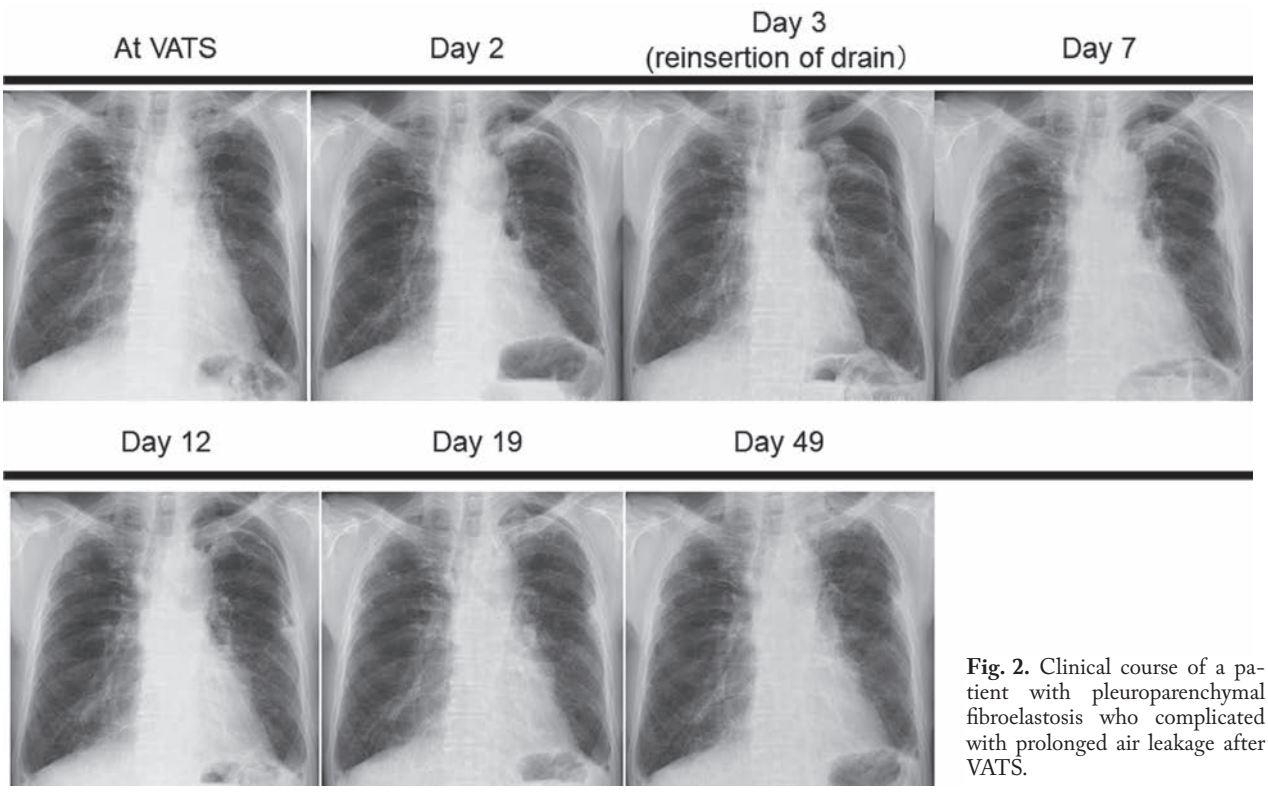


Fig. 2. Clinical course of a patient with pleuroparenchymal fibroelastosis who complicated with prolonged air leakage after VATS.

Table 2. Clinical characteristics of patients who developed postoperative acute exacerbation

No.	Age	Sex	%FVC	%DLco	PaO_2	Operation time (Days)	Pathological diagnosis	Duration from onset of AE	Cause of death	Survival time (days)
1	70	F	49.1	55.4	95.2	51	Unclassified (fibrotic NSIP + irregular fibrosis)	2	2nd AE	76
2	71	F	72.2	61.2	67.3	99	Unclassified (UIP + irregular fibrosis)	5	2nd AE	656

F; Female, FVC; forced vital capacity, DLco; diffusing capacity for carbon monoxide, PaO_2; partial pressure of arterial oxygen, NSIP; non-specific interstitial pneumonia, UIP; usual interstitial pneumonia, AE; acute exacerbation

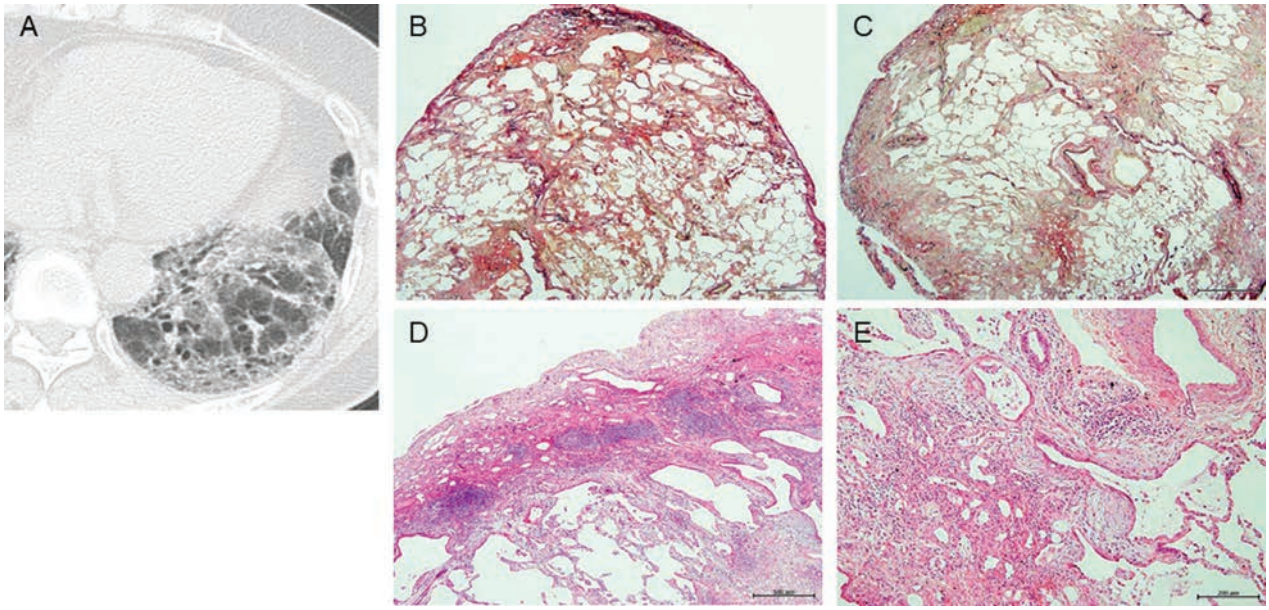


Fig. 3. (A) Chest CT scan shows diffuse reticulation and widespread ground-glass opacity accompanied by traction bronchiectasis predominantly in the bilateral lower lobes. (B) Lung biopsy specimens from the left lingula obtained by VATS reveals prominent uniform thickening of alveolar septa by fibrosis (nonspecific interstitial pneumonia; NSIP pattern) (Elastic van Gieson stain) (Scale bar = 1 μm). (C) Lung biopsy specimens from the left lower lobe reveal subpleural and perilobular fibrosis adjacent to relatively normal alveoli (usual interstitial pneumonia; UIP pattern) (Elastic van Gieson stain) (Scale bar = 1 μm). (D) There are marked inflammatory cell infiltration and lymphoid hyperplasia within dense collagen fibrosis (Hematoxylin-Eosin stain) (Scale bar = 200 μm). (E) Fibroblastic foci are sporadically present in dense collagen fibrosis and lymphocytes and plasma cells infiltration is mainly observed (Hematoxylin-Eosin stain) (Scale bar = 200 μm)

Discussion

In this single center retrospective study, we aimed to assess the usefulness and postoperative complications of VATS in patients with ILD at our institution. The usefulness of SLB for the purpose of diagnosis of ILD remains still controversial not only because of the development of the chest HRCT but also because of the high morbidity and mortality associated with the procedure. Moreover, in several institutions, SLB has been replaced with cryobiopsies; however, according to the latest IPF guideline, in cases without typical UIP pattern on chest HRCT, VATS is recommended (conditional recommendation) to be performed for a confident diagnosis (12).

The biopsy number and site may affect the diagnostic efficacy of VATS. In the present study, the site of biopsy was determined by the abnormalities on chest CT images of major lesions and intermediate or minor lesions. There is histologic discordance between lobes in 13-26% of patients with IIPs (13, 14). Therefore, a single site of biopsy cannot be suffi-

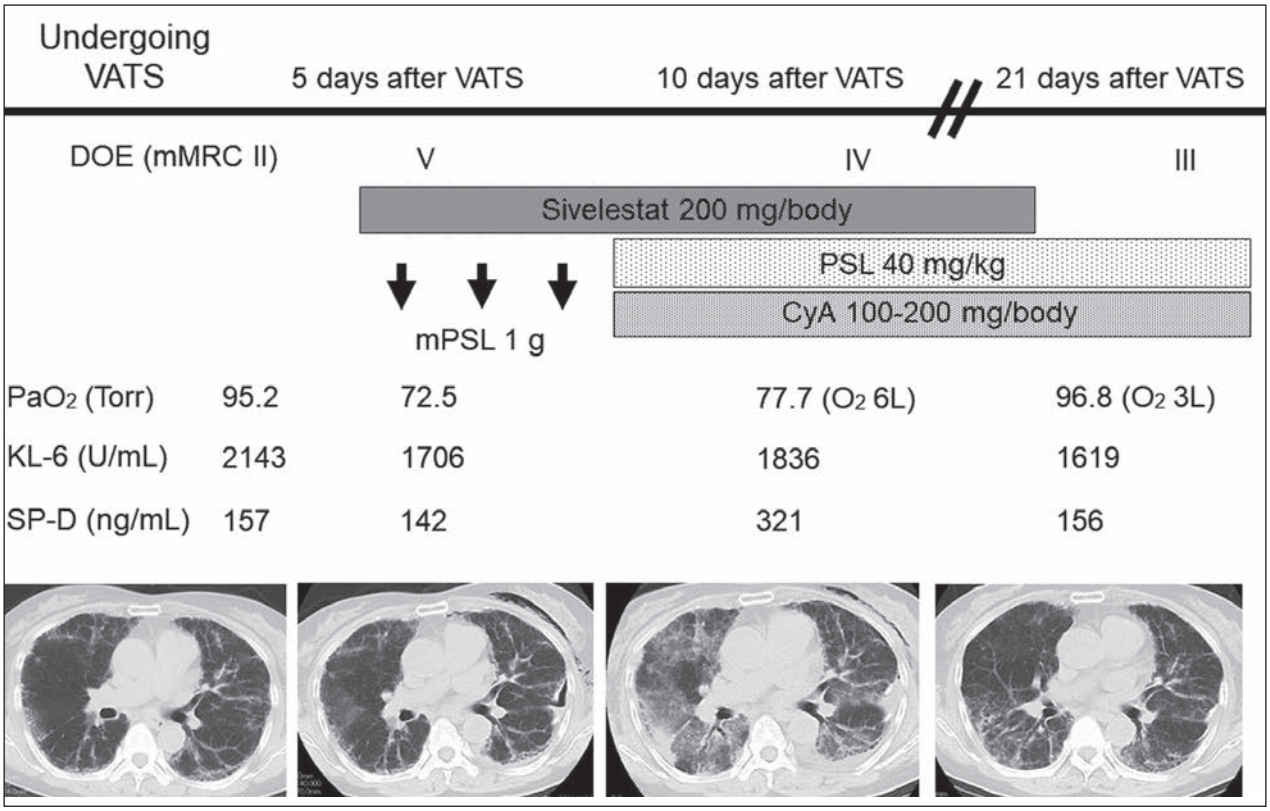


Fig. 4. Clinical course of a patient with idiopathic unclassifiable interstitial pneumonia who developed acute exacerbation after VATS

Table 3. Postoperative complications and operative mortality

Variable	Number	%
Postoperative complications	18	12.6
Pneumothorax after discharge	5	3.5
Prolonged air leakage (≥5 days)	4	2.8
Wound infection	3	2.1
Atelectasis	2	1.4
Acute exacerbation of interstitial pneumonia	2	1.4
Hemothorax	1	0.7
Pneumonia	1	0.7
Operative mortality		
30 days	0	0
60 days	0	0
90 days	0	0

Table 4. MDD of ILD

Variable	Number	%
Idiopathic interstitial pneumonia	75	52.4
Idiopathic pulmonary fibrosis	31	21.7
Nonspecific interstitial pneumonia	22	15.4
Cryptogenic organizing pneumonia	2	1.4
Unclassifiable interstitial pneumonia	15	10.5
Pleuroparenchymal fibroelastosis	5	3.5
Interstitial pneumonia related collagen vascular disease	49	34.3
Chronic hypersensitivity pneumonitis	11	7.7
IgG4 related lung disorder	2	1.4
Sarcoidosis	1	0.7
Langerhans cell histiocytosis	1	0.7
Leukemia	1	0.7
Diffuse panbronchiolitis	1	0.7
Common variable immunodeficiency	1	0.7
HTLV-I associated bronchiolo-alveolar disorder	1	0.7

MDD; multidisciplinary discussion diagnosis, ILD; interstitial lung disease, HTLV-1; Human T-cell leukemia virus type 1

cient to obtain a definite diagnosis for ILD patients. In this study, 2 or 3 biopsies were obtained from approximately 90% of our patients. More than half of our patients (61.5%) underwent a biopsy on both the upper and lower lobes.

It has been reported that in general, risk factors of SLB are male sex, increasing age, increasing comorbidity, an unstable condition such as rapidly progressive ILD requiring for mechanical ventilation, severely impaired pulmonary function, coexisting of pulmonary hypertension in patients with ILD, undergoing open lung biopsy (OLB), and a provisional

Table 5. Comparison between chest HRCT images and histological findings in IIPs

Histologic pattern	Chest HRCT pattern		MDD
	Possible UIP (n = 55)	Inconsistent with UIP (n = 21)	
UIP	25 (45%)	2 (10%)	IPF
NSIP	14 (25%)	7 (33%)	NSIP
OP	0 (0%)	2 (10%)	COP
Unclassifiable	16 (29%)	5 (24%)	Unclassifiable IP
PPFE	0 (0%)	5 (24%)	PPFE

HRCT; high resolution computed tomography, IIPs; idiopathic interstitial pneumonias, UIP; usual interstitial pneumonia, NSIP; nonspecific interstitial pneumonia, OP; organizing pneumonia, PPFE; pleuroparenchymal fibroelastosis, MDD; multidisciplinary discussion diagnosis, IPF; idiopathic pulmonary fibrosis, COP; cryptogenic organizing pneumonia

diagnosis of IPF or connective tissue disease-related ILD (5-8). In the present study, 30-, 60-, and 90-day mortality were 0%. However, %FVC of predicted of ILD patients with postoperative complication tend to decrease (79.2±20.7% vs. 88.2±21.1%). Moreover, one patient of postoperative AE had lower %FVC of predicted of 49.1% and %DLco of predicted of 55.4%, and the other had component of histological UIP. VATS is generally considered as a safe procedure to provide enough lung tissue samples for definitive histological diagnosis. However, postoperative complications may outweigh the potential benefits in patients with ILD because postoperative AE or prolonged air leakage is one of the particularly critical and significant complications. Sigurdsson et al. (15) reported that a 30-day mortality for SLB in 73 ILD patients was 2.7% and its complication rate was 16%. Moreover, Kreider et al. (16) demonstrated that a morbidity rate was 19% and a 60-day mortality rate was 4.4% in 68 patients with ILD undergoing VATS lung biopsy. According to a comprehensive literature review by Nguyen and Meyer (9), 9.6% of patients with ILD who underwent VATS experienced one or more postoperative complications. Additionally, the overall 30-day mortality was 2.1% for VATS lung biopsy. In this study, the 30-day mortality was 0%. Importantly, the low mortality rate may be related to appropriate patient selection criteria and operation by trained chest surgeons. The postoperative complication rate was 12.6%, including 5 cases of pneumothorax after discharge (3.5%), 4 cases of prolonged air leakage (2.8%), and 2 cases of acute exacerbation (1.4%). Three of 9 cases (33.3%) were complicated by either pneumothorax after discharge or prolonged air leakage, which were resected specimens of PPFE lesions. Probably, we should avoid resection of specimens of PPFE lesions in the upper lobes. As reported by Kreider et al. (16), 3 patients with pathological UIP and a low DLco value (19, 27, 30%) died after VATS lung biopsy. Postmortem examination in 2 of these 3 patients revealed diffuse alveolar damage on a background of UIP, consistent with AE of UIP. Thus, patients with definite UIP pattern on chest HRCT were excluded in this study. Interestingly, 2 cases developed postoperative AE in our study were histologically diagnosed with unclassifiable IP with significant irregular dense fibrosis and numerous fibroblastic foci. Unfortunately, it was impossible to predict these findings before undergoing VATS. Incidences of postoperative AE were too few to compare characteristics of ILD patients with or without AE.

Sverzellati et al. (3) reported that 34 out of 55 patients diagnosed as IPF on biopsy had received a diagnosis of NSIP, CHP, or sarcoidosis on chest CT. In addition, Morris et al. (4) described that only 54% of patients who received a consensus diagnosis of usual UIP after VATS lung biopsy had received a diagnosis of probable UIP on chest HRCT. In contrast, Raghu et al. (17) reported that the positive predictive value of possible UIP pattern on chest CT was 94% for the finding of histologic UIP. However, there has been a selection bias noticed in this study because the entire cohort of patients had histologic UIP and previously selected for participation in a clinical trial of IPF. Fell et al. (18) found that in patients without honeycomb change on HRCT, older age and a higher HRCT interstitial score are highly predictive of a diagnosis of IPF. A recent study by Gruden et al. (19) showed that a pattern of patchy heterogeneous basilar-predominant reticular abnormality without honeycombing was strongly associated with histologic UIP. In our study, approximately 50% of patients with possible UIP on chest HRCT was consistent with UIP; however, about 30% of patients was diagnosed with idiopathic unclassifiable IP. Some patients, who were considered as unclassifiable IP at initial diagnosis would ultimately be diagnosed as IPF through the clinical course under MDD. Therefore, we believe that MDD will be vitally necessary to make a more precise diagnosis for ILD.

The limitations of this study are as follows. Firstly, this study included retrospectively short

follow-up period and small number of patients at a single center. Therefore, our results may not represent the usefulness and safety of VATS in the entire ILD population. In the future, longer and larger observation prospective studies are needed to confirm our results. Secondly, patients with IP related to connective tissue diseases usually do not need to undergo a biopsy under VATS in clinical setting. However, we suppose that this attempt is useful to select whether anti-fibrotic agents or anti-inflammatory agents such as corticosteroid or immunosuppressants should be applied. Thirdly, patients with a definite UIP pattern on chest HRCT were excluded from indication of VATS based on their risk of AE-IPF in this study. However, we should pay attention to referring patients with possible UIP pattern for VATS, because many patients with possible UIP could have histologic UIP as reported by Raghu et al. (17) and because patients with a provisional diagnosis of IPF including patients with possible UIP pattern also have an increased mortality after SLB, as described by Hutchinson et al. (8).

In conclusion, although VATS is not required in patients with suspected IPF who had typical UIP pattern on chest HRCT, in other ILD patients, VATS is safely performed to obtain a confident diagnosis for appropriate treatment strategies.

Acknowledgments

We would like to thank Dr A. Kurosaki for interpretation of radiological findings (Department of Radiology, Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan), Dr T. Uekusa for analysis of pathological findings (Department of Pathology, Labor Health and Welfare Organization Kanto Rosai Hospital, Kanagawa, Japan).

This study is partially supported by a grant from Ministry of Health, Labor and Welfare of Japan awarded to the Study Group on Diffuse Pulmonary Disorders, Scientific, Research/Research on intractable diseases.

Author Contributions:

KSu, SH: study design, data analysis, manuscript preparation, guarantor of paper

KSu, HO, TM, YM, YN, KM, KSi: data collection, data analysis

KSu, HO, YM, YN, KM, YA, TM, AI, KSi, SH: manuscript preparation and review

All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Lynch DA, Sverzellati N, Travis WD, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. Lancet Respir Med 2018; 6: 138-53.
2. Jacob J, Hansell DM. HRCT of fibrosing lung disease. Respirology 2015; 20: 859-72.
3. Sverzellati N, Wells AU, Tomassetti S, et al. Biopsy-proved idiopathic pulmonary fibrosis: spectrum of nondiagnostic thin-section CT diagnoses. Radiology 2010; 254: 957-64.
4 . Morris D, Zamvar V. The efficacy of video-assisted thoracoscopic surgery lung biopsies in patients with Interstitial Lung Disease: a retrospective study of 66 patients. J Cardiothorac Surg 2014; 10; 9: 45.
5. Utz JP, Ryu JH, Douglas WW, et al. High short-term mortality following lung biopsy for usual interstitial pneumonia. Eur Respir J 2001; 17: 175-9.
6. Park JH, Kim DK, Kim DS, et al. Mortality and risk factors for surgical lung biopsy in patients with idiopathic interstitial pneumonia. Eur J Cardiothorac Surg 2007; 31: 1115-9.
7. Bando M, Ohno S, Hosono T, et al. Risk of acute exacerbation after video-assisted thoracoscopic lung biopsy for interstitial lung disease. J Bronchology Interv Pulmonol 2009; 16: 229-35.
8. Hutchinson JP, Fogarty AW, McKeever TM, et al. In-hospital mortality after surgical lung biopsy for interstitial lung disease in the United States. 2000 to 2011. Am J Respir Crit Care Med 2016; 193: 1161-7.
9. Nguyen W, Meyer KC. Surgical lung biopsy for the diagnosis of interstitial lung disease: a review of the literature and recommendations for optimizing safety and efficacy. Sarcoidosis Vasc Diffuse Lung Dis 2013; 30: 3-16.
10. Raghu G, Collard HR, Egan JJ, et al; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. 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.
11. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. Collard HR, Ryerson CJ, Corte TJ, et al. Am J Respir Crit Care Med 2016; 194: 265-75.
12. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med 2018; 198: e44-e68.
13. Sumikawa H, Johkoh T, Fujimoto K, et al. Usual interstitial pneumonia and nonspecific interstitial pneumonia: correlation between CT findings at the site of biopsy with pathological diagnoses. Eur J Radiol 2012; 81: 2919-24.
14. Flaherty KR, Travis WD, Colby TV, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med 2001; 164: 1722-7.
15. Sigurdsson MI, Isaksson HJ, Gudmundsson G, Gudbjartsson T. Diagnostic surgical lung biopsies for suspected interstitial lung diseases: a retrospective study. Ann Thorac Surg 2009; 88: 227-32.
16. Kreider ME, Hansen-Flaschen J, Ahmad NN, et al. Complications of video-assisted thoracoscopic lung biopsy in patients with interstitial lung disease. Ann Thorac Surg 2007; 83: 1140-4.
17. Raghu G, Lynch D, Godwin JD, et al. Diagnosis of idiopathic pulmonary fibrosis with high-resolution CT in patients with little or no radiological evidence of honeycombing: secondary analysis of a randomised, controlled trial. Lancet Respir Med 2014; 2: 277-84.
18. Fell CD, Martinez FJ, Liu LX, et al. Clinical predictors of a diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2010; 181: 832-7.
19. Gruden JF, Panse PM, Gotway MB, Jensen EA, Wellnitz CV, Wesselius L. Diagnosis of usual interstitial pneumonitis in the absence of honeycombing: evaluation of specific CT criteria with clinical follow-up in 38 patients. AJR Am J Roentgenol 2016; 206: 472-80.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 157-166

Cyclophosphamide pulse therapy as treatment for severe interstitial lung diseases

Arik Bernard Schulze[1], Georg Evers[1*], Andreas Kümmel[2], Felix Rosenow[3], Jan Sackarnd[3], Jan Philipp Hering[4], Christoph Schülke[4], Jonas Andreas Engelbertz[5], Dennis Görlich[6], Peter J. Barth[7], Georg Lenz[1,9], Heidemarie Becker[8], Michael Mohr[1#], Lars Henning Schmidt[1#]*

[1] Department of Medicine A, Hematology, Oncology and Pneumology, University Hospital Muenster, Muenster, Germany; [2] Department of Hematology, Medical Oncology and Pneumology, University Medical Center Mainz, Mainz, Germany; [3] Department of Cardiovascular Medicine, Internal Intensive Care Medicine, University Hospital Muenster, Muenster, Germany; [4] Department of Clinical Radiology, University Hospital Muenster, Muenster, Germany; [5] Unit of Cytostatic Reconstitution, Hospital pharmacy, University Hospital Muenster, Muenster, Germany; [6] Institute of Biostatistics and Clinical Research, Westfaelische Wilhelms-Universitaet Muenster, Muenster, Germany; 7 Gerhard-Domagk-Institute of Pathology, University Hospital Muenster, Muenster, Germany; [8] Department of Medicine D, Nephrology, Rheumatology and Hypertensiology, University Hospital Muenster, Muenster, Germany; [9] Cluster of Excellence EXC 1003, Cells in Motion, Muenster, Germany

Abstract. *Introduction:* Besides invasive or non-invasive ventilation, treatment of severe forms of interstitial lung diseases (ILD) includes immunosuppressive medication. In case of refractory organ- or life-threatening courses of disease, cyclophosphamide pulse therapy can serve as a rescue treatment option. *Objectives:* To investigate therapeutic and prognostic effects of cyclophosphamide for the treatment of severe forms of ILD on intensive care unit (ICU) we performed this analysis. *Methods:* Between 2009 and 2017 we identified 14 patients, who were treated on intensive care unit (ICU) with severe forms of ILD. Retrospectively, clinical, radiologic and prognostic data were collected and evaluated. *Results:* Our analysis demonstrated a prognostic impact of cyclophosphamide on the ILD in general. Whereas pulmonary manifestations of both systemic sclerosis (SSc) and ANCA-associated vasculitis had an improved outcome, a reduced overall survival was found for Goodpasture syndrome (GPS), dermatomyositis (DM), cryptogenic organizing pneumonia (COP) and drug reaction with eosinophilia and systemic symptoms (DRESS; p=0.040, logrank test). Besides, additional plasmapheresis and initiation of cyclophosphamide within ten days following initial diagnosis of ILD were associated with improved prognosis. *Conclusion:* Positive prognostic effects of cyclophosphamide pulse therapy in ICU treated patients suffering from severe respiratory failure due to pulmonary manifestations of both SSc and ANCA-associated-vasculitis were observed. Further prognostic and therapeutic data are needed for cyclophosphamide for this indication in order to prevent patients from its toxic side-effects, who most likely will not benefit from its application. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 157-166)*

Received: 23 August 2018
Accepted after revision: 24 February 2019
Correspondence: Dr. med. Arik Bernard Schulze
Department of Medicine A
University Hospital Muenster
Albert-Schweitzer-Campus 1, Building A1
48149 Muenster, Germany
Tel. +49-251-83-44827
E-mail: ArikBernard.Schulze@ukmuenster.de

Key words: intensive care medicine, interstitial lung disease, treatment, chemotherapy, computed tomography, exacerbation

[*] these authors are contributed equally to this work as first authors
[#] these authors are contributed equally to this work as senior authors

Introduction

The term "interstitial lung diseases" (ILD) encompasses both acute and chronic parenchymal lung diseases (1). The origin of ILD can be reactive to toxic agents, idiopathic (*i.e.* idiopathic interstitial pneumonia) or associated with systemic diseases such as granulomatous disorders, connective tissue diseases (CTD) or vasculitis (2, 3). For the final diagnosis anamnesis, clinical and functional data as well as radiologic ILD patterns and histopathological results are taken into consideration (2, 3, 5-8).

In general, treatment of acute exacerbations and progressive courses of ILDs is difficult. Often, immunosuppressive regimens are initiated with corticosteroids (3, 9). To intensify immunosuppressive treatment, addition of rituximab or cyclophosphamide is recommended only for progressive ILD forms due to either connective tissue disorders (CTD) or to vasculitides (10-12). As a rescue option, the British Thoracic Society (BTS) suggests the application of cyclophosphamide for the treatment of refractory and progressive ILD forms other than idiopathic pulmonary fibrosis (IPF) (13). However, only few data exist upon the prognostic and therapeutic effects of cyclophosphamide in critically ill patients.

For chronic ILD forms, Schupp et al. evaluated the impact of cyclophosphamide pulse therapy in n=26 patients. According to their analysis, prognostic outcome was improved for patients with lymphocytic interstitial pneumonia (LIP) and non-specific interstitial pneumonia (NSIP) following cyclophosphamide application. In contrast, patients with p-ANCA positive vasculitis had the worst prognosis. However, patients who had less than 3 infusions of cyclophosphamide and who were treated on ICU were not included in their study (14).

Since many ICU patients with severe ILD forms require invasive ventilation and sedation (15), it is often impossible to obtain patients' consent. Consequently, considering toxic side effects (16), the indication to initiate additional cyclophosphamide is met by interdisciplinary teams (7, 8).

To investigate the impact of cyclophosphamide pulse therapy in patients requiring ICU treatment due to respiratory failure caused by severe ILD forms, we performed this retrospective analysis with focus on radiologic ILD patterns and other clinical factors.

Material and Methods

Study population

First, approval of the ethical committee Muenster was obtained (Ref. 2017-599-f-S). In total, n=14,421 ICU patients were treated on our ICUs between 2009 and 2017. Among these patients, we identified n=14 patients suffering from different forms of ILD, who received at least one course of intravenous cyclophosphamide as rescue therapy (Table 1).

Data collection was performed retrospectively. Besides clinical data, therapeutic information (*e.g.* cyclophosphamide cycles, dosage, first-line immunosuppression, ventilation mode, ventilation duration, P/F ratio [*i.e.* Horowitz index=arterial oxygen partial pressure (p_aO_2 in mmHg)/fraction of inhaled oxygen (FiO_2 in %)], additional antibiotics, extracorporal membrane oxygenation and plasmapheresis) was gathered, too. Due to varying dosage schedules (mg/kg *vs.* mg/m^2 vs. mg as absolute dosage), all analyses considered the absolute cyclophosphamide dosage. Of interest, cyclophosphamide was given exclusively to patients with severe respiratory failure (as defined by a partial oxygen pressure in [mmHg]/oxygen saturation in inhaled gas [%]) <200) due to various forms of ILD, who required further immunosuppressive therapy. In contrast, if an infectious origin was suspected, cyclophosphamide was not applied. However, additional antibiotic prophylaxis was initiated before cyclophosphamide was given as prophylactic treatment in all patients.

Radiologic examination

With focus on the radiologic ILD patterns, thoracic computed axial tomography (CAT) scans of the identified patients were categorized as non-specific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), organizing pneumonia (OP), diffuse alveolar damage (DAD) and lymphocytic interstitial pneumonia (LIP) (17). To determine the morphologic extent of the underlying disease, the involvement of the affected lobes (*i.e.* upper left lobe including lingula, lower left lobe, upper right lobe, middle lobe and lower right lobe) was evaluated.

Table 1. Baseline characteristics of the study cohort. Age [years], cyclophosphamide dosage [mg], PaO_2/FiO_2 ratio [mmHg/%], ventilation period, delay from ILD diagnosis to first cyclophosphamide administration, survival since cyclophosphamide administration and follow-up period [days] are presented as mean with standard deviation (SD) and median with interquartile range (Q1-Q3). Sex, diagnoses, pathologic laboratory values, supportive therapy, ventilation mode, veno-venous extra corporal membrane oxygenation (ECMO), laboratory values and survival status are presented with the absolute and relative (in %) proportions

	N_{total} = 14	% of non-missing variables
Age [years]		
o mean (± SD)	52.4 (± 18.8)	
o median (Q1-Q3)	58 (35-65)	
Sex, N		
• male	7	50
• female	7	50
Diagnoses, N		
• Granulomatosis with polyangiitis (GPA)	4	29
• Microscopic polyangiitis (MPA)	3	21
• Goodpasture Syndrome (GPS)	2	14
• Dermatomyositis (DM)	2	14
• Systemic sclerosis (SSc)	1	7
• Cryptogenic organizing pneumonia	1	7
• DRESS	1	7
Pathologic laboratory parameters, *N*		
• c-ANCA/ anti-PR3	5 /14	36
• p-ANCA/ anti-MPO	3 /14	21
• RF	1 / 8	13
• ANA	4 /14	29
• anti-GBM	2 / 4	50
Supportive therapy, N		
• steroids, all tapered	14	100
- methylprednisolone 1 g/ 3d	6	43
- methylprednisolone 0.5 g/ 3d	2	14
- prednisolone 250 mg/ 3d	4	29
- prednisolone 100 mg/ 7-14d	2	14
• antibiotics	14	100
- aminopenicillin +β-lactamase-inhibitor	10	71
- cephalosporin ≥2. gen	2	14
- carbapenem	2	14
+ fluoroquinolon	2	14
+ macrolide	8	57
• plasmapheresis	7	50
Time from ILD diagnosis to cyclophosphamide treatment [days]		
o mean (± SD)	11.3 (± 11.2)	
o median (Q1-Q3)	6.0 (3.3-22.0)	
First cyclophosphamide dose [mg]		
o mean (± SD)	807 (± 204)	
o median (Q1-Q3)	775 (713-1,000)	
Ventilation mode, N		
• invasive	12	86
• non-invasive	2	14
Ventilation period [days]		
o mean (± SD)	16.9 (± 10)	
o median (Q1-Q3)	16.5 (8-27)	

(continued)

Table 1 ***(continued)*****.** Baseline characteristics of the study cohort. Age [years], cyclophosphamide dosage [mg], PaO_2/FiO_2 ratio [mmHg/%], ventilation period, delay from ILD diagnosis to first cyclophosphamide administration, survival since cyclophosphamide administration and follow-up period [days] are presented as mean with standard deviation (SD) and median with interquartile range (Q1-Q3). Sex, diagnoses, pathologic laboratory values, supportive therapy, ventilation mode, veno-venous extra corporal membrane oxygenation (ECMO), laboratory values and survival status are presented with the absolute and relative (in %) proportions

	N_{total} = 14	% of non-missing variables
PaO_2/FiO_2 ratio [mmHg/%]		
o mean (± SD)	91 (± 46)	
o median (Q1-Q3)	73 (53-133)	
ECMO (V/V), N		
• yes	7	50
• no	7	50
Survival status, N		
• censored	6	43
• dead	8	57
Causes of death, *N*		
• respiratory failure	2	25
• multi organ failure	5	63
• pulmonary hemorrhage	1	13
Overall survival [days]		
o mean (± SD)	682.2 (± 237.2)	
o median (Q1-Q3)	29 (15 - miss.)	
Follow up [days]		
o mean (± SD)	822.5 (± 255.2)	
o median (Q1-Q3)	946 (363-1007)	

Statistical analyses

Descriptive statistics for mean and standard deviation, median and interquartile range (IQR) as well as frequencies were used to investigate our study population. Two-fold associations between categorical variables were analyzed via Fisher's exact test or Chi-square test. Continuous variables were tested using either unpaired t-test or Mann-Whitney-U tests depending on the normality of the data. Associations of more than two groups (*e.g.* diagnosis variable) with categorial and continuous outcomes were analyzed using Chi-square test or Kruskal-Wallis test if applicable.

Overall survival was defined as time (days) between first cyclophosphamide application on intensive care unit and death or censoring. Survival time was compared between groups using Log rank tests and visualized by Kaplan Meier plots. Both, data collection and calculations were performed using IBM SPSS Statistics® version 24 (released 2016, IBM Corp., Armonk, NY, USA). The local significance level was set to 0.05. An adjustment to multiplicity was not determined as the analysis was explorative.

Results

The study population is characterized in Table 1. The majority of the investigated patients (n=12) displayed autoimmune features. Regardless of the underlying disease, mean time between the first diagnosis of interstitial lung disease and the ICU admission was 4.1 (± 8.3) days. Due to respiratory failure (P/F ratio average 90.6 (± 45.6)) on admission, supportive ventilation (*i.e.* invasive ventilation in n=12 patients; non-invasive ventilation in n=2 patients) was applied in all patients for a period of 16.9 days (± 10.0 days) independent from the underlying disease (p= .134, Kruskal-Wallis test). Additional extracorporal membrane oxygenation (ECMO) support was applied in n=7 patients.

With regard to systemic medication, all patients received both corticosteroids and antibiotics

prior to cyclophosphamide pulse therapy. The time between first ILD diagnosis and the initiation of cyclophosphamide therapy ranged between 1 and 35 days (mean 11.3 days, median 6 days). The applied absolute dosages of cyclophosphamide varied from 400 mg up to 1.000 mg (mean 807.1 mg). In addition, plasmapheresis was performed in five patients concurrently to the first application of cyclophosphamide. In two cases cyclophosphamide was given after or at least one week prior to plasmapheresis.

Overall survival for cyclophosphamide therapy is demonstrated in Figure 1. Cyclophosphamide pulse therapy was associated with an increased overall survival in one patient with systemic sclerosis and in patients with ANCA-associated vasculitis [*i.e.* microscopic polyangiitis; *MPA,* granulomatosis with polyangiitis; *GPA*, eosinophilic granulomatosis with polyangiitis; *EGPA* (10)]. Whereas median overall survival in patients with underlying Goodpasture syndrome (*GPS*) was 29 days, patients suffering from Dermatomyositis (*DM*), Drug reaction with eosinophilia and systemic symptoms (*DRESS*) and cryptogenic organizing pneumonia (*COP*) achieved less than 10 days. Of interest, one PR3 positive GPS patient died within 29 days, whereas one ANCA-negative GPS patient was still alive after 1007 days.

Cyclophosphamide doses >800 mg absolute were associated with a reduced overall survival (p=0.012, log rank test; Table 3). Yet, considering confounders, hemoglobin count, positivity for ANA or ANCA, impaired renal function (eGFR ≤59 ml/min/1.73m^2), ECMO application, ventilation mode, P/F ratio, plasmapheresis or radiologic ILD patterns did not alter cyclophosphamide dosage (p>0.05, Mann-Whitney-U test; Supplementary Table S1).

Regarding ILD patterns, we found possible non-specific interstitial pneumonia (NSIP) pattern in n=9 patients, usual interstitial pneumonia (UIP) pattern in n=2 patients and an organizing pneumonia (OP) pattern in n=6 patients. Of note, multiple radiographic patterns were observed in four patients (Table 2). For none of the radiologic patterns a significant prognostic effect was found (p>0.05, log rank test; Table 3). In n=12 cases all pulmonary lobes were affected by ILD, whereas in two patients only two respectively four lobes were affected. Of interest, overall survival did not correlate with the morphologic extent of ILD on chest CT scan (p=0.557, log

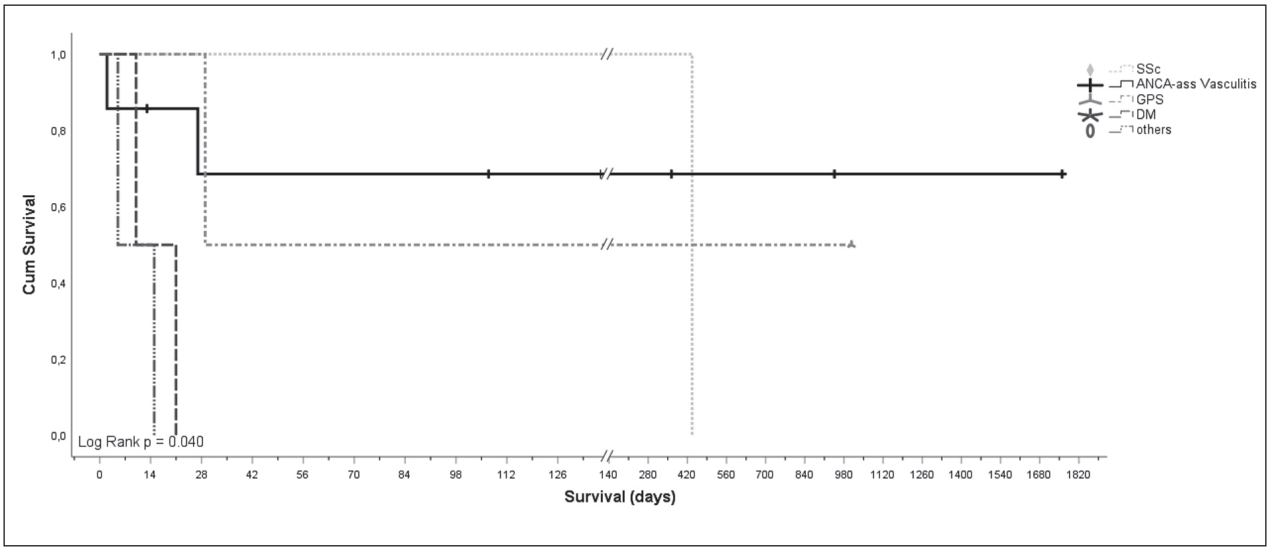


Fig. 1. Overall survival following first application of cyclophosphamide [days] stratified for ANCA-associated vasculitis (*i.e.* GPA, MPA), GPS, DM, SSc and other ILD forms (Log Rank p=0.040).
Legend: SSc: Systemic sclerosis, n=1; ANCA-ass vasculitis: Anti-neutrophil cytoplasmic antibodies-associated vasculitis (*i.e.* Granulomatosis with polyangiitis (GPA), Microscopic polyangiitis (MPA) and Eosinophilic granulomatosis with polyangiitis (EGPA)), n=7; GPS: Goodpasture syndrome, n=2; DM: Dermatomyositis, n=2; others, n=2, containing COP: Cryptogen organizing pneumonia, and DRESS: Drug reaction with eosinophilia and systemic symptoms. Survival axis scale [days] is split (indicated by //) after 140 days and then shows greater intersections.

Table 2. Study collective

No.	Sex	Age	Diagn.	Ventilation mode	Ventilation days	P/F ratio	ECMO	Plasma-pheresis	CAT scan pattern	Survival status	Overall survival [days]
1	M	55	GPA	IV	26	64,4	Yes	Yes	NSIP+ UIP- OP+	Alive	946
2	F	61	DM	IV	18	58,8	No	No	NSIP+ UIP- OP-	Dead	21
3	M	80	GPS	IV	30	141,6	Yes	Yes	NSIP+ UIP- OP+	Dead	29
4	M	64	MPA	IV	2	69,7	No	Yes	NSIP+ UIP- OP+	Alive	363
5	M	60	MPA	IV	6	40,2	Yes	Yes	NSIP+ UIP- OP-	Alive	107
6	F	51	SSc	NIV	15	46,1	No	No	NSIP- UIP- OP-	Dead	437
7	M	36	DRESS	IV	15	54,7	Yes	No	NSIP- UIP- OP-	Dead	15
8	F	67	DM	NIV	18	99,7	No	No	NSIP+ UIP+ OP-	Dead	10
9	M	61	COP	IV	28	130,0	Yes	No	NSIP+ UIP- OP+	Dead	5
10	F	31	GPA	IV	8	130,0	No	No	NSIP- UIP- OP-	Alive	13
11	M	19	GPS	IV	9	169,2	No	Yes	NSIP- UIP- OP+	Alive	1007
12	F	56	MPA	IV	24	152,8	Yes	No	NSIP+ UIP+ OP-	Dead	27
13	F	72	GPA	IV	32	76,0	No	Yes	NSIP- UIP- OP+	Alive	1760
14	M	20	GPA	IV	5	35,0	Yes	Yes	NSIP+ UIP- OP-	Dead	2

Legend: Sex category (M = male, F= female), Age [years], ventilation mode (IV = invasive ventilation, NIV = non-invasive ventilation), absolute period of ventilation [days], the P/F ratio (*i.e.* partial oxygen pressure in [mmHg]/ oxygen saturation in inhaled gas [%]), the use of ECMO and Plasmapheresis, CAT scan patterns, regarding NSIP, UIP and OP as possible (+) or unlikely (-), survival status (alive/ dead) and survival since first cyclophosphamide administration [days]. Of interest, patient no. 10 was lost-to-follow-up after 13 days and discharge from hospital

rank test) or severity of pulmonary failure. Furthermore no differences were found for anti-neutrophil cytoplasmic antibodies, anti-nuclear antibodies, rheumatoid factors, anti-glomerular basal membrane antibodies and blood count (p>0.05, log rank test). Impaired renal function (eGFR in ml/min/1.73 m^2 cutoff >59 *vs.* ≤59) as well as anemia (hemoglobin level in g/dl cutoff ≥11 *vs.* < 11) or leukocytosis (in cells/µl cutoff >10.000 vs. ≤10.000) was not associated with improved or reduced prognosis (p>0.050, log rank test). Among the investigated patients, n=7 patients died on ICU due to the fulminant course of disease. N=2 patients died due to respiratory failure, n=4 died due to multiple organ failure (MOF) and one patient died due to pulmonary hemorrhage. Of interest, one patient died 12 months later following initial ICU admission due to non-septic MOF.

Additional plasmapheresis (p=0.018, log rank test) and application of cyclophosphamide within ten days following first ILD diagnosis (p=0.028, log rank test) had a positive effect on prognosis (Table 3). Moreover, we observed that plasmapheresis was performed more often in patients with hemoglobin levels below 11 g/dl (p=0.029, Fisher's exact test) independent from ANCA- or ANA-positivity, leukocyte count, renal impairment, ECMO use, ventilation mode and radiographic ILD pattern (Fisher's exact test p>0.05 in all calculations, data not shown).

Discussion

Treatment of acute respiratory failure due to various ILD forms is challenging. The therapeutic armamentarium includes supportive ventilation, extracorporal membrane oxygenation, plasmapheresis, antibiotics and immunosuppressive medication. Corticosteroids are often applied as first immunosuppression (10, 13, 18-21). However, there are patients, who require more intensified immunosuppressive regimens to overcome refractory courses of disease. Here, cyclophosphamide or rituximab can serve as therapeutic alternatives especially for ANCA-associated vasculitis (10, 20, 21) or for CTDs (11). With regard to cyclophosphamide, the recommended dosage is 15 mg/kg body weight every 2 to 3 weeks in combination with corticosteroids (22).

Schupp et al. evaluated the prognostic impact of intravenous cyclophosphamide pulsed therapy in n=26 patients with chronic ILD forms, who did not require ICU treatment. Patients were followed up for 18 months. According to their analysis, beneficial effects were demonstrated for patients with both LIP-patterns and NSIP-patterns, whereas p-ANCA associated ILDs did not benefit from additional cyclophosphamide pulsed therapy (14).

In our study, we focused on those ILD patients who received cyclophosphamide as part of a rescue

Table 3. Univariate prognostic models depending on laboratory values, supportive therapies, CAT scan patterns and first absolute cyclophosphamide dosage. Additional plasmapheresis, early cyclophosphamide administration (≤10 days to ILD diagnosis) and an absolute first cyclophosphamide dose ≤800 mg were associated with improved overall survival in ILD patients treated with cyclophosphamide on ICU

Variable	Comparator	n (14)	Median OS[§] [days]	95% CI[§]	Log Rank p
Laboratory values[a]					
ANCA[b]	negative	6	15	1.8-28.2	0.156
	positive	8	n. r.[i]		
ANA[c]	negative	10	27	0.0-531.0	0.538
	positive	4	29	n. e.[k]	
Hemoglobin-level [g/dl]	<11	7	n. r.[i]		0.090
	≥11	7	21	5.6-36.4	
Leukocyte-count [/µl]	≤10.000	9	27	11.3-42.7	0.282
	>10.000	5	n. r.[i]		
eGFR[d] **[ml/min/1.73m²]**	>59	6	15	1.8-28.2	0.075
	≤59	8	n. r.[i]		
Supportive measures					
ECMO[e]	not used	7	n. r.[i]		0.280
	used	7	27	0.0-57.8	
Ventilation	invasive	12	29	n. e.[k]	0.419
	non-invasive	2	10	n. e.[k]	
P/F ratio	<100	9	437	0.0-1261.4	0.773
	≥100 - 199	5	29	9.1-48.9	
Plasmapheresis	not used	7	21	9.1-33.0	**0.018**
	used	7	n.r.[i]		
CAT scan patterns					
NSIP[f]	unlikely	5	437	n. e.[k]	0.286
	possible	9	27	9.5-44.5	
UIP[g]	unlikely	12	437	0.0-1383.2	0.148
	possible	2	10	n.e.[k]	
OP[h]	unlikely	8	21	6.2-35.8	0.072
	possible	6	n.r.[i]		
Treatment					
Cyclophosphamide dose [mg]	≤800	9	437	n. e.[k]	**0.012**
	>800	5	15	7.2-22.8	
Delay ILD diagnosis to Cyc. administration [days]	≤10	9	n.r.[i]		**0.028**
	>10	5	15	4.3-25.7	

Legend: [§] median overall survival in days since first cyclophosphamide administration, [$] 95% confidence interval, [a] laboratory values on ICU admission, [b] Anti neutrophil cytoplasmic antibodies, [c] Anti nuclear antibodies, [d] estimated glomerular filtration rate (CKD-EPI method[35]) in ml/min/1.73m², [e] extracorporal membrane oxygenation, [f] non-specific interstitial pneumonia, [g] usual interstitial pneumonia, [h] organizing pneumonia, [i] not reached, [k] not estimated

immunosuppressive treatment on ICU to overcome respiratory failure. We found superior treatment effects of cyclophosphamide in n=7 patients (50%) suffering from ANCA-associated ILDs compared to other ILDs. Similar observations were reported by Novack et al. in 1971 (23). A possible explanation for the results of Schupp et al. might be due to the fact, that in chronic (stable) ILD the possible life threatening side effects of cyclophosphamide exceed the benefit. Since plasmapheresis was applied in five out of seven patients (71%) with ANCA-associated vasculitis, it is difficult to interpret the sole effect of cyclophosphamide in this context.

For sure, one major limitation of our study is its small study population. However, it is difficult to perform prospective studies with larger study populations with focus on the risk-benefit analysis for cyclophosphamide for this indication. Among n=14,421 ICU patients treated between 2009 and 2017 on our ICUs, we identified only n=14 ILD patients, who were treated with cyclophosphamide due to this indication. To our knowledge, the underlying study is

the first study, which investigates the impact of cyclophosphamide for critically ill ILD patients suffering severe respiratory failure and requiring ICU therapy.

Acute treatment of various ILD forms is summarized in Figure 2. As demonstrated, the application of cyclophosphamide represents a therapeutic alternative for ILD forms. Whereas cyclophosphamide is recommended as first line treatment of SSc (20, 21, 24) and ANCA-associated vasculitis (10), there is less evidence for RA, DM and GPS. Here, cyclophosphamide could be an alternative option for refractory courses (19, 25, 26). Even though favorable effects of cyclophosphamide are reported for the treatment of pulmonary manifestations of GPS (18), there are still patients who died due to respiratory failure (27-29). Likewise, cyclophosphamide application had positive effects on lung function in some DM patients, still the prognosis in general is poor (19, 30). Even more evidence for the application of cyclophosphamide in COP is lacking. Whereas, corticosteroids are recommended for COP treatment (13, 31), less data exist upon the intensified immunosuppression with azathioprine, cyclosporine A or cyclophosphamide (13). With respect to the rather low incidence, one review recommended corticosteroids and intravenous immunoglobulins following the withdrawal of potential causal agents as treatment option for DRESS syndrome (32), whereas additional cyclophosphamide was only suggested by one study (33).

Against this background, we evaluated the therapeutic impact of cyclophosphamide for severe respiratory failure due to various ILD forms. Corresponding to the published recommendations, we observed positive effects of cyclophosphamide in those ILD patients with systemic sclerosis (SSc) (20) and ANCA-associated vasculitis (10). Especially in these patients, early administration of cyclophosphamide within ten days and a starting dosage of less than 800 mg had favorable effects. In consideration of the toxic

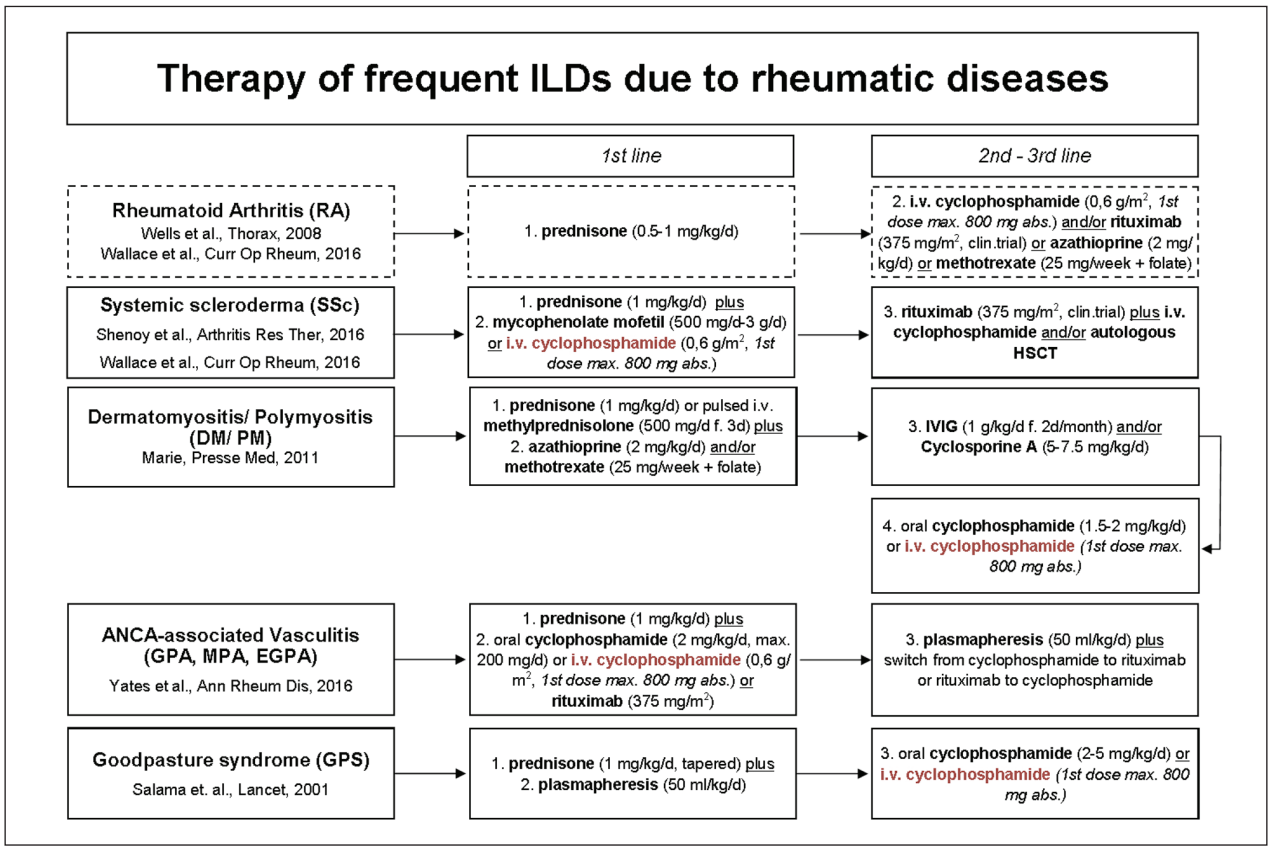


Fig. 2. Possible therapeutic options for the therapy of interstitial lung diseases (ILDs)

side effects (*e.g.* anemia, thrombocytopenia, leukocytopenia, hemorrhage cystitis) of cyclophosphamide further studies should investigate the optimal dosage (34). Without doubt, additional plasmapheresis (10) might have improved the clinical status. Since patients suffering from DM/PM did not benefit from additional cyclophosphamide therapy in our analysis, we cannot support its use for this specific indication.

In conclusion cyclophosphamide pulse therapy for the treatment of respiratory failure due to ILD can be a beneficial therapy option for some patients with underlying SSc and ANCA-associated vasculitis. When considering cyclophosphamide in these patients, treatment should be initiated early, the dosage should be below 800 mg and plasmapheresis should be added. However, there is less evidence for the application of cyclophosphamide in those patients with various ILD patterns due to GPS, DM, COP or DRESS. In consideration of the available literature and our analysis we cannot support the application of cyclophosphamide as first line treatment for ILD forms other than ANCA-positive vasculitis or SSc associated ILDs. Even though cyclophosphamide is still applied for this indication, it is one of the most toxic routinely prescribed immunosuppressive agents (13). Consequently, the identification of those patients who might benefit from this therapy is crucial. Here, the investigation of larger study collectives with focus on ICU patients with severe ILD forms could serve for further therapeutic stratification. Whether less toxic agents or targeted therapies will be available in future is unclear.

Authorship Statement: LHS and MM designed the study. LHS, ABS and GE performed research, collected data and analyzed the data. LHS and ABS wrote the paper. FR, AK and JS collected data and gave relevant input upon treatment on ICU. JPH and CS performed retrospective CAT scan analysis. JAE detected cyclophosphamide treated patients on ICU and gave relevant input on cyclophosphamide dosage and treatment schedules. DG planned, controlled and supported the statistical analysis. PJB, GL, MM, LHS and HB gave relevant input on underlying disease. Every author read and approved the corrected manuscript.

References

1. Travis WD, 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.
2. Demedts M, Costabel U. ATS/ERS international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. Eur Respir J 2002; 19: 794-6.
3. Fischer A, Du Bois R. Interstitial lung disease in connective tissue disorders. Lancet 2012; 380: 689-698.
4. Sambataro, G. et al. State of the art in interstitial pneumonia with autoimmune features: a systematic review on retrospective studies and suggestions for further advances. Eur Respir Rev an Off J Eur Respir Soc 27, 2018; 148: 170139
5. Fischer A, Lee JS, Cottin,V. Interstitial lung disease evaluation: Detecting connective tissue disease. Respiration 2015; 90: 177-184.
6. Robles-Perez A, Molina-Molina M. Treatment considerations of lung involvement in rheumatologic disease. Respiration 2015; 90: 265-274.
7. Tomassetti S, Piciucchi S, Tantalocco P, Dubini A, Poletti V. The multidisciplinary approach in the diagnosis of idiopathic pulmonary fibrosis: A patient case-based review. Eur Respir Rev 2015; 24, 69-77.
8. Bonini M, Fiorenzano G. Exertional dyspnoea in interstitial lung diseases: the clinical utility of cardiopulmonary exercise testing. Eur Respir Rev 2017; 26: 235-8.
9. Mathai SC, Danoff SK. Management of interstitial lung disease associated with connective tissue disease. BMJ 2016, h6819. doi:10.1136/bmj.h6819
10. Yates M, et al. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis 2016; 75: 1583-1594.
11. Saunders P, et al. Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial. Trials 2017; 18: 275.
12. Stone JH, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010; 363: 221-32.
13. Wells AU, Hirani N. Interstitial lung disease guideline. Thorax 2008; 63: v1-v58.
14. Schupp JC, Köhler T, Müller-Quernheim J. Usefulness of Cyclophosphamide Pulse Therapy in Interstitial Lung Diseases. Respiration 2016; 91: 296-301.
15. Xu Y. et al. Prone position ventilation support for acute exacerbation of interstitial lung disease? Clin Respir J 2017; 12: 1732-1380. doi:10.1111/crj.12665
16. Hengstler JG, et al. Induction of DNA crosslinks and DNA strand lesions by cyclophosphamide after activation by cytochrome P450 2B1. Mutat Res - Fundam Mol Mech Mutagen 1997; 373: 215-223.
17. de Lauretis A, Veeraraghavan S, Renzoni E. Review Series: Aspects of Interstitial lung disease: Connective tissue disease-associated interstitial lung disease: How does it differ from IPF? How should the clinical approach differ? Chron Respir Dis 2011; 8: 53-82.
18. Salama AD, Levy JB, Lightstone L, Pusey CD. Goodpasture's disease. Lancet 2001; 358: 917-920.
19. Marie I, Mouthon L. Therapy of polymyositis and dermatomyositis. Autoimmun Rev 2011; 11: 6-13.
20. Wallace B, Vummidi D, Khanna D. Management of connective tissue diseases associated interstitial lung disease. Curr Opin Rheumatol 2016; 28: 236-245.
21. Shenoy PD, Bavaliya M, Sashidharan S, Nalianda K, Sreenath S. Cyclophosphamide versus mycophenolate mofetil in scleroderma interstitial lung disease (SSc-ILD) as induction therapy: a single-centre, retrospective analysis. Arthritis Res Ther 2016; 18: 123.
22. de Groot K, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med 2009; 150: 670-80.
23. Novack SN, Pearson CM. Cyclophosphamide Therapy in Wegener's Granulomatosis. N Engl J Med 1971; 284: 938-942.
24. Tashkin DP, et al. Cyclophosphamide versus Placebo in Scleroderma Lung Disease N Engl J Med 2006; 354: 2655-2666.
25. Kurita T, Yasuda S, Amengual O, Atsumi T. The efficacy of calcineurin inhibitors for the treatment of interstitial lung disease associated with polymyositis/dermatomyositis. Lupus 2015; 24: 3-9.
26. Shimojima Y, Ishii W, Matsuda M, Kishida D, Ikeda S. Effective

Use of Calcineurin Inhibitor in Combination Therapy for Interstitial Lung Disease in Patients With Dermatomyositis and Polymyositis. JCR J Clin Rheumatol 2017; 23: 87-93.
27. Salam N, Rezki H, Fadili W, Hachim K, Ramdani B. Goodpasture's syndrome - four case reports. Saudi J Kidney Dis Transpl 2007; 18: 235-8.
28. Uezu Y, Kiyatake I, Tokuyama K. A case of Goodpasture's syndrome with massive pulmonary hemorrhage ameliorated by cyclophosphamide pulse therapy. Nihon Jinzo Gakkai Shi 1999; 41: 499-504.
29. Kojima K, et al. Two cases of Goodpasture's syndrome--clinicopathological studies and relapse. Nihon Jinzo Gakkai Shi 1993; 35: 89-96.
30. Kameda H, Takeuchi T. Recent advances in the treatment of interstitial lung disease in patients with polymyositis/dermatomyositis. Endocrine Metab Immune Disord - Drug Targets 2006; 6: 409-415.
31. Lazor R, et al. Cryptogenic organizing pneumonia. Characteristics of relapses in a series of 48 patients. The Groupe d'Etudes et de Recherche sur les Maladles 'Orphelines' Pulmonaires (GERM"O"P). Am J Respir Crit Care Med 2000; 162: 571-7.
32. Cacoub P, et al. The DRESS syndrome: A literature review. Am J Med 2011; 124: 588-597.
33. Laban E, et al. Cyclophosphamide Therapy for Corticoresistant Drug Reaction With Eosinophilia and Systemic Symptoms (DRESS) Syndrome in a Patient With Severe Kidney and Eye Involvement and Epstein-Barr Virus Reactivation. Am J Kidney Dis 2010; 55: e11-e14.
34. Jayne D. Review article: Progress of treatment in ANCA-associated vasculitis. Nephrology 2009; 14; 42-48.
35. Levey AS, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604-12.

Supplementary Table S1. Mann Whitney U test analysis for equal variances of not normally distributed absolute cyclophosphamide dose regarding laboratory values, supportive therapeutic measures and CAT scan patterns.

Variable	Comparator	n (14)	mean 1st CYC[$] dose [mg]	standard-deviation	p[$]
Laboratory values[a]					
ANCA[b]	negative	6	675	± 282	0.287
	positive	8	813	± 171	
ANA[c]	negative	10	820	± 224	0.069
	positive	4	588	± 143	
Hemoglobin-level [g/dl]	<11	7	786	± 165	0.645
	≥11	7	721	± 285	
Leukocyte-count [/µl]	≤10.000	9	811	± 247	0.149
	>10.000	5	650	± 154	
eGFR[d] [ml/min/1.73m²]	>59	6	758	± 294	0.894
	≤59	8	750	± 183	
Supportive measures					
ECMO[e]	not used	7	693	± 262	0.392
	used	7	814	± 184	
Ventilation	invasive	11	786	± 201	0.336
	non-invasive	3	633	± 321	
Plasmapheresis	not used	7	807	± 262	0.357
	used	7	700	± 189	
CAT scan patterns					
NSIP[f]	unlikely	5	730	± 277	0.784
	possible	9	767	± 211	
UIP[g]	unlikely	12	713	± 219	0.091
	possible	2	1000	n. e.[k]	
OP[h]	unlikely	8	813	± 252	0.126
	possible	6	650	± 148	
overall			**754**	**± 227**	

Legend: [$] mean cyclophosphamide dose in mg absolute, [$] Mann-Whitney-U test, asymp. significance (2-tailed), [a] laboratory values on ICU admission, [b] Anti neutrophil cytoplasmic antibodies, [c] Anti nuclear antibodies, [d] estimated glomerular filtration rate (CKD-EPI method[17]) in ml/min/1.73m², [e] extracorporal membrane oxygenation, [f] non-specific interstitial pneumonia, [g] usual interstitial pneumonia, [h] organizing pneumonia, [k] not estimated

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 167-171

Atypical presentation of isolated orbital Langerhans cell histiocytosis

Nikisha Q. Richards[1], Matthew Young[1], Kasey Pierson[1], John Le[1], Yuan Rong[2]
[1] Department of Ophthalmology, Virginia Commonwealth University, Richmond, VA; [2] Department of Pathology, Virginia Commonwealth University, Richmond, VA

Abstract. *Background:* A 9-year old female presented with one month of waxing and waning upper eyelid swelling. An excisional biopsy via anterior orbitotomy was performed. *Objective:* To describe a patient presenting atypically with symptoms concerning for orbital cellulitis who was diagnosed with Langerhans cell histiocytosis (LCH). *Methods:* Description of case report. *Results:* We report a case of a 9-year old female with one month of periorbital edema and erythema suspected to be orbital cellulitis. A complete ophthalmological exam, subsequent imaging, and an excisional biopsy revealed the diagnosis of LCH. With a confirmed diagnosis, the patient started chemotherapy indicated by the Histiocyte Society Evaluation and Treatment Guidelines. *Conclusion:* Langerhans cell histiocytosis (LCH) embodies a spectrum of diseases with the primary pathologic process being the abnormal proliferation of polyclonal Langerhans cells. In children with isolated bony involvement, the most common presenting symptom is pain. Rarely is orbital involvement with associated periorbital edema and erythema the primary presentation. *(Sarcoidosis Vasc Diffuse Lung Dis 2019; 36 (2): 167-171)*

Key words: Langerhans cell histiocytosis, orbital tumors, pediatric tumors, bone lesions

Introduction

Langerhans cell histiocytosis (LCH) is a term used to describe a spectrum of diseases characterized by clonal proliferation of Langerhans cells which can manifest as either a unisystem or multisystem disease (1). It had previously been classified as a group of conditions under the general term "histiocytosis X". Due to improved histologic classification of LCH, it is now recognized as a disease which presents along a spectrum of severity ranging from benign isolated bone lesions to aggressive multisystem disease (2). For instance, eosinophilic granuloma presents as a solitary or multifocal lesion predominantly in older children. When characterized by lytic bone lesions of the skull, proptosis, and diabetes insipidus, it is known as Hand-Schuller-Christian disease. In children, disease characterized by multiple organ involvement associated with rash, hepatosplenomegaly, anemia, and lymphadenopathy is known as Abt-Letterer-Siwe disease and has the worst prognosis (3-5).

The typical age of onset of LCH is 1-4 years old with a median age at diagnosis of 3 years old (4), although it can be seen at any age (2, 6). The most common presenting symptom is bony pain (7). Presenting with primary symptoms arising exclusively from the orbit is uncommon (8-10). Those diagnosed when less than one year old have a worse prognosis (2). The incidence ranges from 2.6-8.9/1,000,000 children per year and 1-2/1,000,000 adults per year (1-5). It is more common in boys than girls, and

Received: 27 August 2018
Accepted after revision: 5 November 2018
Correspondence: Nikisha Q. Richards, MD
Department of Ophthalmology,
Virginia Commonwealth University
401 N 11th St, Suite 439, Nelson Clinic 4th floor
Richmond, VA 23298 USA
Tel. (804) 828-9315
Fax: (804) 828-1010
E-mail: nrichards101521@gmail.com

Caucasians are the most frequently affected. Multisystem involvement and hemophagocytic syndrome are associated with a less favorable outcome (11).

The decision regarding whether LCH represents a true neoplasm versus an inflammatory disease is controversial. There is evidence that it may represent an abnormal maturation of Langerhans cells and an aberrant immune system reaction, however no specific infectious or autoimmune etiologies have been identified (1, 5). Support for LCH being a neoplastic process is provided by the co-occurrence of LCH with other malignancies such as myelodysplastic syndrome (5, 12). Histologically, LCH is characterized by clonal proliferation of Langerhans cells that resemble tissue macrophages rather than dendritic cells found in the skin (1). The infiltrate is often accompanied by eosinophils and, less frequently, lymphocytes, plasma cells, and polymorphonuclear leukocytes. Focal necrosis and fibrosis may also be present in more chronic lesions. Immunohistochemistry is used to aid in the diagnosis of LCH, with Langerhans cells being immunopositive for S100, CD1a, and CD207 (langerin) (1, 4, 5, 13). On electron microscopy, rod and tennis racket shaped bodies, called Birbeck granules, are found in the cytoplasm of Langerhans cells and are considered pathognomonic for the disease (1, 4, 12). Identification of the BRAF V600E gene mutation in LCH has become an effective tool to aid in diagnosis (1, 4, 14). Bone is the most frequently involved tissue, but skin, lymph nodes, spleen, lung, liver, brain and gastrointestinal tract involvement is also seen (2). Of all bony lesions, the most common site is the skull, followed by the femur, mandible, and pelvis (4). Here, we present a case report of LCH involving the orbit in a pediatric patient.

Case Report

All patient health information was collected and evaluated in a HIPAA-compliant manner, and this research adhered to the tenets of the Declaration of Helsinki.

A nine year old African-American female was transferred to the Virginia Commonwealth University (VCU) Medical Center for further management of presumed orbital cellulitis. The patient and mother reported a one month history of waxing and waning left upper eyelid swelling that did not resolve with warm compresses or a 10 day course of oral cefdinir prescribed for preseptal cellulitis. The patient denied any pain or tenderness. The patient's history was significant for allergic rhinitis and a recent dental surgery with placement of a palate expander. Despite treatment with a bactericidal antibiotic, her upper eyelid edema did not resolve. She was therefore brought to an outside hospital emergency room where computed tomography (CT) of the head was obtained and showed findings concerning for frontal sinusitis with extension of inflammation into the left superior orbit. CT imaging was repeated upon transfer to VCU and is presented in Figure 1.

At presentation, the patient was afebrile. Upon examination, her Snellen visual acuity without correction at near was 20/20. Ishihara color plates were full in both eyes. No relative afferent pupillary defect was appreciated, and intraocular pressures obtained with tonopen were 15 mmHg on the right and 16 mmHg on the left. She had minimal periorbital swelling of the left upper eyelid with no erythema or tenderness to palpation. Hertel exophthalmometry measurements were equal at 16 mm bilaterally at a base of 92 mm indicating no proptosis. Her CRP was 0.5 mg/dl (reference range 0-0.5 mg/dl) and ESR was 3 mm in 1 hr (reference range 0-10 mm). No leukocytosis was present. Blood cultures were negative for bacteria or fungi.

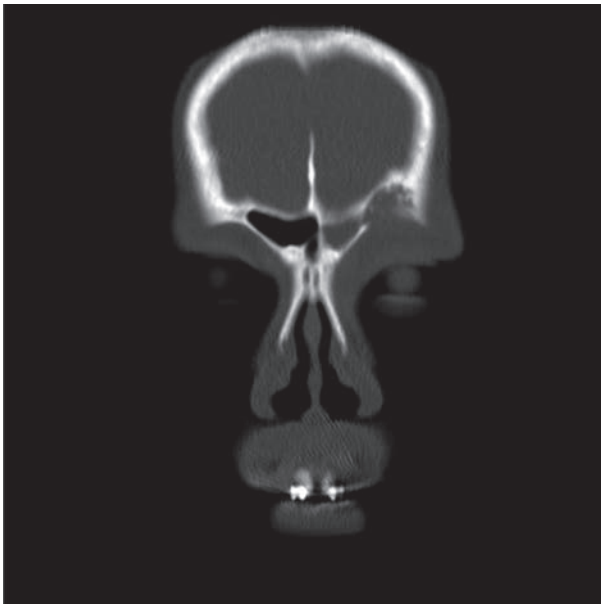


Fig. 1. Coronal view of CT of the head showing left frontal sinusitis with superior orbital extension and bony erosion

The repeat CT scan obtained upon admission with the addition of contrast suggested a soft tissue lesion of the left superior orbit and redemonstrated the osseous remodeling and destruction demonstrated on outside imaging. Magnetic resonance imaging (MRI) of the head and orbit, both with and without gadolinium, was then obtained to better characterize the lesion. MRI showed a 9 x 17 x 12 mm left superior orbital lesion with adjacent thickening and enhancement of the dura but no further extension into the brain parenchyma (Figures 2, 3). The decision was made to perform an excisional biopsy via an anterior orbitotomy without bone flap. Intraoperatively, a bony defect measuring approximately 17 mm in an anterior-posterior direction and 15 mm horizontally was explored with excision of a friable lesion with initial frozen sections revealing chronic inflammatory cells. The lesion directly communicated with both the frontal sinus and frontal lobe dura and was bluntly dissected free, keeping the dural plane intact. The edges of the bony defect were malleable and thinned.

The diagnosis of LCH was confirmed by pathology, discussed further in the next section. It was confirmed the patient only had involvement of the aforementioned sites with a negative chest radiograph, complete skeletal survey, and laboratory evaluation. Despite the fact that lytic bone lesions only become apparent when at least a third of the density is lost, radiography remains the gold standard of the diagnostic and staging procedure. The patient followed up with pediatric hematology-oncology who started her on vinblastine and prednisone according to the LCH III protocol. Since initiation of treatment, the patient's orbital symptoms of eyelid swelling and erythema have completely resolved. She has maintained 20/20 best corrected Snellen visual acuity and full Ishihara color plates. Repeat MRI eight months after starting treatment displayed no recurrence of the mass and the patient has not had a BRAF gene mutation investigation.

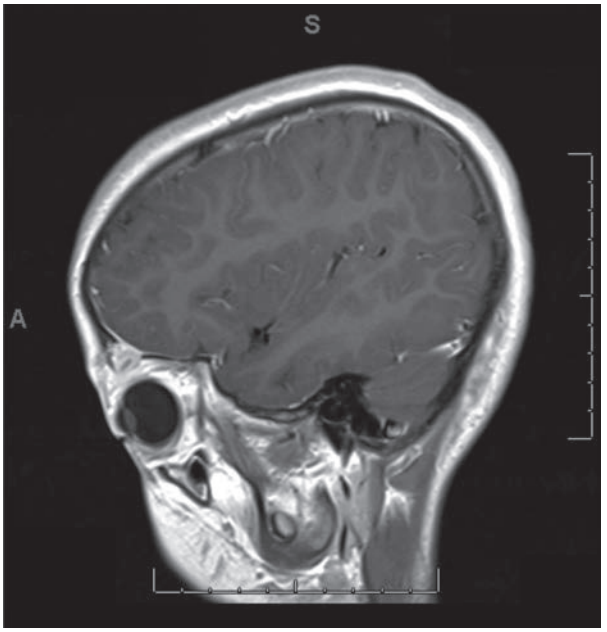


Fig. 2. Transverse cut of brain MRI showing left superior orbital mass with adjacent dural enhancement

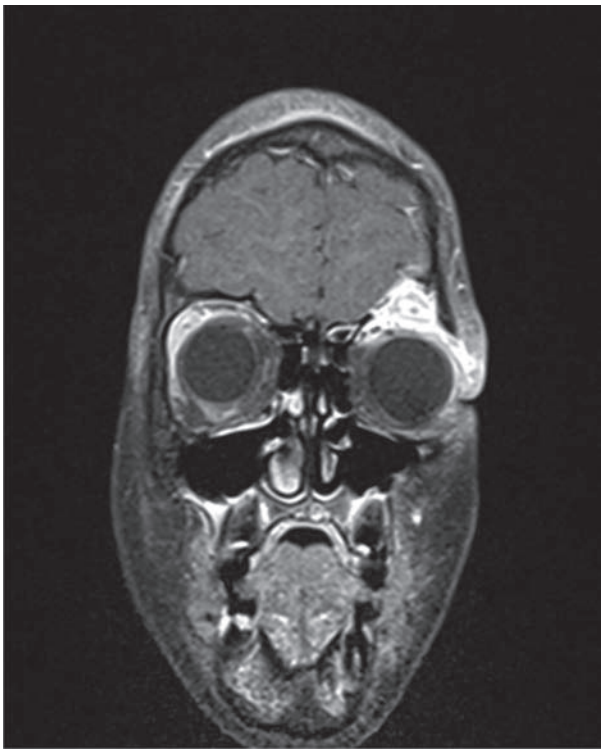


Fig. 3. Coronal cut of Brain MRI showing superior orbital lesion

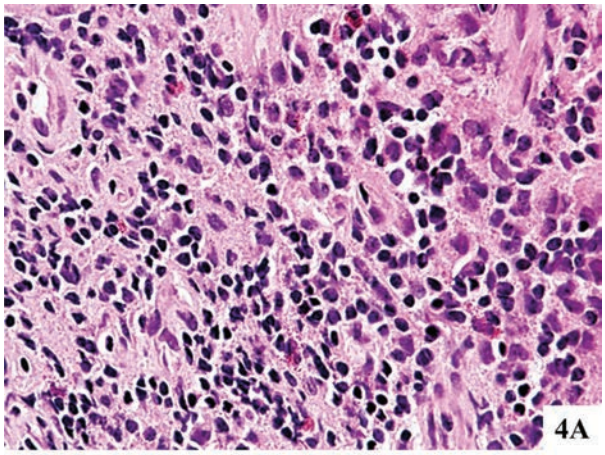
Histopathology

Microscopically there are sheets of neoplastic cells with scattered eosinophils, plasma cells and multinucleated giant cells. The neoplastic cells have elongated or clefted nuclei with occasional nuclear grooves and inconspicuous nuclei (Figures 4A-4B). Mitotic activity is low (1-2 mitoses/10HPF). No necrosis is present. The neoplastic cells are strongly pos-

itive for CD1a (Fig. 4C) and focally positive for s100 (Fig. 4D). These features are most consistent with a diagnosis of Langerhans cell histiocytosis. Several other entities that may have overlapping morphological appearance should be considered in the differential diagnoses, which include Rosai-Dorfman disease, Erdheim Chester disease, and non-specific chronic inflammation (inflammatory pseudotumor). Rosai-Dorfman disease shows large and pale histiocytes with empripolesis (engulfment of lymphocytes) and the histiocytes are usually s100 positive and CD1a negative. There are no Langerhans cells in Erdheim Chester disease and the histiocytes in this entity are negative for both CD1a and s100. For non-specific chronic inflammation (inflammatory pseudotumor), there is usually a mixed population of inflammatory cells without CD1a positive Langerhans cells.

Discussion

The reported incidence of orbital involvement in all forms of LCH is variable but has been reported to be as high as 37.5% and is usually seen in patients with chronic lesions (2, 4, 5). The most common ocular adnexal manifestation of LCH is an isolated orbital infiltrate and is typically unilateral (1). Involved structures may include the eyelid, conjunctiva, caruncle, choroid, and the optic nerve. Orbital apex syndrome, cavernous sinus syndrome, and sarcoidosis



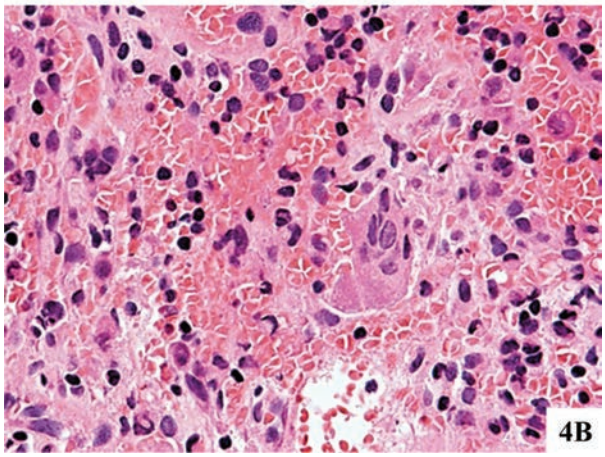
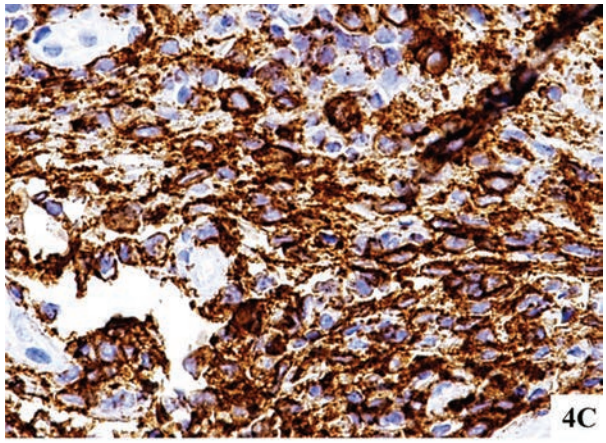


Fig. 4A-4B. H&E sections of the orbital lesion. Magnification 4A: 10X; 4B: 20X. H&E sections show sheets of neoplastic cells with scattered eosinophils, plasma cells and multinucleated giant cells. The neoplastic cells have elongated or clefted nuclei with occasional nuclear grooves and inconspicuous nuclei



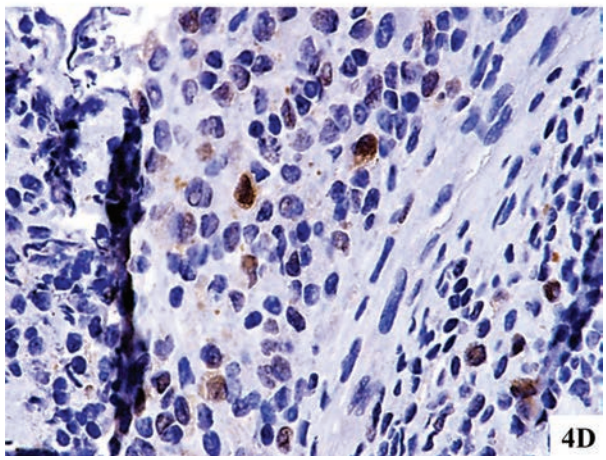


Fig. 4C-4D Immunohistochemical staining. 4C: CD1a; 4D: s100. Magnification at 20X. The neoplastic cells are strongly positive for CD1a and focally positive for s100

are also possible (15, 16). Orbital LCH typically presents as an isolated bone lesion and soft tissue mass, most typically superiorly or superotemporally along the orbital roof. This may lead to proptosis as well as periorbital erythema and edema, mimicking an infectious process. Additional symptoms may include blurred vision, diplopia, and potentially the development of amblyopia in children. Orbital imaging classically shows "punched out" lytic lesions of the bone with an associated soft tissue lesion (17).

The differential for orbital LCH includes periorbital or orbital cellulitis, a ruptured dermoid cyst, sarcoidosis, idiopathic orbital inflammatory syndrome, and other malignant processes such as leukemia, neuroblastoma, and rhabdomyosarcoma (1). The absence of any history of pulmonary sequelae, ophthalmoplegia, diplopia, ptosis, pupillary changes, and other neurological manifestations makes sarcoidosis and neurosarcoidosis less likely (16). The diagnosis of LCH is established by incisional or excisional biopsy. Due to the rarity of the condition, treatment often varies on an individual case basis, however there are treatment algorithms available (18). For isolated caruncular and eyelid lesions, excision may successfully treat the disease. Excision in combination with intralesional steroids for isolated orbital lesions have also been used effectively, as high doses of steroids are thought to inhibit osteolysis (19). Chemotherapy can be used for multifocal disease or for orbital lesions with dural involvement, with the most common agents being vinblastine, prednisone, etoposide, and methotrexate. Radiation can be used to treat disease recurrence. Lastly, bone marrow transplant and immunoglobulin therapy have been used for uncontrolled disease recurrences and CNS involvement (1). After treatment, disease recurrence is most common within 12-18 months, although there are case reports of recurrence over 10 years later (20). The prognosis for patients with limited orbital involvement is favorable (1, 2).

LCH is an important consideration for patients presenting with a chronic orbital process. Here, we have discussed a pediatric patient with an initial presentation concerning for an infectious process, however the chronicity and lack of response to initial, bactericidal antibiotic therapy raised the suspicion for a non-infectious process. As discussed earlier, the most common presenting symptom in children with isolated bony involvement is pain, however this case was unique in that the patient denied any pain or tenderness. This case demonstrates the importance of maintaining a high index of suspicion for alternate diagnoses when evaluating patients for presumed orbital infections.

References

1. Herwig MC, Wojno T, Zhang Q, Grossniklaus HE. Langerhans cell histiocytosis of the orbit: five clinicopathologic cases and review of the literature. Surv Ophthalmol 2013 Jul-Aug; 58(4): 330-40.
2. Vosoghi H, Rodriguez-Galindo C, Wilson MW. Orbital involvement in Langerhans cell histiocytosis. Ophthal Plast Reconstr Surg 2009 Nov-Dec; 25(6): 430-3.
3. Margo CE, Goldman DR. Langerhans cell histiocytosis. Surv Ophthalmol 2008 Jul-Aug; 53(4): 332-58.
4. Monsereenusorn C, Rodriguez-Galindo C. Clinical Characteristics and Treatment of Langerhans Cell Histiocytosis. Hematol Oncol Clin North Am 2015 Oct; 29(5): 853-73.
5. Zinn DJ, Chakraborty R, Allen CE. Langerhans Cell Histiocytosis: Emerging Insights and Clinical Implications. Oncology (Williston Park) 2016 Feb; 30(2): 122-32, 139.
6. Hamre M, Hedberg J, Buckley J, et al. Langerhans cell histiocytosis: an exploratory epidemiologic study of 177 cases. Med Pediatr Oncol 1997 Feb; 28(2): 92-7.
7. Buckwalter JA, Brandser E, Robinson RA. The Variable Presentation and Natural History of Langerhans Cell Histiocytosis. The Iowa Orthopaedic Journal 1999; 19: 99-105.
8. Anton M, Holousová M, Rehurek J, Habanec B. Histiocytosis X and the orbit in children. Cesk Oftalmol 1992; 48: 176-80.
9. Bhanage AB, Katkar AD, Ghate PS. Langerhans cell histiocytosis with presentation as orbital disease. J Pediatr Neurosci 2015 Apr-Jun; 10(2): 162-5.
10. Anton M, Holousová M, Rehůrek J, Habanec B. Histiocytosis X and the orbit in children. Cesk Oftalmol 1992 May; 48(3): 176-80. Czech.
11. Favara BE, Jaffe R, Egeler RM. Macrophage activation and hemophagocytic syndrome in langerhans cell histiocytosis: report of 30 cases. Pediatr Dev Pathol 2002 Mar-Apr; 5(2): 130-40.
12. Collin M, Bigley V, McClain KL, Allen CE. Cell(s) of Origin of Langerhans Cell Histiocytosis. Hematol Oncol Clin North Am 2015 Oct; 29(5): 825-38.
13. Picarsic J, Jaffe R. Nosology and Pathology of Langerhans Cell Histiocytosis. Hematol Oncol Clin North Am 2015 Oct; 29(5): 799-823.
14. Rollins BJ. Genomic Alterations in Langerhans Cell Histiocytosis. Hematol Oncol Clin North Am 2015 Oct; 29(5): 839-51.
15. Gross FJ, Waxman JS, Rosenblatt MA, Tabibzadeh SS, Solodnik P. Eosinophilic granuloma of the cavernous sinus and orbital apex in an HIV-positive patient. Ophthalmology 1989 Apr; 96(4): 462-7.
16. Rosini, F, Bennett, D, Cerase, A, Volterrani, L, Federico, A, et al. Neurol Sci 2017; 38: 517.
17. Azouz EM, Saigal G, Rodriguez MM, Podda A. Langerhans' cell histiocytosis: pathology, imaging and treatment of skeletal involvement. Pediatr Radiol 2005 Feb; 35(2): 103-15. Epub 2004 Jul 28.
18. Bezdjian A, Alarfaj AA, Varma N, Daniel SJ. Isolated Langerhans Cell Histiocytosis Bone Lesion in Pediatric Patients: Systematic Review and Treatment Algorithm. Otolaryngol Head Neck Surg 2015 Nov; 153(5): 751-7.
19. Woo KI, Harris GJ. Eosinophilic granuloma of the orbit: understanding the paradox of aggressive destruction responsive to minimal intervention. Ophthal Plast Reconstr Surg 2003 Nov; 19(6): 429-39.
20. Wladis EJ, Tomaszewski JE, Gausas RE. Langerhans cell histiocytosis of the orbit 10 years after involvement at other sites. Ophthal Plast Reconstr Surg 2008 Mar-Apr; 24(2): 142-3.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2019; 36 (2); 172-173

The mystery of Black Pete make-up: a sarcoid-like foreign-body reaction

Marjolein Drent[1, 2, 3], *Marcel Veltkamp*[1, 3, 4], *Aalt Bast*[2, 3, 5]

[1]ILD Center of Excellence, St. Antonius Hospital, Nieuwegein, the Netherlands; [2]Dept of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Science, Maastricht University, Maastricht, the Netherlands; [3]ild care foundation research team, Ede, the Netherlands, [4]Division of Heart and Lungs, University Medical Center, Utrecht, the Netherlands, [5]Venlo Campus, Maastricht University, Venlo, the Netherlands

The cause of sarcoidosis remains unknown but is likely to depend on both genetic and environmental factors (1, 2). Here, we describe the flare of systemic sarcoidosis in a patient associated with an unusual trigger of the disease.

A 34-year-old male was diagnosed with sarcoidosis in October 2016. At presentation he had a classic Lofgren's syndrome. At that time there also were signs of activity (increase of soluble interleukin 2 receptor (sIL2R)). Within a couple of months his clinical condition and clinical features of disease activity improved. In January 2018 he came back and recalled a flare of skin lesions in his face (figure 1a). Fascinatingly, he had a rather extraordinary explanation why this relapse occurred. At the end of November he volunteered at a traditional Dutch feast of Sinterklaas which celebrates the name day of Saint Nicholas on December, 6th. Sinterklaas is assisted by many mischievous helpers with black faces and colorful Moorish dresses. These companions are called Zwarte Piet ("Black Pete"). The patient was one of the Black Pete's. He used make-up (figure 1b). Both make-ups contain talc, but the blue make-up also consisted of magnesium aluminium silicate.

Received: 26 March 2019
Accepted after revision: 17 April 2019
Correspondence: Marjolein Drent
[1]ILD Center of Excellence, St. Antonius Hospital, Nieuwegein, the Netherlands;
E-mail: m.drent@ildcare.nl

Just one week later he developed skin lesions in his face (figure 1a) and some weeks later also on his legs. Further investigation showed again an increase of his sIL2R and enlarged mediastinal lymph nodes.

Foreign body granulomas in the skin have been described frequently and may have various causes. Talc or talcum (hydrous magnesium silicate) is a clay mineral composed of hydrated magnesium silicate. Beside an industrial mineral, talc is an ingredient in various cosmetic and personal hygiene products. Talc

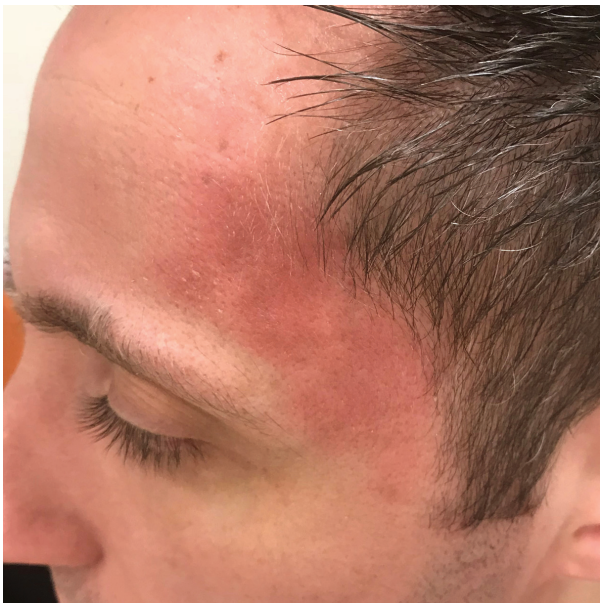


Fig. 1a. Flare-up of sarcoidosis: erythematous plaques most prominent in the left temporal region

Fig. 1b. Face of the same patient was colored with make-up one week before the skin lesions appeared (figure 1a)

may cause some adverse effects such as local inflammation, infection, and allergic reactions on the skin and even systemic adverse effects such as sarcoid like reactions. Most commonly, erythematous nodules are seen. Tattoo pigments may migrate to regional lymph nodes and may cause a sarcoid like granulomatous reaction (3).

Among others talc is believed to trigger an exaggerated immune response, with macrophages mediating antigen processing and presentation, leading to an influx of T cells, a polarized T helper 1 (Th1) cytokine response, and ultimately, to granuloma formation (4). Several susceptibility genes have been identified in sarcoidosis, with the strongest associations to date in the major histocompatibility (MHC) class II gene alleles, which coordinate antigen presentation. These findings support the role of a dysregulated response to antigens in development of sarcoidosis (5).

A flare up of systemic sarcoidosis in a 34-year-old male with a history of sarcoidosis first presenting with Lofgren's syndrome, who demonstrated spontaneously remission half a year after the first submission (April 2017), was presented. December 2017 he developed skin lesions in his face, just after he used make-up. This make-up contains talc. This undeniably exceptional case shows that the trigger of a sarcoid-like reaction might be make-up containing talc. It emphasizes that talc exposures should be considered in case of skin lesions as well as other manifestations of sarcoidosis or sarcoid-like reactions.

Acknowledgement

We wish to thank the patient for his consent to publish the pictures and accompanying text.

References

1. Lodha S, Sanchez M, Prystowsky S. Sarcoidosis of the skin: a review for the pulmonologist. Chest 2009; 136: 583-596.
2. Valeyre D, Prasse A, Nunes H, et al. Sarcoidosis. Lancet 2014; 383: 1155-1167.
3. Anolik R, Mandal R, Franks AG, Jr. Sarcoidal tattoo granuloma. Dermatol Online J 2010; 16: 19.
4. Zissel G. Cellular activation in the immune response of sarcoidosis. Semin Respir Crit Care Med 2014; 35: 307-315.
5. Wolin A, Lahtela EL, Anttila V, et al. SNP Variants in major histocompatibility complex are associated with sarcoidosis susceptibility: a joint analysis in four European populations. Front Immunol 2017; 8: 422.