Antinuclear Antibodies in Systemic Sclerosis

Subjects: Rheumatology

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Systemic sclerosis is a systemic autoimmune rheumatic disease characterized by immune abnormalities, leading to vasculopathy and fibrosis. Autoantibody testing has become an increasingly important part of diagnosis and prognostication. Clinicians have been limited to antinuclear antibody (ANA), antitopoisomerase I (also known as anti-Scl-70) antibody, and anticentromere antibody testing. ANA are common in the general population, occurring in up to 20% of women. The presence of an ANA is not necessarily suggestive of a pathologic process, particularly at low titers.

systemic sclerosis scleroderma antibodies

1. Antinuclear Antibodies (ANA)

ANA are common in the general population, occurring in up to 20% of women. The presence of an ANA is not necessarily suggestive of a pathologic process, particularly at low titers [1]. Rather, low-titer ANA are thought to reflect a state of benign autoimmunity. However, a subset (5–8%) of these individuals will progress to develop a systemic autoimmune rheumatic disease (SARD), such as systemic sclerosis (SSc), Sjogren's syndrome, or systemic lupus erythematosus [1]. ANA-positive individuals that subsequently develop a SARD have significantly increased T and B cell activation and increased $LAG3^+$ T regulatory cells and TGF-ß1 [2][3][4][5]. Immunoregulation usually prevents development of rheumatic disease in ANA-positive individuals. In contrast, immunoregulation becomes impaired in individuals who progress to develop a SARD, resulting in an imbalance favoring inflammation and fibrosis.

Since the 1960s, it has been recognized that ANA are common in individuals with SSc [6][7]. ANA have been reported to occur in 75–95% of patients with SSc, with a sensitivity of 85% and specificity of 54% on immunofluorescence [8]. The antigen substrate that is utilized for the assay affects the specificity and sensitivity of ANA differently. An indirect immunofluorescence assay using HEp-2 cells (HEp-2 IFA) is the gold standard technique. The presence of ANA as a result of HEp-2 IFA is reported as a titer and a pattern. A clinically relevant ANA titer is 1:80 or more [9].

The staining pattern reported with ANA testing by HEp-2 IFA can also be informative. The presence of anti-Scl-70 and anti-U1-RNP antibodies in the sera creates a speckled pattern, while anti-Th/To, anti-fibrillarin (anti-U3RNP) and anti-PM/Scl antibodies create a nucleolar staining pattern. Anti-RNAP I antibodies result in nucleolar staining, while antibodies against RNAP II and III give a speckled appearance or no fluorescence [8]. With the identification of over 30 staining patterns that span many diseases, an international consensus on antinuclear antibody patterns

(ICAP) has proposed a classification system to standardize the interpretation and reporting of staining patterns [10]. While the presence of ANA and staining patterns is helpful, their absence should be interpreted with caution. For example, the anti-RNAP antibodies demonstrate nucleolar staining only 30–44% of the time [11][12]. Thus, ANA staining patterns should not be used as the sole screening test for SSc-specific antibodies. ANA-negative SSc patients exist and may reflect a subset of SSc who have delayed progression of nailfold microangiopathy, defined by an early nailfold capillary NVC pattern [13].

2. Anti-Topoisomerase I Antibodies

Since anti-topoisomerase I antibodies (ATA) respond to immunoblots with a 70 kDa protein, they were originally known as anti-Scl-70 antibodies. Further research revealed that Scl-70 was a breakdown product of the full-length 100 kDa protein; thus, it was found that the name Scl-70 was misleading. ATA was detected in 15–42% of SSc patients, with 90–100% specificity [14][15]. ATA has sensitivity of 34% [8]. ATA has a poor prognosis and is highly associated with diffuse cutaneous SSc (dcSSc). Patients with limited cutaneous SSc (lcSSc) and other SARD have also been noted to have ATA. The risk of severe pulmonary fibrosis and cardiac involvement is increased in SSc patients with ATA. Additionally, tendon friction rubs, the development of digital ulcers, and joint involvement have all been associated with ATA [14][15][16]. An association with scleroderma renal crisis was reported but was not found consistently across all SSc cohorts. Furthermore, the presence of ATA in patients with Raynaud's phenomenon is associated with a higher risk of developing SSc [16].

3. Anticentromere Antibodies

Anti-anticentromere antibody (CENP) antibodies, also known as anticentromere antibodies, were first reported in 1980. Several CENP proteins have been identified (CENP-A, CENP-B, CENP-C, and others), but CENP-B is thought to be the primary target of the anti-CENP B cell response in SSc [17]. Anti-CENP is the most commonly detected autoantibody in SSc cohorts, with a detection frequency of 20 to 38% [14][15][18]. Anti-CENP antibodies are specific to SSc and are reported to have specificity of 99.9% and sensitivity of 33% [19][20]. They occur less frequently in individuals of Afro-Caribbean descent compared to Caucasians [21]. Additionally, primary biliary cirrhosis, Sjogren's syndrome, Raynaud's phenomenon, and systemic lupus erythematosus have all been linked to anticentromere antibodies [22]. Patients with Raynaud's phenomenon are at high risk of developing SSc if they have anti-CENP antibodies. [9][17]. When compared to other SSc-related antibodies, anti-CENP antibodies are typically associated with limited cutaneous SSc and have a better prognosis [23][24]. In this clinical subgroup of individuals, anti-CENP is associated with a higher risk of pulmonary arterial hypertension, peripheral neuropathy, and mortality [16][25]

4. Anti-Ribonucleic Acid Polymerase I, and III Antibodies

Anti-ribonucleic acid polymerase (anti-RNAP) antibodies were first described in the 1990s. Anti-RNAP I and III antibodies almost always coexist and are considered to be highly specific to SSc [14]. Anti-RNAP II antibodies are

not only seen in SSc but also in systemic lupus erythematosus and overlap syndromes. Since ELISA and LIA are now more frequently used for their detection, the nucleolar speckled immunofluorescence pattern normally associated with anti-RNAP is not a sensitive tool for detecting these autoantibodies. [14][26]. The frequency of anti-RNAP I and III varies between 5% and 31% of SSc patients. In a recent meta-analysis, the pooled overall prevalence of anti-RNAP III was 11% [26]. RNAP antibodies consist of two subunits: the largest RP-155 and RP-11 [18]. RP-155 is associated with dcSSc and a higher risk of renal crisis. These patients may also be at higher risk of tendon friction rubs, synovitis, myositis, joint contractures, and the risk of developing malignancies. Despite the prevalence of renal involvement, survival is better in patients with anti-RNAP than in those with ATA or anti-U3RNP [16]. Although they have 100% specificity, autoantibodies against the RP-11 subunit of RNAP III are less sensitive than anti-RP-155 antibodies and do not seem to improve the diagnostic utility of anti-RP-155.

5. Anti-Fibrillarin (Anti-U3RNP) Antibodies

Bernstein et al. published the first report on anti-fibrillarin (anti-U3RNP) antibodies in 1982. Anti-U3-RNP antibodies specifically target a 34 kDa component of the small nucleolar ribonucleoprotein, which is located in the fibrillar area of the nucleolus and is implicated in pre-RNA processing [27]. Anti-U3RNP antibodies are detected in 41% of SSc patients. It is considered relatively specific to SSc and is mutually exclusive from CENP, ATA, and anti-RNAP [14] [28]. They are found more frequently in African American than in Caucasian patients [29]. Anti-U3 RNP is associated with male sex, Afro-Caribbean descent, younger age at diagnosis, and higher risk of developing PAH and gastrointestinal involvement [30]. Regardless of demographics or disease type, anti-fibrillarin antibody positivity is associated with poorer survival. [31].

6. Anti-Th/To Ribonucleoprotein (Anti-Th/To) Antibodies

Anti-Th/To ribonucleoprotein antibodies (anti-Th/To) mainly bind to two mitochondrial RNA processing (MRP) proteins and the ribonuclease P complexes. They are present in 1–13% of SSc patients [32]. Anti-Th/To antibodies have high specificity (99%) for SSc. However, anti-Th/To antibodies have been observed in patients with rheumatoid arthritis, systemic lupus erythematosus, polymyositis, and Sjogren's syndrome. Despite reports of up to 21% of anti-Th/To positive patients having dcSSc, the majority of these patients have lcSSc [33]. Anti-Th/To-positive SSc patients often develop pulmonary hypertension and interstitial lung disease but experience less involvement of joints and muscles [34]. Anti-Th/To antibody is a predictor for a worse prognosis [28].

7. Anti-U11/U12 RNP Antibodies

Low concentrations of macromolecular U11/U12 RNP complexes are present in eukaryotic cells, where they function as spliceosome components and catalyze the splicing of pre-messenger RNA into pre-mRNA introns [35]. The prevalence of anti-U11/U12 -RNP antibodies is 3.2% [36]. Anti-U11/U12 RNP antibodies have been associated with gastrointestinal manifestations, Raynaud's phenomenon, pulmonary fibrosis, and an increased risk of mortality

[37][38]. The presence of anti-U11/U12 RNP autoantibodies may indicate a subset of patients who are more likely to develop cancer when SSc first appears [39].

8. Anti-Ro/SSA Antibodies

Anti-SSA/Ro52 antibodies occur with a prevalence of 20% in SSc patients [40]. Anti-Ro52 antibody is a risk factor for a serious pulmonary outcome [41]. While one study found no correlation between the presence of anti-Ro52 antibodies and Raynaud's phenomenon, sclerodactyly, digital ulcers, gangrene, calcinosis cutis, telangiectasia, or esophageal dysmotility [41], anti-Ro52 antibody is predictor of poor survival in SSc [42].

9. Anti-Ku Antibodies

Initially discovered in individuals with scleroderma–polymyositis overlap syndrome, anti-Ku antibodies were first reported in 1981 by Mimori et al. Ku is a DNA-binding protein involved in DNA repair, which is important for the non-homologous end-joining pathway's ability to repair double-stranded DNA breaks [43]. In a recent international cohort, anti-Ku antibodies were rarely found in only 1.1% of SSc patients. Anti-Ku is more commonly detected in limited SSc patients with overlap disorders (myositis or lupus) [44][45][46]. Anti-Ku positivity is associated with myositis and interstitial lung disease (ILD), while vascular involvement is less prevalent [47]. With regard to prognosis, no survival difference has been associated with this autoantibody [47].

10. Anti-PM/Scl Antibodies

Anti-PM/Scl antibodies are a heterogeneous group of autoantibodies directed to several proteins of the nucleolar PM/Scl macromolecular complex. The two main autoantigenic protein components were identified and named PM/Scl-75 and PM/Scl-100, based on their molecular weights [48]. Anti-PM/Scl have sensitivity of 12.5% and specificity of 96.9% for SSc [49].

11. Anti-PM75 Antibodies

Anti-PM/Scl 75 antibodies occur with a prevalence of 10.4% [49]. Anti-PM75 antibodies are associated with high rates of calcinosis cutis and gastrointestinal manifestations, including gastroesophageal reflux disease, dysphagia, small intestinal bacterial overgrowth, and fecal incontinence. ILD was also prevalent in SSc patients with anti-PM75, second only to ATA-positive patients. Pulmonary hypertension is reported to be the clinical feature most commonly associated with the anti-PM75 antibody [50].

12. Anti-PM100 Antibodies

Anti-PM/Scl 100 antibodies have a prevalence of 7.1% [49]. Anti-PM100 is more associated with calcinosis rather than gastrointestinal manifestation. ILD was also less frequent compared to the anti-PM75 [50]. Patients with anti-

PM100 antibodies had higher survival rates [50].

13. Anti-hUBF/NOR-90 Antibodies

The anti-NOR90 antibody, a nucleolar type of ANA, is found in 6.1% of SSc patients [51]. However, this antibody tends to be less specific for SSc and is reported in other SARD, such as systemic lupus erythematosus, Sjogren's syndrome, and rheumatoid arthritis [52]. Anti-NOR90 antibodies may be a biomarker for idiopathic interstitial pneumonia with features of systemic sclerosis. Anti-NOR90 antibodies are associated with the occurrence of arthritis/arthralgia, sicca symptoms, and Raynaud's phenomenon [53][54]. Systemic sclerosis with anti-NOR90 antibodies can be complicated by interstitial lung disease and cancer [55]. Anti-NOR-90 antibodies may be associated with a favorable prognosis [51].

14. Anti-RuvBL1 and RuvBL2 Antibodies

RuvBL1/2 is an important modulator of transcriptional activation and protein assembly and is essential for cell proliferation. It is located in the nucleus but can also be present in the cytoplasm [56]. Although only 1–2% of patients have anti-RuvBL1/2, it is highly specific to SSc. The relationship of anti-RuvBL1 and RuvBL2 with older onset age, more frequent diffuse skin and skeletal muscle involvement, male sex, and overlap myositis are its distinguishing features [57].

15. Platelet-Derived Growth Factor Stimulatory Antibodies (PDGFs)

Platelet-derived growth factor stimulatory (PDGF) antibodies are the primary mitogens for cells of mesenchymal and neuroectodermal origin. PDGF, first described in the 1970s as a serum factor that stimulates smooth muscle cell proliferation, is now one of the best-characterized growth factor receptor systems [58]. SSc appears to have a distinctive signature that stimulates autoantibodies against PDGFR. Their biological effect on fibroblasts may contribute to the pathogenesis of the disease. [59].

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