

Leaders

Autoantibodies and overlap syndromes in autoimmune rheumatic disease

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Abstract

Many patients diagnosed with autoimmune rheumatic disease cannot be categorised easily into one of the established clinical entities such as systemic lupus erythematosus, dermatomyositis, or systemic sclerosis. The term “overlap syndrome” has been increasingly used to identify such patients and is useful in terms of clarifying prognosis and facilitating disease management. This article reviews overlap syndrome in autoimmune rheumatic disease, with particular emphasis on the associated serological markers.

(*J Clin Pathol* 2001;54:340–347)

Keywords: autoantibodies; overlap syndromes; autoimmune rheumatic disease

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Accepted for publication
20 September 2000

Table 1 Overlap syndromes in autoimmune rheumatic disease and associated autoantibodies^{7,8}

Overlap syndrome	Autoantibody directed to
Mixed connective tissue disease	nRNP; hnRNP(A2/RA33 complex); U1 RNA
tRNA synthetase syndrome	Jo-1, PL-7, PL-12, OJ, EJ
Polymyositis/pulmonary fibrosis	KJ
Polymyositis/systemic lupus erythematosus	56 kDa nRNP
Polymyositis/scleroderma	Ku; PM-Scl; U2 RNP; DNA-PK
Secondary Sjögren's syndrome	Ro/SSA; La/SSB; Ki/SL
Rheumatoid arthritis/systemic lupus erythematosus	None identified
Scleroderma (CREST)/primary biliary cirrhosis	Centromere; pyruvate dehydrogenase complex
Systemic lupus erythematosus/autoimmune hepatitis	tRNA

hnRNP, heterogeneous nuclear ribonuclear protein; PM-Scl, polymyositis-scleroderma overlap syndrome.

proteins. This article will consider, primarily, overlap syndromes associated with autoimmune rheumatic disease; specific reference will be made to autoantibody profiles and ramifications for diagnosis and pathogenesis will be discussed. It should be noted that overlap between organ specific autoimmune syndromes, such as myasthenia gravis, Hashimoto's thyroiditis, and insulin dependent diabetes mellitus, is frequently seen; however, a detailed discussion of these conditions is beyond the scope of this paper.

Autoimmune rheumatic diseases are classified using internationally accepted criteria, which frequently incorporate the detection of specific autoantibodies as unique diagnostic markers. Examples include: antidouble stranded DNA (anti-dsDNA) and anti-Smith antigen (anti-Sm) for establishing a diagnosis of systemic lupus erythematosus (SLE)¹; the detection of rheumatoid factor in the diagnosis of rheumatoid arthritis (RA)²; anticentromere, anti-DNA topoisomerase I, and anti-RNA polymerase I and III antibodies as markers for the scleroderma spectrum of disease; and a group of antibodies including antiliver/kidney microsomes (anti-LKM), antismooth muscle antigen (anti-SMA), and antinuclear (ANA), included in diagnostic criteria for autoimmune hepatitis.³

However, many patients cannot be assigned to a single disease category. This difficulty has led to the concept of “overlap syndrome”, where symptoms from two or more autoimmune conditions are identified in the same patient. Overlap syndromes are commonly associated with autoimmune rheumatic disease, where up to 25% of patients with early stage or mild variant disease fall into this category.^{4,5} Overlap syndrome has also been described in patients with autoimmune liver diseases, including autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis (table 1).⁶

Clinically, it is useful to define overlap syndromes to clarify prognosis and facilitate disease management.⁹ Two approaches can be made when categorising such conditions. The first is by the identification of a pattern of clinical features, and a good example is the tRNA synthetase syndrome. The second is by the initial detection of a unique autoantibody profile combined with specific clinical findings—for example, mixed connective tissue disease (MCTD).¹⁰

Autoimmune rheumatic disease

Connective tissue diseases (CTDs) are characterised by considerable clinical diversity and heterogeneity. Characteristic clinical features and the detection of autoantibodies help to define these disorders and facilitate diagnosis and appropriate treatment. The CTD overlap syndromes are distinguished by the concomitant occurrence of clinical and serological features of the component diseases. Any CTD can be a partner in an overlap disorder—for example, RA is a common partner to SLE, systemic sclerosis, or Sjögren's syndrome (SS).^{11–12}

In some cases, overlap syndromes have not been clearly circumscribed or widely accepted and the term “undifferentiated” or “transitional” CTD has been used, implying that they are incompletely developed “classic” CTD, rather than distinct entities with overlapping features.⁴ Disease in patients with undifferentiated CTD might evolve into a more recognisable syndrome, most commonly SLE, myositis, or scleroderma,^{13–15} although one report suggested that most remain undifferentiated.^{16–17} Overall, the picture of overlap syndromes with respect to CTD is complex and heterogeneous. Observer bias might play a role in disease classification, so the presence of specific autoantibody profiles might be a useful tool in the diagnosis and management of such patients.

Mixed connective tissue disease

The existence of MCTD has been the subject of much debate^{10–15–18–20} (reviewed by Smolen and Steiner²¹). A detailed discussion is beyond the scope of this review, but suffice to say this syndrome was originally described in 1972 by Sharp and colleagues.²² The syndrome is a combination of features typically found in patients with SLE, scleroderma, polymyositis/dermatomyositis (PM/DM), or RA. The presence of high titre ANA with speckled pattern and antibodies to uridine rich U1 small nuclear ribonucleoprotein (snRNP) differentiates this MCTD from other CTDs. Three points provide a focus for controversy: first, patients with MCTD may ultimately develop an established CTD, commonly, systemic sclerosis, SLE, or myositis.^{13–15} Second, anti-U1 snRNP antibodies are not confined to MCTD and are found in patients with other CTDs, especially SLE,^{19–23} and, finally, the definition of MCTD is confusing. Although originally described as a benign disorder, considerable organ involvement can accompany this condition and the prognosis may be poor.¹⁰

Several classification criteria exist for MCTD^{22–24–25}; all regard the presence of high titre speckled ANA and anti-U1 snRNP as essential for diagnosis. However, other autoantibodies are seen, specifically antibodies to heterogeneous nuclear ribonucleoprotein (hnRNP),²⁶ U1 RNA,²⁷ phospholipid,²⁸ rheumatoid factor, and endothelial cells.²⁹ Antibodies to dsDNA and Sm, both specific markers for SLE, are rare.²¹

It is well established that snRNPs are a group of protein particles associated with small RNA molecules in the nucleus of all eukaryotic cells.

The most abundant snRNPs are denoted U1, U2, U4/U6, and U5 and, together with hnRNP, they form a major component of the spliceosome, where pre-messenger RNA is processed into mature mRNA. The snRNP spliceosomal complex has several associated common proteins that might also be autoantigenic. Common proteins binding to U1 snRNP particles are represented as U1–70K, U1A, and U1C. Antibodies can be directed to several components of the spliceosome, although with different specificities in different diseases.^{21–29} Antibody specificity is normally determined by western blot analysis. Autoantibodies binding to spliceosomal snRNPs (U1, U2, U4/U6, and U5 complexes) are termed anti-Sm antibodies and are associated with SLE. Antibodies recognising the U1 snRNP complex alone are termed anti-nRNP and are considered the hallmark of MCTD (reviewed by Klein Gunnewiek and colleagues³⁰). Anti-nRNP antibodies recognise primarily the U1 snRNP specific proteins U1–70K, U1A, and U1C and U1 snRNA. There are conflicting reports detailing the role of anti-nRNP antibodies in predicting disease severity and progression. On balance, evidence suggesting that U1 snRNP antibody titres correlate with clinical events is unconvincing.³¹ Alternatively, antibodies to U1 RNA have been shown to be associated with progressive disease and exacerbation.²⁷ Epitope spreading, whereby an immune response to an epitope expands to include associated epitopes on the same molecule, is implicated in the immune response to snRNA and the production of possibly pathogenic antibodies associated with disease. Recent reports suggest that secondary light chain rearrangements in U1-RNA reactive B cells, possibly as a result of crossreactivity with viral immunogens, can lead to changing specificity and affinity of anti-RNA antibodies (epitope spreading).³² It is interesting to note that long term follow up of clinical and serological findings in patients with MCTD has demonstrated intramolecular spreading of autoantibody reactivity against snRNP polypeptides. This was followed by “epitope contraction” and the ultimate disappearance of anti-snRNP antibodies during prolonged remission in some patients.³³

Spliceosomal hnRNP, a collection of 30 proteins termed hnRNP A1 to U, is also a common target for autoantibodies in several CTDs, namely, SLE, MCTD, and RA.^{30–34} Autoantibodies to hnRNP A/B (RA33 complex)²⁶ are found in sera from patients with RA, SLE, and MCTD (table 2). In each disease, diverse hnRNP complex epitopes are

Table 2 hnRNP, Sm, and U1 snRNP reactivities of autoantibodies in patients with SLE, RA, and MCTD (adapted from Smolen and Steiner²¹)

Autoantibody	MCTD	RA	SLE
Anti-hnRNP	+	+	+
Anti-Sm	–	–	+
Anti-U1 snRNP	+	–	+

hn, heterogeneous nuclear; MCTD, mixed tissue connective disease; RA, rheumatoid arthritis; RNP, ribonuclear protein; SLE, systemic lupus erythematosus; Sm, Smith antigen; sn, small nuclear.

recognised—for example, in MCTD, sera appear to recognise a distinctive private epitope on the hnRNP A2 protein.³⁵

It has been mentioned previously that a diagnostic feature of MCTD is the production of large quantities of autoantibodies. An imbalance in cytokine synthesis could be an important factor in driving the immune response, resulting in excessive autoantibody production. Hassan and colleagues³⁶ have reported an increase in both type 1 and type 2 cytokines in patients with MCTD, including an increase in the cytokines interleukin 10, interferon γ , and tumour necrosis factor α .

Immunogenetic studies have established a linkage between antigens of the major histocompatibility complex (MHC), human leucocyte antigens (HLA) DR4 and DR2, and the presence of autoantibodies to U1 snRNP and U1 snRNA, with the core clinical features of MCTD.^{18 37} In the future, genetic studies might be able to predict those patients with overlap syndrome who will eventually develop a classically defined CTD such as scleroderma, SLE, or myositis.

Polymyositis, dermatomyositis, and overlap syndromes

Autoimmune myositis has two major classifications, polymyositis (PM) and dermatomyositis (DM). Both cause weakness of the proximal muscles and patients have a high frequency of specifically associated autoantibodies to nuclear and cytoplasmic antigens, termed myositis specific antibodies (MSA) (reviewed by Mimori³⁸). Myositis specific antibodies are of fundamental importance: they are a tool for diagnosis and patient classification and provide an insight into the pathogenesis of these conditions (for reviews see Targoff^{39 40}). MSA are found almost exclusively in patients with DM/PM and associated overlap syndromes. Autoantibodies detected in myositis associated overlap syndromes also include anti-U1 RNP, anti-Ro/SSA, anti-La/SSB, and anti-Sm. However, it is recognised that when autoimmune myositis is associated with another CTD, the full criteria for the concomitant condition may not be met.⁴¹

tRNA synthetase syndrome

The tRNA synthetase syndrome is characterised by myositis (PM or DM) (83–100%), interstitial lung disease (50–80%), and Raynaud's phenomenon (60–93%).⁴² There is some doubt that this syndrome should be regarded as a true overlap.⁴³ The features of this syndrome may be confused with those of SLE, scleroderma or RA⁴⁴; however, tRNA synthetase syndrome is rarely observed as an overlap syndrome associated with other well defined CTDs.^{45 46} tRNA synthetase syndrome is determined serologically by the presence of antibodies against aminoacyl-tRNA synthetases, and diagnosis depends upon the detection of the appropriate autoantibody. The tRNA synthetases are a series of 20 cytoplasmic enzymes that attach tRNA to its corresponding amino acid during the assembly of polypeptides. Antibodies against five different

aminoacyl-tRNA synthetases have been described: anti-Jo-1 (histidyl), PL-7 (threonyl), PL12 (alanyl), OJ (isoleucyl), and EJ (glycyl). Each antibody is independently associated with a distinctive clinical syndrome exhibiting similar clinical features; the synthetase antibodies share immunochemical properties including inhibition of enzymatic function; they do not crossreact with other synthetases; only one antisynthetase antibody will occur in any one patient and they have strong immunogenetic associations. The most predominant antibodies are directed towards Jo-1 (IgG1 subclass); patients with these antibodies tend to have a relatively young age of disease onset compared with other myositis conditions, arthritis is a more common feature, and these antibodies are inclined to be associated with PM rather than DM.^{40 42}

Mechanisms of autoimmunity are focused around the concept that MSA play a fundamental role in the disease process. Antisynthetase antibodies each inhibit the enzyme of their antigen specificity, indicating the highly selective nature of the autoimmune response. Enzyme inhibition is proportional to the amount of antibody detected in patients' sera and there are some reports to suggest that antibody titre correlates with disease activity. The autoimmune response is antigen driven, possibly initiated by antigen mimicry, with infectious agents mimicking host antigens and inducing an immune response capable of crossreaction with host proteins (molecular mimicry). Various infectious agents have been implicated including enteroviruses, such as Coxsackie virus, picornaviruses, and retroviruses. Host and viral protein or RNA could interact forming altered host proteins that are immunogenic; picornaviruses have been implicated in this mechanism. Alternatively, Plotz⁴⁷ suggested that anti-idiotypic mechanisms might drive the autoimmune response in myositis associated syndromes. Anti-idiotypic antibodies directed towards the binding site of antibodies to viral tRNA might crossreact with synthetases. Finally, a genetic background conducive to the development of MSA is required. HLA associations have been established between B8, DR3, DRw52, and tRNA synthetase syndrome.^{39 40}

Other myositis overlaps

SLE is associated with polymyositis in 4–16% of cases and some reports have indicated that this overlap syndrome follows a benign course. However, a recent review of cases by Garton and Isenberg⁴⁸ has indicated that no significant difference is seen between overlap and non-overlap patients. Antibodies to Jo-1 were of low prevalence in this group, although anti-56 kDa nRNP antibodies were seen with high frequency. Anti-56 kDa nRNP antibodies are associated with myositis occurring together with SLE; the presence of this antibody can help to predict muscle involvement in this group of patients.⁴⁹ Other overlap associations with myositis have been reported but it is doubtful that these reports reflect true overlap syndromes rather than coincidence.

Table 3 Autoantibodies in scleroderma

Autoantibody	Clinical importance
Anti-DNA topoisomerase I (Scl-70)	Diffuse scleroderma
Anticentromere (proteins A, B C)	Limited scleroderma
Anti-U3RNP (fibrillarin)	Diffuse scleroderma—severe disease
Anti-7-2/8-2 RNP (Th/To)	Limited scleroderma
Anti-RNA polymerases I, II, III	Diffuse scleroderma, renal crisis
Anti-PM-Scl	Scleroderma overlap
Anti-U2 RNP	Scleroderma overlap
Anti-Ku	Scleroderma overlap
Antipyruvate dehydrogenase complex (M2)	Limited scleroderma and scleroderma overlap

Scleroderma and overlap syndromes

Scleroderma or systemic sclerosis is a generalised connective tissue disease involving sclerotic changes in the skin and many other organ systems. The condition encompasses a spectrum of disorders that range from localised forms such as morphea with limited cutaneous involvement (which may involve the internal organs after long periods) to diffuse cutaneous disease invariably accompanied by early internal organ involvement.⁴¹ Patients with scleroderma overlap are frequently referred to dermatologists owing to cutaneous symptoms and may be misdiagnosed as having scleroderma. Thus, to establish diagnosis and treatment for patients with scleroderma overlap syndromes, it is important that the associated clinical and serological features are recognised. Clinically, scleroderma has been described in association with SS, SLE, DM, Hashimoto's thyroiditis, and primary biliary cirrhosis.^{41–50} Scleroderma overlap syndromes are frequently referred to as MCTDs; however, overlaps also occur in patients who do not have antibodies to nRNP and are associated with ANA of nucleolar staining pattern; table 3 details the full range of associated autoantibodies.⁵¹

Raynaud's phenomenon

Raynaud's phenomenon is almost universal in patients with scleroderma and is a feature of many other CTDs and overlap syndromes. Table 4 shows the prevalence of Raynaud's phenomenon in these conditions. It is a disorder characterised by episodic, clearly demarcated, two or three phase colour change of the extremities (usually fingers), in response to cold or emotion. Raynaud's phenomenon occurs in up to 5% of the normal population, with over 90% of sufferers being female; the age of onset is usually below 25 years. Five per cent of patients presenting with this condition eventually develop an autoimmune rheumatic disease. The best predictors of progression to CTD are an asymmetrical pattern of Raynaud's, positive ANA, and abnormal nail fold capillary microscopy.⁴¹

Table 4 Prevalence of Raynaud's phenomenon in autoimmune rheumatic diseases (%) (adapted from Morrow et al)⁴¹

Scleroderma	>95
Sjögren's syndrome	20–50
Myositis	20–40
Systemic lupus erythematosus	20–30
Rheumatoid arthritis	<5

Scleroderma-polymyositis overlap syndrome (PM-Scl)

The term “sclerodermatomyositis” was used originally to define a group of patients with features of both scleroderma and dermatomyositis.⁵² The main features of this syndrome are myalgia or myositis, arthralgia, scleroderma-like cutaneous changes, Raynaud's phenomenon, and an association with specific autoantibodies: anti-PM-Scl, anti-Jo-1, anti-Ku, and anti-U2 RNP. The detection of specific autoantibodies or autoantibody profiles assists accurate diagnosis, allowing provision of optimal treatment. Patients with PM-Scl overlap respond to small doses of corticosteroids and do not require intensive treatment; in contrast, patients with scleroderma may not respond to such treatment.⁵¹

Antibodies to PM-Scl prevail and are found in 40–50% of patients.^{7–8, 53} This antibody was first identified by double immunodiffusion and indirect immunofluorescence, where it produces a characteristic homogeneous nucleolar staining pattern together with weak staining of the nucleoplasm. The PM-Scl antigen is located at the site of ribosome assembly in the granular component of the nucleolus, although its function is unknown; the PM-Scl complex may have a role in ribosomal maturation.^{54–55} It is a compound antigen consisting of up to 16 polypeptides with molecular weights ranging from 20 to 110 kDa. Autoantibodies are directed predominantly against two molecules of 100 kDa (95%) and 75 kDa (50%).^{54–55} However, although anti-PM-Scl antibodies are found in patients with PM-Scl overlap syndrome in Europe and North America,^{7–8, 56} they are not found in Japanese patients. This is probably the result of differences in genetic and environmental backgrounds.⁵⁷ Anti-PM-Scl antibodies are strongly associated with HLA DR3; this HLA marker is found rarely in the Japanese population but is common in the normal population of Europe and North America.

Anti-Ku antibodies were described initially in Japanese patients with PM-Scl overlap, although they have since been described in other patient groups.⁵⁸ The target antigen is a heterodimer of 70 kDa and 80 kDa proteins that acts as a regulatory subunit of DNA dependent protein kinase, an enzyme that catalyses the phosphorylation of nuclear proteins. This enzyme complex is involved in transcription, DNA repair, antigen receptor V(D)J recombination, and apoptosis.⁵⁷ These antibodies also present a nucleolar staining pattern by immunofluorescence. Other associated autoantibodies include anti-DNA protein kinase complex antibodies and anti-U2 RNP antibodies, rare antibodies that usually coexist with anti-U1 RNP.⁸

The aetiology and pathogenic mechanisms of PM-Scl overlap remain unknown. A strong association of anti-PM-Scl antibody with HLA DR3 and an increased frequency of HLA DQA*0501 suggest a genetic influence.⁵⁶ However, these associations are not absolute and it is probable that several independent factors can result in similar pathogenic mechanisms.⁵⁹

Table 5 Autoimmune diseases associated with Sjögren's syndrome (adapted from Morrow et al)⁴¹

Rheumatoid arthritis
Scleroderma
Systemic lupus erythematosus
Polymyositis/dermatomyositis
Primary biliary cirrhosis
Autoimmune hepatitis
Polyarteritis nodosa
Diabetes
Graves' disease
Myasthenia gravis
Coeliac disease

Other scleroderma overlap syndromes

An association between limited scleroderma (CREST syndrome), primary biliary cirrhosis, and SS has been reported.⁵⁰ Patients are characterised serologically by the presence of autoantibodies directed against the pyruvate dehydrogenase complex (M2), centromere, and Ro/SSA and/or La/SSB and genetically by an association with HLA Cw6.⁶⁰

Scleroderma and RA may be seen as part of an overlap syndrome. Rheumatoid factor can be detected together with joint damage, but whether this represents a distinct clinical entity or part of the spectrum of scleroderma is unknown.^{50 61}

SS overlap

SS is a chronic inflammatory autoimmune disorder affecting the lachrymal and salivary glands and other exocrine organs throughout the body. Dryness of eyes and mouth, chronic fatigue, and arthralgias are the most common features of this disease, which may appear solely, as primary SS, or together with other rheumatic disease as secondary SS. Sicca syndrome describes a limited form of SS restricted to dryness of eyes and mouth, and this syndrome is frequently associated with other autoimmune disease but mainly SLE and RA (table 5). Moutsopoulos and Manousakakis⁶² have suggested additional classification groups for patients with SS based on serological and immunogenetic profiles. They have suggested that they can be divided into three major subsets: those with anti-Ro/SSA and La/SSB antibodies; those with no specific autoantibody response; and those with antibodies against autoantigens such as centromere, mitochondria, and thyroperoxidase. An extensive array of both organ and non-organ specific autoantibodies may be detected in patients with SS (table 6), the most prominent serological feature being the production of rheumatoid factor and ANA associated with antibodies to Ro/SSA and La/SSB.⁶³ However, important differences in serological profiles may be found, depending on the nature of the overlap syndrome partner.

Rheumatoid factor is the most common autoantibody found in patients with SS. Differences between SS rheumatoid factor and that produced in patients with RA have been seen, where rheumatoid factor variable genes are close to germ line configurations in SS.^{64 65} Occasionally, very high titres of rheumatoid factor may produce a cryoglobulinaemic vasculitis in these patients.

Anti-Ro/SSA and anti-La/SSB antibodies are not specific for SS but are found commonly in these patients (for a detailed review of these autoantibodies see Scofield and colleagues⁶⁶). The Ro autoantigen is an RNP complex binding to the stem-loop structure of human cytoplasmic RNA (hYRNA). Antibodies directed against two Ro polypeptides have been identified—52 kDa (Ro52) and 60 kDa (Ro60) proteins—and there is evidence that the 52 kDa protein binds to the 60 kDa protein, although this is not certain.⁶⁷ The Ro 52 kDa protein has been isolated and characterised; it contains two zinc finger structures and a leucine zipper motif, indicating a role in DNA and RNA binding and protein-protein interaction. La, which may bind transiently to Ro, is an ATP dependent transcription termination factor for RNA polymerase III; it is a 48 kDa protein that binds polyU stretches at the 3' end of unprocessed polymerase III transcripts. Antibody responses to Ro, La, and other nuclear antigens, such as antiproteasomal antibodies, have been seen to vary between primary SS and SS overlap syndromes.^{68 69} Anti-Ro antibodies have been shown to fluctuate in parallel with disease activity,⁷⁰ although this is the exception rather than the rule in clinical practice. The relation between autoantibody profile and disease pathogenesis is not clear; the penetration of these autoantibodies into living cells is powerful evidence that antibodies to Ro and La are implicated pathogenically in disease. For example, in complete neonatal heart block,⁷¹ a possible mechanism of tissue damage includes the penetration of these autoantibodies into living cells.⁶⁹ In SS, autoantibodies are produced in inflammatory lesions of the salivary glands and the immune response appears to be antigen driven. Infection, resulting in mechanisms such as molecular mimicry, reaction to "altered self", intermolecular and intramolecular spreading might result in antibodies to multiple, linked, self components frequently seen in SS.⁷² Viral infection is strongly implicated as an aetiological factor—for example, Epstein-Barr virus,⁷³ retroviruses such as human T cell leukaemia virus type 1,^{74 75} hepatitis C,⁷⁶ and also organisms such as *Helicobacter pylori*.⁷⁷ HLA associations with secondary SS have been identified; patients with anti-Ro52 and anti-Ro60 have an increased frequency of DR52, in contrast to associations observed in patients with primary SS.⁷⁸ Several studies have reported an association between HLA DQ and anti-Ro and anti-La antibodies.⁷⁹

Table 6 Organ specific and organ non-specific autoantibodies associated with Sjögren's syndrome overlap syndromes (%) (adapted from Morrow et al)⁴¹

Antinuclear antibodies >1/80 (titre)	60–100
Anti-Ro/SSA	5–62
Anti-La/SSB	5–42
Anti-Sm	<5
Anti-RNP	5–23
Anti-dsDNA	5–56
Rheumatoid factor	60–100
Antismooth muscle	30
Antisalivary duct	50–60
Antithyroperoxidase	10–20
Antigastric parietal cell	25–30

ds, double stranded; RNP, ribonuclear protein; Sm, Smith antigen

Alternatively, HLA associations in secondary SS might reflect that of the associated disease. Splitting patients into subgroups based upon HLA associations, autoantibody profiles, and clinical features might provide insight into the pathogenesis of SS overlaps.⁶²

RA/SLE overlap

The existence of patients with a combination of features representing both RA and SLE is acknowledged and termed 'Rhus'.^{41–80} Arthropathy is associated with 5% of patients with lupus and is typically non-erosive. Occasionally, patients with SLE develop erosive arthritis with nodules suggesting an overlap with RA. This is seen more frequently as patients become menopausal, when long standing SLE evolves into a more rheumatoid picture, with erosive joint disease. These conditions are rare and there is a paucity of information regarding autoantibody profiles and immunogenetics. It is debatable whether these conditions represent true overlap, rather than chance occurrence of both conditions in the same patient, or evolution of one disease into another.⁴³

PBC overlap syndromes

Primary biliary cirrhosis (PBC), a chronic, inflammatory, cholestatic disease of the liver results in progressive and irreversible destruction of small interlobular and septal bile ducts and liver failure.⁸¹ The principal treatment for patients with PBC is ursodeoxycholic acid, although advanced disease is treated by liver transplantation. The disease has an autoimmune aetiology and is frequently associated with other autoimmune conditions (table 7).^{82–83} The presence of antimicrobial antibody (AMA) is a hallmark of PBC and these antibodies are found in 95% of patients. AMA are present very early in the natural history of the disease, suggesting an intimate role in its pathogenesis. The major antigen termed M2 is located on the inner mitochondrial membrane and is composed of at least five determinants. The major autoantigen is the E2-subunit of the pyruvate dehydrogenase complex (PDC-E2). However, patients with PBC frequently display antibodies to other mitochondrial antigens, such as the E1 α and E1 β subunits of PDC, protein X of the PDC, and the E2 component of the branched chain α -ketoacid dehydrogenase and α -ketoglutarate dehydrogenase complex. The detection of AMA is the most important diagnostic test for PBC, although these antibodies do not appear to contribute directly to the pathogenesis of the disease.⁸² Recent reports have suggested that biliary epithelial cells are the

Table 7 Autoimmune syndromes associated with primary biliary cirrhosis

Myasthenia gravis
Autoimmune thyroid disease
Mixed connective tissue disease
Systemic lupus erythematosus
Pernicious anaemia
Polymyositis
Limited scleroderma (CREST)
Rheumatoid arthritis
Sjögren's syndrome

autoimmune target in PBC. PDC-E2 epitopes recognised by T cells have been isolated and aberrant expression of MHC class II molecules, intercellular adhesion molecules, and lymphocyte costimulatory molecules has been described.⁸⁴ Antibodies to nuclear antigens are found in up to 50% of patients with PBC and are useful tools that aid the diagnosis of AMA negative PBC (autoimmune cholangitis). These antibodies can also act as markers of overlaps with autoimmune rheumatic diseases and autoimmune hepatitis (AIH).^{6–85} PBC specific ANA include antibodies to gp210, nucleoporin p62, and Sp100⁸² and are detected by immunofluorescence.

PBC/CREST overlap

The association of PBC with limited cutaneous systemic sclerosis or CREST syndrome is well documented.⁸⁶ Patients present with concomitant features of both conditions, although it has been suggested that patients with overlap exhibit a mild form of PBC. Anticentromere antibody (ACA) is seen in 50–80% of patients with CREST syndrome, but is reported in patients with other autoimmune disease, including up to 29% of patients with PBC.⁸⁷ There are three major antigenic polypeptides recognised by ACA termed CENP-A, CENP-B, and CENP-C, with an increased reactivity to CENP-C reported in PBC/CREST overlap syndrome. However, a role for these antibodies in disease pathogenesis has not been ascertained.⁸⁸ AMA are also seen in this overlap population; recently, antibodies to the autoantigens E1 β and E1 α subunit of PDC have been suggested as possible serological indicators for the development of PBC in patients with CREST syndrome.^{83–89} Weak reactivity of almost all sera from patients and controls to the E3 subunit of PDC may shed some light on the mechanism by which immunological tolerance is broken in PBC. An E3 component exists on the membrane of archaeobacteria and eubacteria.⁸³ Unidentified viral infection has been implicated in the production of clonally expanded CD8 positive T cells in these patients; the function of these T cell clones remains unknown but a role in pathogenesis is possible.⁹⁰

Akimoto and colleagues⁸⁸ showed that 75–91% of patients with PBC/CREST overlap syndrome display features of SS. An overlap of SS with PBC alone has also been reported.⁹¹

Autoimmune hepatitis (AIH) overlap syndromes

AIH is associated with a range of autoantibodies including anti-SMA, ANA, anti-LKM, and anti-asialoglycoprotein receptor. Variant forms of AIH are common, including combinations of AIH, PBC, primary sclerosing cholangitis,⁹² or chronic viral hepatitis.⁹³ A detailed discussion of autoimmune hepatitis is beyond the scope of this review (for an overview of associated autoantibodies see Manns,⁶ Czaja,⁹⁴ Chazouilleres *et al*,⁹⁵ and Lohse and colleagues⁹⁶).

Liver disease is also recognised as an important but very rare clinical problem in some patients with SLE, so called lupoid hepatitis.

Sato and colleagues⁹⁷ reported a new auto-antibody to a tRNA related antigen that appears to be specific for a clinical subset of patients who have a mild form of SLE associated with AIH.

Primary sclerosing cholangitis and AIH, both chronic liver diseases with a probable autoimmune background, have been reported to appear as an overlap. Patients fill the diagnostic criteria for both conditions and autoantibodies associated with both conditions are detected; namely, ANA and anti-SMA associated with AIH, together with perinuclear antineutrophil cytoplasmic antibodies associated with primary sclerosing cholangitis.⁹⁵

Conclusion

In a clinical setting, autoantibodies are used to establish diagnosis, estimate prognosis, follow disease progression, and monitor treatment regimens. Some are specific disease markers and play a key role in recognised diagnostic criteria. In some cases, it is the profile of autoantibodies, together with other clinical features, that aids diagnosis.

The identification of overlapping clinical features in a given patient is important because treatment might need to be directed specifically at some of these features. The development of myositis in a patient with apparent scleroderma is a useful example where the myositis may respond to active treatment. It is in overlap patients that autoantibody profiles and possibly genetic associations might be most useful in predicting response to treatment and long term prognosis.

Approximately 50 predominantly nuclear proteins act as autoantigens in autoimmune rheumatic diseases; most autoantigens are multiprotein or nucleoprotein complexes with important functions in cell regulation, such as RNA splicing. The basis of pathogenesis is important to determine because it becomes the focus for possible therapeutic intervention. Characterisation of antigens recognised by autoantibodies associated with non-organ specific autoimmune disease, together with the use of molecular biology technology, will help to clarify similarities and differences between various CTDs and overlap syndromes. This might lead to the development of new diagnostic and therapeutic strategies.^{98 99}

The authors thank Professor D Isenberg for his advice and support.

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