Systemic Sclerosis (Scleroderma)

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Diffuse scleroderma is a chronic systemic disease that targets the skin, lungs, heart, gastrointestinal tract, kidneys, and musculoskeletal system. Three features characterize the disorder: (1) tissue fibrosis, (2) vascular damage, and (3) generation of specific autoimmune response associated with autoantibodies. In diffuse scleroderma, there is widespread skin involvement at onset, including areas proximal to the elbows or knees and/or the trunk, with rapid progression and early visceral involvement.

In limited scleroderma, the skin changes are restricted to the face, neck, and areas distal to the elbows and/or knees sparing the trunk. Visceral involvement occurs late; and hence, patients generally follow a more benign disease course than do patients with the diffuse disease. CREST syndrome (cutaneous calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, [scleroderma limited to the fingers], and telangiectasia) is a form of limited scleroderma.

Overlap syndromes, defined as features of 2 or more rheumatic diseases occurring in the same patient, frequently include findings suggestive of scleroderma. Mixed connective tissue disease (MCTD) is an overlap syndrome with features of scleroderma, polymyositis, lupus-like rashes, and rheumatoid-like polyarthritis.

A smaller group of patients, mostly children, present with a largely benign, self-limiting, non-systemic skin disease with manifestations confined to the skin and subdermal tissues. This localized scleroderma can be divided into 3 subtypes: linear scleroderma, en coup de sabre, and plaque morphea.

Pathophysiology

The precise cause of systemic sclerosis remains unknown; however, the current understanding proposes that an unidentified
antigen recruits CD4+ T cells to the skin, where they are activated to secrete cytokines, resulting in the recruitment and activation of mast cells and macrophages. Together these cells produce a variety of inflammatory mediators including: interleukin-1 (IL-1), IL-2, tumor necrosis factor, tumor growth factor-β (TGF-β), platelet derived growth factor (PDGF), IL-13, histamine, and heparin. More importantly, TGF-β, IL-13, and PDGF are well known to stimulate the expression of collagen and extracellular matrix proteins from fibroblasts resulting in excessive fibrosis, a feature characteristic of scleroderma. Molecular analysis of the antigen receptors of the infiltrating T cells suggests that the accumulated CD4+ T cells are oligoclonal, and their expansion is antigen driven. Since microvascular damage presents itself early in systemic sclerosis, it is postulated that inflammatory mediators such as granzyme A, a protease released by inflammatory cells, could initiate this microvascular insult. Damaged/activated endothelium can release PDGF, TGF-β, and chemotactic factors that cause the recruitment of fibroblasts and trigger periadventitial fibrosis. Eventually widespread narrowing of the microvasculature leads to ischemic injury and further scarring.

B-cells from these patients show hyper-responsiveness and autoantibody formation, a facet in its pathology that has been well exploited in laboratory diagnosis of systemic sclerosis. One such group of antibodies are the anti-nuclear antibodies (ANAs). This large group of autoantibodies are directed against specific nuclear antigens and may be seen in 85% to 95% of patients with this disease. Specific anti-nuclear antibodies are important in the diagnosis of systemic sclerosis as described below.

**Laboratory Diagnosis of Systemic Sclerosis**

**Specific Anti-Nuclear Antibodies (ANAs)**

**Anti-topoisomerase-I (Scl-70) antibodies.** Although not very sensitive, these autoantibodies are quite specific (100% compared to normal control subjects and 99.5% compared to patients with other connective tissue diseases). Anti-topoisomerase-I antibodies are usually indicative of diffuse cutaneous scleroderma, although no pathogenic role has been identified. Patients with these antibodies often develop peripheral vascular disease, pulmonary fibrosis, with cardiac involvement, and coexisting malignancies. Thus, titers for anti-topoisomerase-I IgG fluctuate with disease activity and skin score and this may be a useful marker of disease activity.

**Anti-Centromere Antibodies (ACAs)**

These are highly specific for scleroderma, in particular the subset of limited scleroderma referred to as CREST syndrome. Anti-centromere antibodies have been found to be associated with an increased prevalence of ischemic digital loss in patients with limited cutaneous involvement.

**Other Autoantibodies**

Small nucleolar ribonucleoprotein particles (Rnp) are important factors in ribosomal RNA processing and modification. There are 3 types of antibodies to small nucleolar ribonucleoprotein particles: anti-Th/To antibodies, antiU3-snoRNP, and anti-U1-snRNP antibodies. For a more in depth discussion on these, autoantibodies the reader can refer to references. Anti-nuclear antibodies occur in 15% to 40% of patients with scleroderma, they can also occur in autoimmune diseases such as systemic lupus erythematosus, polymyositis or dermatomyositis, and rheumatoid arthritis. In a patient with appropriate clinical findings, the identification of ANAs strongly suggests systemic sclerosis. However, this test is not very specific because a number of other connective tissue autoimmune diseases may present with ANAs [such as systemic lupus erythematosus (SLE), mixed connective tissue disease, Sjögren's syndrome, polymyositis, and rheumatoid arthritis]. Thus, the investigation for ANAs has become part of the general screening procedure in the diagnosis of connective tissue autoimmune diseases.

**Detection of ANAs by Indirect Immunofluorescence**

In our laboratory, the presence of ANAs in a patient's serum is investigated using an indirect fluorescent antibody technique that employs human epithelial cells (HEp-2) as the antigenic substrate. Cultured HEp-2 cells have been shown to yield greater sensitivity and sharper mitotic patterns than the originally used mouse kidney tissues. The presence of autoantibodies, the titer, and the pattern of immunofluorescence is reported.

Patients, who exhibit clinical symptoms of systemic sclerosis, initially have their sera screened for ANAs at 2 dilutions: 1:80 and 1:160. Briefly the procedure consists of overlaying HEp-2 cells with serially diluted patient sera followed by an incubation period to allow specific binding of the autoantibodies in the sera to cell nuclei antigens. After a washing step to remove non-specifically bound autoantibodies, the HEp-2 cells with their specifically bound autoantibodies, are incubated with fluorescein conjugated goat anti-human antibodies that bind to the autoantibodies and allow visualization of the complex by a fluorescent microscope (Image 2). Samples that stain positively at a titer of 1:160 are now repeated with the sample serially diluted from 1:80 to 1:2,560. A positive reaction at 1:160 is recognized as clinically significant however, there will always be a small healthy population of individuals (~5%) that will stain positive for ANAs. A definitive diagnosis must include consideration of the clinical data. The immunofluorescence test for ANAs is a labor intensive, subjective test that requires extensive quality control measures. In many laboratories, ANAs are detected by ELISA or multiplex bead technology.

**Detection of Specific ANAs by ELISA**

Anti-nuclear antibody-positive individuals have their serum investigated for the particular type of autoantibody using an ELISA technique. This involves incubating the microtiter wells coated with various nuclear antigens (SSA, SSB, Sm, Scl-70, RnP) with diluted serum samples. If IgG antibodies against any of these antigens are present in the samples they will bind to the respective antigens forming antigen-antibody complexes. Alkaline phosphatase labeled anti-human IgG is added and will bind to these complexes. Substrates of the enzyme develop and color is detected by the analyzer indicating positivity.

**Autoantibodies Not in Routine Clinical Usage**

Several autoantibodies have been identified whose presence, although not routinely analyzed in the laboratory for clinical diagnosis, is implicated in disease pathogenesis.

**Anti-endothelial antibodies,** present in 28% to 78% of patients with systemic sclerosis, are clinically associated with ischemic digital infarcts and pulmonary arterial hypertension. Recently, it was shown that if anti-endothelial cell antibody-
positive sera were introduced into normal chick embryos, endothelial cell apoptosis was induced in vivo. However, the absence of a sclerodermatous phenotype indicated the involvement of other factors in the disease process. Anti-fibroblast antibodies have also been seen in systemic sclerosis and in vitro studies have demonstrated their ability to induce activation of fibroblasts. This response is also associated with increased extracellular matrix production and increased expression of intracellular adhesion molecule-1, known to be important in leukocyte recruitment to sites of inflammation.

The matrix metalloproteinases (MMPs) are a family of molecules important in degradation the extracellular matrix, an important process in homeostasis. MMP-1 is known to be responsible for the breakdown of collagen I, II, III and, interestingly, sera from patients with systemic sclerosis, have high levels of anti-MMP-1 immunoglobulin (Ig) G. Such studies suggest that anti-MMP-1 IgG inhibit MMP-1 mediated collagenase activity. The elastic microfibrils of the extracellular matrix are made up of fibrillin. The tight skin mouse represents a genetic model for human scleroderma, mainly because a defect within the fibrillin-1 gene results in a phenotype similar to human scleroderma, with increased collagen deposition and thickening of the skin. Furthermore, the tight skin mouse also generates anti-fibrillin antibodies, suggesting their involvement in the pathogenesis. Moreover, anti-fibrillin-1 antibodies are present in 80% of systemic sclerosis patients belonging to the Choctaw American Indian and Japanese ethnicities, compared to less than 50% of Caucasian patients.

**Treatment**

Unfortunately, there is no drug that can alter the underlying disease process in scleroderma. The natural course of the disease is highly variable and the strategy for the most effective therapy targets disease in the specific organs. The most popular drug is D-penicillamine, which is thought to work as an antifibrotic and immunosuppressive agent. Other therapies used in this disease include low-dose, weekly methotrexate, colchicines, potassium paraminobenzoate, interferon, antithymocyte globulin, cyclosporin, tacrolimus (FK-506), dimethyl sulfoxide, relaxin, photopheresis, and corticosteroids.

**Prognosis**

For patients with diffuse scleroderma, the 5-year and 10-year survival rate is 80% and 60%, respectively. Patients with limited scleroderma have a normal survival rate unless there is severe pulmonary hypertension. Finally, patients with older age at onset, diffuse skin disease, presence of tendon friction rubs, and anti-topoisomerase antibody have a worse prognosis.

**Summary**

In summary, systemic sclerosis is a chronic inflammatory disease that can affect both the subcutaneous tissue and viscera. Clinical symptoms due to tissue fibrosis, vascular damage, and the generation of antinuclear antibodies. Laboratory diagnosis requires the detection ANA and anti-Scl-70 antibodies.
While the etiology remains unknown, better understanding of the disease, have improved the treatment and overall disease prognosis. 

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