Cyclophosphamide Responsive Interstitial Lung Disease in “Overlap Syndrome”

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Case Presentation

A 58-year-old Korean woman presented with 3 weeks of worsening shortness of breath and dyspnea on exertion.

The patient's rheumatologic history dated back to 9 years prior to presentation. At that time, she had pain and swelling in both the second and third proximal interphalangeal joints (PIPs) and a positive rheumatoid factor and

was diagnosed with rheumatoid arthritis (RA). She was treated with nonsteroidal anti-inflammatory medications with control of her symptoms. One and a half years prior to presentation, the patient developed bilateral knee pain and swelling. She was started on prednisone 50 mg orally (PO) daily and infliximab 3 mg/kg intravenously (IV) with significant improvement. Infliximab was stopped after 1 month secondary to an allergic rash, and azathioprine 25 mg PO daily was started. At this time, she self-discontinued the prednisone. Over the next 6 months, she developed shortness of breath with exertion. The patient had multiple hospital admissions for fevers, cough, and shortness of breath and was treated each time for presumed pneumonia with antibiotics and steroids followed by a slow steroid taper.

Two weeks prior to presentation, she had pulmonary function testing which revealed a forced vital capacity (FVC) of 60% predicted (normal >80% predicted); total lung capacity, 45% predicted; and vital capacity, 59% predicted. There was also a reduction in diffusing capacity in the lung with carbon monoxide (DLCO) measured at 15% predicted (31% after adjusted for alveolar volume). These results were consistent with a severe restrictive ventilatory defect with restrictive lung disease.

On presentation to our clinic, the patient complained of bilateral metacarpophalangeal (MCP) joint pain and swelling and could walk only two blocks and climb one flight of stairs secondary to shortness of breath. Review of systems was significant for Raynaud's phenomenon that developed 4 years earlier, a 10-pound weight loss over 1 month, dry eyes and mouth, and burning epigastric pain.

The patient's medications included azathioprine 25 mg PO daily, celecoxib 200 mg PO two times daily, prednisone 7.5 mg PO daily (as part of a steroid taper), alendronate 70 mg PO daily, atovaquone 5 cm³ PO two times daily, lansoprazole 40 mg PO daily, calcium/vitamin D PO daily, acetaminophen 650 mg PO daily as needed for pain. Allergies included penicillin and trimethoprim-sulfamethoxazole. Past medical history was significant for gastroesophageal reflux disease and

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Each author certifies that his or her institution has approved the reporting of this case, that all investigations were conducted in conformity with ethical principles of research.

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hypertension. Social history was significant for absence of tobacco use, alcohol use, illicit drug use, or environmental exposures. Family history was not significant.

Physical examination was remarkable for blood pressure 100/60 mmHg, respiration rate 24 breaths per minute, heart rate 110 beats per minute, and oxygen saturation 89% on room air. The patient was thin and ill appearing, in moderate respiratory distress and had tightened perioral skin. She was tachycardic without any murmurs and had no jugular venous distension. She had bilateral coarse crackles up a third of the lung fields from the bases without wheezing. Her abdomen was soft with mild epigastric tenderness. Extremities were warm, had trace bilateral lower extremity edema and two plus dorsalis pedis pulses. Her skin demonstrated telangiectasias on the left and right upper chest wall and dorsum of both hands, multiple areas of calcinosis over both hands, and one area of calcinosis at the left anterior knee. There was periungual erythema, clubbing, and pitting of the pads of several finger tips as well as slightly tightened skin distal to the wrists. On musculoskeletal examination, the wrists were warm and tender with limited dorsiflexion and synovitis bilaterally. There was moderate swelling and warmth of both 2nd and 3rd MCP and PIP joints. On neurological exam, cranial nerves were intact, her strength was 5/5 in all extremities and sensation was grossly intact.

The patient was admitted, placed on oxygen, and started on broad-spectrum antibiotics (vancomycin 1 g IV every 12 h, levofloxacin 750 mg IV daily, and aztreonam 1 g every 8 h) as well as methylprednisolone 16 mg PO three times a day.

Results of initial laboratory studies were significant for a white blood cell count of $9.5 \times 10^9/\mu\text{L}$, hemoglobin 11.5 g/dL, amylase 162 U/L, lipase 812 U/L, erythrocyte sedimentation rate 95 mm/h, C-reactive protein 5.3 mg/dL, creatine kinase

904 U/L, lactate dehydrogenase 1,288 U/L, and aldolase 18.5 U/mg (Table 1). Serological studies revealed rheumatoid factor 122 U/ml, anti-cyclic citrullinated antibody 157, and antinuclear antibody 2+ in a cytoplasmic pattern. Double-stranded DNA antibody, anti-Ro antibody, anti-La antibody, anti-Smith antibody, anti-RNP antibody, anti-Scl-70 antibody, anti-centromere antibody, RNA polymerase III, anti-Jo1 antibody, and anticardiolipin antibody were negative. Complement levels were normal and RPR negative.

Bilateral hand X-rays had no evidence of erosions, subluxations, soft tissue calcifications or joint space narrowing. Chest X-ray demonstrated a small right pleural effusion, moderate cardiomegaly, and diffusely increased interstitial markings (Fig. 1). A high-resolution computed tomography (HRCT) of the chest (Fig. 2) demonstrated diffuse ground glass opacification of the lower lobes and interstitial lung disease (further discussed below). Bronchoalveolar lavage revealed 72% macrophages, 20% neutrophils, and 8% lymphocytes. The cytology was negative for malignant cells. Acid fast stain, silver stain, fungal, and bacterial cultures were all negative. Transthoracic Doppler echocardiography (TTE) demonstrated normal left ventricular ejection fraction but an elevated estimated pulmonary systolic pressure of 59 mmHg (normal <35 mmHg) consistent with moderate to severe pulmonary hypertension. Given the finding of severe pulmonary hypertension on TTE, the patient underwent a left and right cardiac catheterization which showed normal coronary arteries, and mild pulmonary hypertension (resting mean pulmonary artery pressure of 26 mmHg) and no intracardiac shunt.

Radiographic Findings and Discussion

The ground glass opacities began in the mid to lower lung fields becoming more extensive in the lower lung fields with early bleb formation (Fig. 2a and b). There was pleural thickening at the left base and bronchiectasis, likely from traction and fibrosis, as well as enlarged lymph nodes (largest 12 mm in diameter) in the upper mediastinum and a small pericardial effusion (Fig. 3a, b). There also was dilatation of the esophagus (Fig. 4).

High-resolution computed tomography is currently the gold standard for radiographic diagnosis of interstitial lung disease and can be used early in the assessment of patients with suspected pulmonary involvement [7]. In both RA and systemic sclerosis (SSc), HRCT may demonstrate interstitial fibrosis and alveolar airspace infiltrates, also known as ground glass opacities. In both diseases, there is usually basilar predominance, with subpleural reticular infiltrates seen early in disease and fibrotic changes, honeycombing and bronchiectasis later in the course. In RA, nodules can be seen in up to 30% of cases, and pleural and pericardial effusions are often present. In SSc, cardiomegaly is more common than in RA, esophageal dilatation is often present, and pleural and pericardial effusions are generally not seen.

Table 1 Initial laboratory results

	Result	Normal values
White blood cell	9.8	$4.0\text{--}10.0 \times 10^9/\mu\text{L}$
Hemoglobin	11.5	12–16 g/dL
Hematocrit	32.5	35–45%
MCV	95.3	80–100 fL
Platelet	418	$150\text{--}350 \times 10^9/\mu\text{L}$
AST	51	15–41 U/L
ALT	19	14–54 U/L
Alkaline phosphatase	54	25–95 U/L
Total bilirubin	0.2	0.3–1.2 mg/dL
Total protein	7.4	6.1–7.9 g/dL
Albumin	2.9	3.5–4.8 g/dL
Amylase	162	27–131 U/L
Lipase	812	22–51 U/L
ESR	95	0–30 mm/h
CRP	5.3	<1 mg/dL
Creatine kinase	904	38–234 U/L
Lactate dehydrogenase	1,288	105–333 U/L
Aldolase	18.5	4 U/mg

AST aspartate aminotransferase, ALT alanine aminotransferase, ESR erythrocyte sedimentation rate, CRP C-reactive protein

Fig. 1. Admission posterior–anterior and lateral chest radiography reveals moderate cardiomegaly, small right pleural effusions, and diffuse interstitial markings

Differential Diagnosis and Rheumatologic Discussion

This patient presented with features of different systemic autoimmune diseases. In addition to her previous diagnosis of RA, on admission she had sclerodactyly, facial skin tightening, calcinosis, Raynaud's phenomenon, telangiectasias, and esophageal dysmotility, which were most consistent with limited systemic sclerosis (CREST syndrome). Her dyspnea on exertion and lung examination were suggestive of interstitial lung disease, which was confirmed by pulmonary function testing and HRCT. In addition to having lung disease,

she had laboratory evidence of myositis manifested by elevated creatine kinase and aldolase.

Both RA and SSc patients may develop inflammatory myositis and/or interstitial lung disease. Furthermore, patients with anti-synthetase syndrome, which is a subgroup of idiopathic inflammatory muscle diseases, can present with interstitial lung disease and arthritis. Although, muscle enzymes were elevated in our patient, she did not exhibit any weakness or muscle tenderness. Thus, especially given that she had features of CREST syndrome, specific anti-synthetase antibodies other than anti-Jo-1 were not ordered. Of note, although SSc serologies (anti-Scl-70 antibody, anti-centromere antibody, and RNA polymerase III) and anti-RNP antibody were negative, serologic testing is neither perfectly sensitive nor specific for the diagnosis of any connective tissue disease.

In addition to connective tissue disorders, infectious etiologies such as atypical pneumonia, tuberculosis, or pneumocystis pneumonia were considered as the cause of her lung disease but were ruled out by bronchoscopy. Drug-induced lung injury such as azathioprine-associated interstitial pneumonitis was another consideration; however, this was less likely to be the cause of her pulmonary symptoms as she had been treated with low-dose azathioprine for a relatively short period and her symptoms continued despite cessation of the medication.

In summary, clinically, this patient can be best classified as having an “overlap syndrome” with rheumatoid arthritis (seropositive for rheumatoid factor and anti-CCP antibody), limited SSc (CREST syndrome), interstitial lung disease, and laboratory evidence of inflammatory myositis.

Fig. 2. Initial high-resolution computed tomography of the chest reveals diffuse ground glass opacities most prominent at lower lung fields (*white dashed arrows*), traction bronchiectasis (*white arrow*), early bleb formation (*thick white arrow*), and enlarged hilar lymph nodes (*curved white arrow*, Fig. 1b)

Diagnostic Discussion—Pulmonary

Of all of the rheumatologic diseases, SSc has the highest rate of pulmonary involvement. Pulmonary involvement has emerged as the primary cause of mortality in SSc,

Fig. 3. **a, b** Initial high resolution computed tomography of the chest demonstrates a small pericardial effusion (*white arrow, a*), pleural thickening (*white curved arrow, b*), and extensive ground glass opacities in the posterior lower lobes (*white dashed arrows*)

accounting for 60% of deaths, with pulmonary hypertension and interstitial lung disease each contributing equally [20]. Post-mortem analysis indicates that 70–100% of patients with SSc have pulmonary involvement [7]. Pulmonary disease usually occurs after skin involvement but may also occur as the presenting manifestation; the presenting symptom is often dyspnea. The differential diagnosis of dyspnea in SSc includes pulmonary arterial hypertension, interstitial pulmonary fibrosis, aspiration pneumonitis, pleuritis, pericarditis, bronchiolitis, COPD, and muscle weakness. Recurrent aspiration of gastric contents is especially common in SSc and is thought to be secondary to impaired esophageal motility and gastroesophageal reflux. Often more than one of these processes contributes to poor pulmonary status.

Pulmonary arterial hypertension (PAH) in SSc may be present with or without interstitial lung disease (ILD). PAH without ILD is a result of an arteriopathy characterized by plexiform lesions in the pulmonary vascular bed. Ten percent of patients with limited SSc have isolated PAH. PAH can also occur secondary to interstitial fibrosis. The interstitial lung disease of SSc is thought to be due to inflammation, although the response to immunosuppressive drugs is poor. The most common histological pattern seen on the lung biopsy of SSc patients is nonspecific interstitial pneumonitis (NSIP), characterized by a homogeneous distribution of fibrosis, interstitial inflammation, and hyperplasia of type II pneumocytes. Less commonly, patients may develop usual interstitial pneumonia (UIP) in which there is a more patchy distribution of fibrosis [18]. As the ILD in SSc progresses, the alveolar septa become thickened, airspaces are destroyed and honeycombing occurs. This impairs gas exchange and can worsen secondary pulmonary hypertension.

The initial workup of ILD in SSc should include a chest X-ray, HRCT, and pulmonary function tests (PFT). Both HRCT and PFT are used to assess the severity of interstitial lung disease and monitor the progression of the disease overtime. Many patients will have some abnormality on pulmonary testing even in the absence of symptoms. PFTs in patients with ILD usually demonstrate a restrictive pattern with diminished FVC and reduced DLCO. (Normal spirometry with an isolated reduction in DLCO should raise a concern for PAH. In such an instance, TTE should be performed [11]). If interstitial lung disease is found, HRCT should be performed every 6–12 months to monitor progression of disease. The data on the natural progression of radiographic computed tomography (CT) findings and PFTs over time demonstrate that most people progress in 3 to 4 years despite treatment. Bronchoalveolar lavage (BAL) is occasionally used in patients with interstitial lung disease to evaluate the presence of alveolitis. Those with increased neutrophils on BAL may progress more rapidly and some recommend more aggressive therapy. There is conflicting

Fig. 4. Initial high-resolution computed tomography of the chest reveals a dilated esophagus (*white arrow*)

data on the utility of BAL in SSc because there is no clear correlation between the initial cellular analysis, the change in BAL cellular profiles with treatment and the clinical prognosis of patients [8].

Open lung biopsy is seldom performed during the course of ILD in SSc due to the invasive nature of the procedure and the potential for morbidity and mortality. In addition, a study by Bouros et al. which included surgical lung biopsies from 80 patients with fibrosing alveolitis associated with SSc, demonstrated no significant correlation between the severity of the histological lesions (NSIP versus UIP) and the progression of the ILD [3].

In summary, interstitial lung disease is the most common clinical pulmonary manifestation of SSc; one should maintain a high suspicion for pulmonary involvement when these patients present with clinical symptoms of shortness of breath and dyspnea on exertion. Screening with CXR, HRCT, and PFTs should be done and monitoring for progression and early treatment is important.

Therapeutic Discussion

While there have been major advances in treating specific organ system complications of SSc, there is no therapy proven to reverse or prevent fibrosis associated with the illness. Potential targets of treatment include the fibroblast which causes tissue fibrosis, the endothelial cell which leads to changes in the small arteries and arterioles, B cells which produce autoantibodies, and T cells which are responsible for cellular infiltration and cytokine/growth factor dysregulation.

Potentially “disease modifying” interventions currently being used or under investigation include cyclophosphamide or immunoablative therapy with bone marrow transplant salvage. Results with milder immunosuppressive agents such as methotrexate and corticosteroids or with antifibrotic agents such as penicillamine and colchicine have been disappointing. There have been two randomized double-blinded placebo-controlled trials studying the effects of cyclophosphamide for SSc-associated ILD. In the scleroderma lung study, Tashkin et al. [22] investigated the effects of 12 months of oral cyclophosphamide on patients with SSc and active alveolitis and inflammatory interstitial lung disease, as indicated by abnormal BAL and/or groundglass opacities on HRCT. One hundred and fifty-eight patients were randomized to oral cyclophosphamide 2 mg/kg daily versus placebo for 1 year. The primary outcome at 12 months was change in FVC, as expressed as the percentage of the predicted value. Secondary outcomes included DLCO, disability indexes (HAQ, SF-36), and skin index. The results favored cyclophosphamide over placebo in that there was less of a decline in FVC, although it was not a clinically meaningful difference. There was no improvement in FVC or DLCO with treatment. Interestingly, the benefit was most pronounced in patients with greater fibrosis at baseline (and not in those with more alveolitis). Secondary outcomes, which included the SF36 “vitality domain,” favored cyclo-

phosphamide, as did skin scores in patients with diffuse disease. The cyclophosphamide arm of the trial had more adverse events and withdrawals and 33% of subjects did not complete the trial. Follow-up data demonstrated that the beneficial effects of cyclophosphamide were persistent up to 18 months but no longer present at 24 months [23].

A second multi-center, randomized double-blind placebo-controlled trial was conducted by Hoyles et al. which investigated the effects of intravenous (IV) cyclophosphamide followed by azathioprine treatment in pulmonary fibrosis in SSc [10]. Forty-five patients with SSc and diffuse or limited interstitial lung disease were randomized to receive six monthly infusions of cyclophosphamide and low-dose prednisolone followed by oral azathioprine, versus placebo. Primary outcome measures were change in percent predicted FVC and change in DLCO. Secondary outcome measures included changes in appearance on HRCT and dyspnea scores. At 6 months, there were no statistically significant improvements in the primary or secondary end points in the active treatment group versus the group receiving placebo [10].

The results of these two trials suggest that cyclophosphamide has at best a modest benefit in the treatment of SSc related ILD. Clinicians must carefully consider the potential toxicity of this approach prior to beginning therapy. Further research is needed to better elucidate the subsets of patients who are more likely to benefit from cyclophosphamide. New therapeutic strategies for SSc are being explored.

Immunopathologic Discussion

The pathogenesis of SSc is very complex, involving vascular injury, endothelium cell activation, tissue hypoxia, innate and adaptive immunity, autoantibodies, fibroblast activation, and matrix accumulation with collagen components.

Vascular injury is perhaps the earliest event in SSc, and leads to endothelial cell activation and dysfunction. Multiple mechanisms have been proposed including damage from anti-endothelial cell autoantibodies, viruses such as CMV and reactive oxygen radicals [1, 2, 15, 24]. Activated endothelial cells demonstrate increased expression of adhesion molecules that attract and activate leukocytes [19]. Activated T cells and monocytes produce inflammatory cytokines. There may be an imbalance between TH1 and TH2 cytokines in systemic sclerosis with increased production of TH2 cytokines such as IL-4 and decreased production of TH1 cytokine such as IFN- γ [14]. Activation of type I IFN has been also observed in SSc and might promote disease by activating autoimmunity similar to systemic lupus erythematosus [12, 21].

B cells also appear to be important in the pathogenesis of SSc. Many patients with SSc express autoantibodies against centromere, topoisomerase-I (SCL-70), and RNA-polymerases.

Serum antibodies isolated from SSc patients can induce fibroblast activation by upregulating adhesion molecules

and proinflammatory cytokines [4]. Henault et al. reported that the antifibroblast antibodies in patients with SSc correlate with anti-topoisomerase antibodies [9].

Fibroblasts, after receiving the stimuli from the above inflammatory and autoimmune processes, become activated and differentiate into myofibroblast. Overproduction of collagen-rich extracellular matrix by fibroblast is the characteristic feature of SSc. Multiple DNA-binding transcription factors, such as SP1, SMAD3, SP3, regulate the expression of various collagen proteins [17]. Transforming growth factor- β (TGF- β) is considered the most important mediator in the fibrogenesis pathway [16]. TGF- β is secreted by platelets, fibroblast, and monocytes. It is a potent stimulator of collagen production and extracellular matrix accumulation. It is secreted in the latent inactive form and is activated during tissue remodeling. Activated TGF- β binds to TGF- β receptor, which in turn results in phosphorylation and activation of SMAD2 or SMAD3 signaling pathway. Activated SMADs then migrate to the nucleus. They recruit transcription factors and result in transcription of gene such as type 1 collagen [13]. Alternatively, TGF- β can lead to collagen and extracellular matrix production in non-SMAD pathways for example via the Abelson tyrosine kinase (Abl) [6]. This pathway is the target for Abl inhibitor, Imatinib (Gleevec), currently used in clinic trials for treating SSc. In addition to TGF- β , platelet-derived growth factor (PDGF) also plays an important role in the pathogenesis of SSc. PDGF is produced by macrophages, endothelial cells and fibroblasts. It is also a chemoattractant for fibroblasts. Baron et al. reported that serum from SSc patients contains stimulatory autoantibodies to PDGF receptor [1]. This interaction induces tyrosine phosphorylation and reactive oxygen species production, resulting in type I collagen-gene expression and myofibroblast phenotype conversion from fibroblasts. Thus it suggests that PDGF receptor induced fibroblast activation plays an important role in the pathogenesis of SSc.

Of note, there is still some controversy in the literature with regard to whether or not such PDGF receptor stimulating antibodies exist in SSc as other laboratories have been unable to reproduce these findings [5].

Hospital Course and Long-term Follow-up

Following a course of broad-spectrum antibiotics and methylpredisone, the patient's creatine kinase trended down to normal over the course of 5 days as did her amylase and lipase. Her acute respiratory symptoms improved but she remained dependent on 3 l of oxygen by nasal cannula with exertion and severely disabled from her interstitial lung disease. In addition to continuing corticosteroids with a slow taper, the patient was treated with monthly intravenous (IV) cyclophosphamide. After the fourth IV cyclophosphamide treatment, she had marked improvement in her dyspnea, and was no longer oxygen dependent. She moved out of a nursing home and was able to walk 1 mile per day without shortness of breath. After she completed 6 months of cyclophosphamide, her pulmo-

Fig. 5. a, b Follow-up high resolution computed tomography of the chest at 9 months after initiation of cyclophosphamide

nary function test revealed improvement in DLCO from 31% to 60% after adjusted for alveolar volume. A follow-up high-resolution chest CT scan 9 months later showed a markedly decreased ground glass appearance (Fig. 5a, b). She had a pulmonary relapse 18 months after the last dose of cyclophosphamide and was treated with a high dose corticosteroid taper. Her azathioprine was switched to mycophenolate for maintenance therapy and she has remained on prednisone 4 mg PO daily with stabilization of her interstitial lung disease. Her inflammatory muscle disease resolved with the initial implementation of corticosteroids.

Conclusion

In summary, we present a patient with a connective tissue disease characterized by features of rheumatoid arthritis, limited systemic sclerosis (CREST syndrome), and laboratory evidence of inflammatory myositis (“overlap syndrome”) associated with ILD, who demonstrated a significant response to intravenous cyclophosphamide therapy in combination with corticosteroids. It is possible that the patient's response to therapy is related to her diagnosis of an overlap syndrome, given the overall poor response of pure SSc patients to immunosuppressive therapy. This case emphasizes the importance of accurate and early diagnosis,

close surveillance of disease progression and aggressive treatment in the prognosis of patients with connective tissue disease-associated ILD.

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