

## Environmental triggers of autoimmunity in anti-synthetase syndrome: the lungs under the spot light

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### ABSTRACT

*The prevalence of interstitial lung involvement in anti-synthetase syndrome (anti-SS) may be as high as 70% and is a major contributor to morbidity and mortality. Histidyl-tRNA synthetase (Jo-1) is the most common autoantigenic target among the aminoacyl-tRNA synthetases.*

*We report two well documented anti-SS cases where it was observed significant exposure to a known inhaled offending antigen, development of a lymphocytic interstitial lung disease (ILD) and negative auto-antibodies, interpreted at first as hypersensitivity pneumonitis. Only after 14 and 30 months, respectively, the development of systemic symptoms compatible with anti-SS and anti-Jo-1 was observed. A growing body of evidence suggests that the lungs are the environment in which Jo-1 autoimmunity may be initiated and propagated. The description of the clinical and laboratorial evolution of these patients together with accumulated evidence of biological plausibility support the hypothesis that anti-SS can follow an episode of lung inflammation secondary to inhaled antigen exposure.*

### Introduction

Anti-synthetase syndrome (anti-SS) is a clinical entity associated with interstitial pneumonia, myositis, arthritis, Raynaud's phenomenon, mechanic's hands, fever, and the production of autoantibodies against aminoacyl-tRNA synthetases (1, 2). Histidyl-tRNA synthetase (Jo-1) is the most common autoantigenic target among the aminoacyl-tRNA synthetases (3, 4). Of particular significance is that the prevalence of interstitial lung disease (ILD) in Jo-1 anti-SS may reach 70% and that ILD is a major contributor to morbidity and mortality (5).

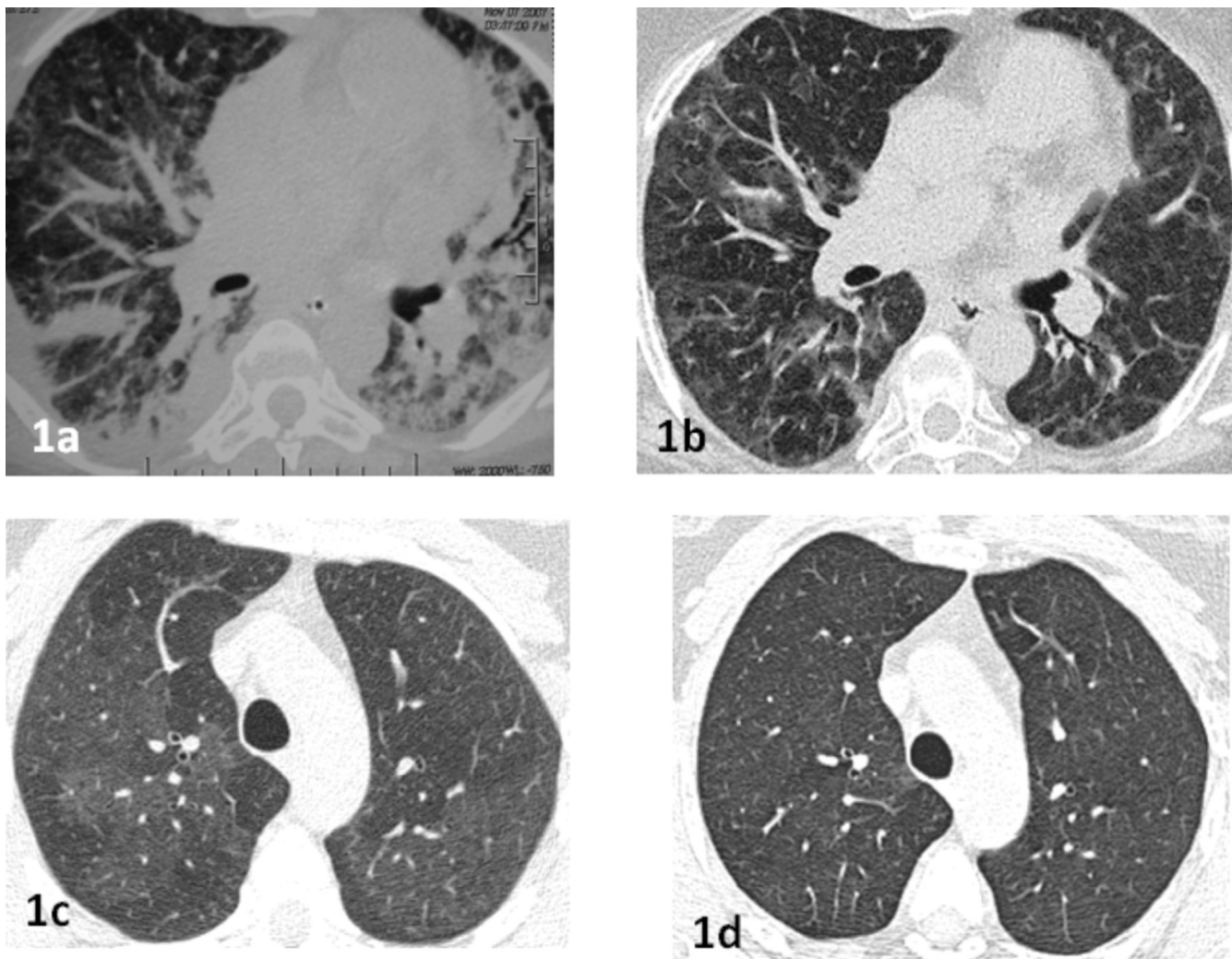
The prevailing hypothesis states that Anti-SS ILD begins as a cellular inflammatory process that fails to terminate appropriately and progresses to a fibroproliferative condition (2). ILD precedes the onset of myositis in up to 70 percent of patients with the anti-SS (5) and there is also evidence in the literature to suggest that the lungs are more than just a commonly involved

organ in Jo-1 anti-SS and rather serve as the environment in which Jo-1 autoimmunity may be initiated and propagated.(6)

### Case 1

A 56-year-old female patient who was a bird breeder presented with a two-month history of progressive dyspnea, dry cough, weight loss and fever. On physical examination, she presented with inspiratory crackles and progressive hypoxaemia. A high-resolution computed tomography (HRCT) of the chest revealed areas of airspace consolidation and diffuse ground-glass opacities (Fig. 1a). At that time, no microorganisms, such as fungi, mycobacteria or bacteria were found in blood or bronchoalveolar lavage (BAL) fluid through direct examination or culture. BAL PCR for respiratory viruses, Mycobacterium tuberculosis, cytomegalovirus, herpesvirus, and pneumocystis jiroveci were negative. Auto-antibody tests were also negative, including Jo-1 (ELISA, Quanta-lite Jo-1 kit). There was a BAL lymphocytosis of 30%. The case was interpreted as hypersensitivity pneumonitis (HP), and she was treated with methylprednisolone at 1 g/day for 3 days followed by prednisone at 40 mg/day, which resulted in significant clinical and radiological improvements. She was advised to quit breeding birds, which she did not consider. During corticosteroid tapering, she presented recrudescence of pulmonary symptoms and radiographic opacities, which reverted with prednisone at 40 mg/day plus azathioprine at 150 mg/day. Six months later, during corticosteroid tapering, pulmonary disease recurred now associated with arthralgias, mechanic's hands, an elevation of creatine phosphokinase to 550 IU/L (normal range: 26–192 IU/L) and anti-Jo-1 positivity. Anti-nuclear antibodies (ANA) were negative. She was given a diagnosis of anti-SS. Cyclophosphamide at 100 mg/day was initiated, prednisone at 40 mg/day was resumed, and the patient quit bird breeding. Subsequently, she presented significant clinical, functional and radiological improvements with no additional disease flare-ups (Fig. 1b). She has been

Competing interests: none declared.



**Fig. 1.** Chest HRCT of anti-SS cases. (1a) Case 1, areas of airspace consolidation and diffuse ground-glass opacities. (1b) Case 1, radiological improvement after treatment. (1c) Case 2, diffuse ground-glass opacities and mosaic attenuation. (1d) Case 2, resolution of the ground glass opacities after treatment.

followed for three and a half years. Cyclophosphamide was switched to cyclosporine at 200 mg/day after 12 months, and low-dose prednisone was maintained.

#### Case 2

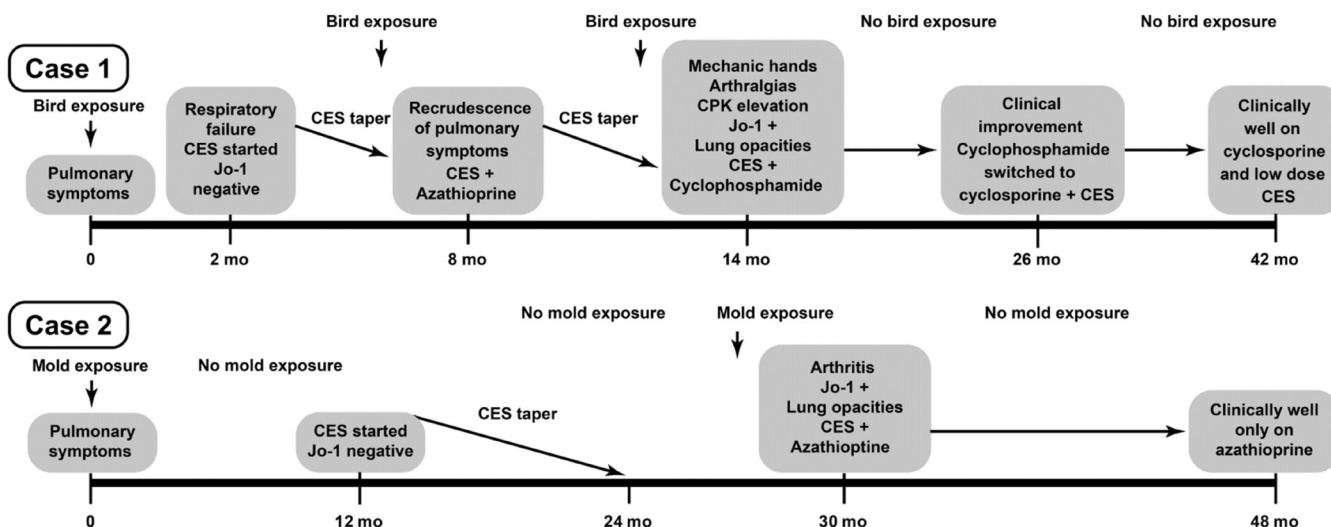
A 57-year-old female patient presented with a 1-year history of dyspnea and cough. She reported significant mold exposure at home. At first evaluation, inspiratory crackles were noted, and chest HRCT revealed diffuse ground-glass opacities and mosaic attenuation (Fig. 1c). Auto-antibody tests were negative, including Jo-1 (ELISA, Quanta-lite Jo-1 kit). BAL was analysed and revealed 35% lymphocytes with a CD4/CD8 ratio of 0.19. No microorganisms such as fungi, bacteria or mycobacteria were recovered from

BAL fluid through direct examination or culture. BAL PCR for *Mycobacterium tuberculosis*, cytomegalovirus, herpesvirus, and *pneumocystis jiroveci* were negative. Transbronchial lung biopsy displayed fibrotic enlargement of alveolar septa and mild peribronchiolar lymphocytic inflammation. At that time, HP was considered the most suitable diagnosis. The patient was started on prednisone at 40 mg/day and advised to avoid mold exposure. The remission of symptoms and image findings was observed, and the prednisone was successfully tapered during the following 12 months; however, 6 months after the complete withdrawal of prednisone, the patient reported re-exposure to mold, which was temporally associated with new migratory ground-glass opacities, arthritis of the wrists, distal and

proximal interphalangeal joints and anti-Jo-1 positivity. Anti-nuclear antibodies (ANA) were negative. The patient then received a diagnosis of anti-SS. Azathioprine at 150 mg/day was initiated, and prednisone 40 mg/day was resumed. Subsequently, she presented clinical and radiological improvements (Fig. 1d) and avoided mold exposure. The patient has been followed for 18 months, and her immunosuppression is being tapered. She is currently on azathioprine at 100 mg/day and has not experienced disease flare-ups.

#### Discussion

These two cases exemplify a common evolution of pulmonary manifestations until the diagnosis of anti-SS was possible (Fig. 2). These patients first presented with pulmonary involvement in



**Fig. 2.** Timeline of anti-SS cases: clinical course, timing of diagnosis, evolution of treatment and relation to bird or mold exposures. CES: corticosteroids. mo: month.

the context of a significant environmental exposure to known antigens capable of triggering an inflammatory reaction in the lungs. These cases were initially interpreted as HP based on clinical-radiological-laboratorial evaluation: lymphocytosis in BAL associated to the exposure to a known offending antigen, recrudescence of symptoms after re-exposure, inspiratory crackles, focal areas of ground-glass opacities, consolidation and mosaic attenuation in case 2 (7, 8). Additionally, in case 2, the histopathological findings were compatible with an initial diagnosis of HP, involving CD8 lymphocytosis and a peribronchiolar lymphomonocytic interstitial pneumonia (9). None of the cases initially presented with evidence of extrapulmonary manifestations or auto-antibodies; however, during follow-up and corticosteroid tapering, extrapulmonary manifestations and anti-Jo1 positivity was detected.

A growing body of evidence suggests a pathogenetic role for anti-Jo-1 in anti-SS: First, anti-Jo-1 autoantibodies undergo spectrotypic broadening, class switching and affinity maturation to the antigen (10). Second, Jo-1 has been shown to demonstrate proinflammatory properties (11). Third, immune complexes containing anti-Jo-1 are able to induce the production of interferon alpha *in vitro*, which promotes immune activation (12). Fourth, mice immunised with murine Jo-1 generate

B and T cells targeting Jo-1 epitopes and subsequently develop a combination of muscle and lung inflammation (13). Collectively, these studies suggest a role for anti-Jo-1 in the initiation and propagation of disease.

Jo-1 is a ubiquitous cytoplasmic antigen, however, Jo-1 expressed in the lungs is more immunogenic due to its higher susceptibility to cleavage by granzyme B, a cytotoxic serine protease found in the cytoplasmic granules of CD8 T lymphocytes and natural killer (NK) cells. Jo-1 cleavage by granzyme B has the capacity to generate fragments that have not been tolerated and therefore have the potential to become autoantigens (6, 14).

An initiating inflammatory stimulus that is capable of exposing and cleaving Jo-1 is then a necessary preliminary step in the sequence of events leading to Jo-1 autoimmunity. Indeed, the longitudinal observation of these two anti-SS patients, specifically the history of exposure to inhaled organic antigens, recrudescence of symptoms after re-exposure, pulmonary manifestations preceding extra-pulmonary symptoms, supports the hypothesis that anti-SS could follow an episode of lung inflammation secondary to inhaled antigen exposure. The inhalation of certain antigens may cause an inflammatory reaction, specifically, hypersensitivity pneumonitis, which is classically characterised by the presence of NK cells

and CD8<sup>+</sup> lymphocytes, known to be rich in granzyme B (15, 16). Through the inflammatory lymphocytic process of HP, we speculate that the exposure and cleavage of cryptic Jo-1 within alveolar septa takes place and, in genetically susceptible individuals, this HP could lead to an autoimmune process against Jo-1.

During the initial inflammatory process in the lungs, anti-Jo-1 was not detected in these two cases. Only after full development of anti-SS it was detected. In both patients Jo-1 was tested twice: at the beginning of pulmonary disease and then at development of systemic symptoms. Therefore, we cannot precise the exact moment when anti-Jo-1 appeared. Nevertheless, this delay between ILD and appearance of anti-Jo-1 argues in favor of the hypothesis of a HP triggering autoimmunity against Jo-1. Autoimmune processes may have many different possible triggers of autoimmunity in susceptible individuals. Future studies aimed at understanding the mechanisms underlying the initiation of autoimmunity in anti-SS are necessary to clarify this issue. Meanwhile, in Jo-1 anti-SS, active questioning about exposure to potential offending inhaled agents and formally advising against exposure may be prudent given its low risk-benefit ratio and the potential to inhibit anti-Jo-1 generation and, consequently, anti-SS activity.

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