



The Role of Autoantibody Testing in Modern Personalized Medicine

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Abstract

Personalized medicine (PM) aims individualized approach to prevention, diagnosis, and treatment. Precision Medicine applies the paradigm of PM by defining groups of individuals with akin characteristics. Often the two terms have been used interchangeably. The quest for PM has been advancing for centuries as traditional nosology classification defines groups of clinical conditions with relatively similar prognoses and treatment options. However, any individual is characterized by a unique set of multiple characteristics and therefore the achievement of PM implies the determination of myriad demographic, epidemiological, clinical, laboratory, and imaging parameters. The accelerated identification of numerous biological variables associated with diverse health conditions contributes to the fulfillment of one of the pre-requisites for PM. The advent of multiplex analytical platforms contributes to the determination of thousands of biological parameters using minute amounts of serum or other biological matrixes. Finally, big data analysis and machine learning contribute to the processing and integration of the multiplexed data at the individual level, allowing for the personalized definition of susceptibility, diagnosis, prognosis, prevention, and treatment. Autoantibodies are traditional biomarkers for autoimmune diseases and can contribute to PM in many aspects, including identification of individuals at risk, early diagnosis, disease sub-phenotyping, definition of prognosis, and treatment, as well as monitoring disease activity. Herein we address how autoantibodies can promote PM in autoimmune diseases using the examples of systemic lupus erythematosus, antiphospholipid syndrome, rheumatoid arthritis, Sjögren syndrome, systemic sclerosis, idiopathic inflammatory myopathies, autoimmune hepatitis, primary biliary cholangitis, and autoimmune neurologic diseases.

Keywords Autoimmune diseases · Diagnosis · Prognosis · Disease phenotype · Precision medicine · Autoantibodies

Introduction

As in the Yang/Ying duality equilibrium, personalized medicine (PM) intertwines with the traditional nosology approach, as the trend for the personalized definition of patients' illnesses coexists with the paradigm of grouping patients with similar clinical presentations under certain

disease labels. Nosology allows the identification of shared clinical patterns and determination of the general response to certain therapeutic options, culminating in the elaboration of diagnostic and therapeutic guidelines. However, this occurs at the expense of obscuring individual peculiarities. On the other hand, PM recognizes that there are relevant differences among patients equally classified under a single disease entity and that the optimal therapeutic approach will be different for each of these patients. By acknowledging that true individual personalization may not be realistic, some have proposed the term Precision Medicine as the practical application of the concept of PM to restricted groups of individuals with similar pathophysiological behavior and sharing favorable outcomes from selected therapeutic approaches.

Although more fashionable in the last two decades, PM is a concept long pursued by physicians and scientists and implies narrowing down the definition of the health

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problems of an individual patient so that the optimal therapeutic approach is devised for that individual. A detailed anamnesis contributes to “personalizing” the assessment of the health status of any given patient by determining the subjective impact (pain, fear, anger, depression, negation) associated with the objective dimension of the disease identified in the physical examination and imaging/laboratory examinations. Information on previous health problems, life habits, response to previous medications, family health problems, religious attitude, and social-financial status is helpful for the understanding of the various relevant aspects of a given patient, fine-tuning her/his current health problem, and defining the most appropriate therapeutic strategy. The goals of PM are comprehensive and extend from identifying an individual’s potential weaknesses, prediction of potential disease(s), fine phenotyping of diseases, and thorough engagement of the social-cultural dimension of the individual [1] (Table 1).

Along the last few decades, important developments potentially relevant to PM have occurred: (1) steady incremental progress in the availability of various biomarkers of health and disease; (2) progressive understanding of the phenotypic relevance of thousands of polymorphic genes; (3) establishment of techniques for determination of the microbiome in distinct body areas and their physiological and pathological significance; (4) comprehension of epigenetic modulation of the genome and its relevance in various biologic states; (5) availability of multiplex assays that allow simultaneous determination of multiple parameters (microarrays, addressable laser bead immunoassays, next-generation sequencing); (6) artificial intelligence and machine learning required to meaningfully extract trends, conjunctions, and focal points in very large datasets. The integrated use of these elements is expected to foster the establishment of a more advanced degree of medicine personalization soon.

Autoimmune diseases are a group of chronic inflammatory conditions of unknown etiology with a putative

breakdown or dysregulation of tolerance for specific sets of autoantigens. In general, it is believed that autoimmune diseases are triggered by environmental and hormonal factors acting on an organism with definable genetic predispositions. Selected inflammatory and immunologic biomarkers are observed in each set of autoimmune diseases, contributing to refining the diagnosis toward PM. Among these, autoantibodies hold a special place in managing patients with autoimmune diseases, contributing to the identification of individuals at risk and disease prediction, disease diagnosis and fine phenotyping, determination of concurrent processes, establishment of prognosis, treatment selection, and monitoring of disease activity and outcomes (Table 2).

One major group of autoimmune diseases are those with circumscribed involvement of the organism and includes organ-specific (e.g., Hashimoto’s thyroiditis), tissue-specific (e.g., Pemphigus Vulgaris), and cell-specific (e.g., immunologic thrombocytopenia) autoimmune diseases. At the other end of the spectrum is a group of systemic autoimmune diseases characterized by widespread involvement of the organism, although some organs and cells may be preferentially targeted. Systemic lupus erythematosus (SLE) is the prototype of this group and possibly the disease with the most heterogeneous autoantibodies [2]. It is noteworthy that disease-specific autoantibodies in circumscribed autoimmune diseases occur at a high frequency, therefore representing biomarkers with high sensitivity. In contrast, autoantibodies in systemic autoimmune diseases most frequently occur at moderate or low frequency, therefore typically representing biomarkers with limited sensitivity (Table 3). Over the last few decades, steady progress has been achieved in the discovery of novel autoantibodies, establishment of fine phenotype associations, and improvement in immunoassays to determine and quantify these biomarkers in several systemic autoimmune diseases.

Following, we will discuss how autoantibodies can contribute to PM, using several examples of circumscribed and systemic autoimmune diseases.

Table 1 The four-P paradigm of Personalized Medicine

P component	Attribute	Useful determinants
Prediction	Classification into high-risk and low-risk tiers	Genetic factors, biomarkers, lifestyle, ethnicity, family history, clinical manifestations
Prevention	Identification of at-risk individuals	Early biomarkers
Personalize	Fine phenotyping of disease manifestations, prognosis and most suitable treatment	Biomarkers, imaging and clinical manifestations
Participation	Patient engagement	Physician–patient rapport, social-cultural background

Adapted from Auffray et al. [1]

Table 2 Examples of relevant roles of autoantibodies in Personalized Medicine

Attribute	Autoantibody specificity	Clinical implication
Identification of individuals at risk	Anti-dsDNA, anti-GAD65, ACPA Anti-mitochondria	Heralds the evolution to SLE, type 1 DM, and RA, respectively Primary biliary cholangitis
Diagnosis	Anti-phospholipase A2 receptor Anti-acetylcholine receptor Anti-RNA polymerase III	Primary membranous glomerulonephritis Myasthenia gravis Increased risk of renal crisis and cancer in SSc patients
Prognosis	ACPA and rheumatoid factor in patients carrying HLA-DRB1-SE Anti-MDA-5	Severe and erosive course in RA patients Rapidly progressive interstitial lung disease in DM patients
Disease fine phenotyping	Anti-SRP	Severe necrotizing myopathy with poor response to immunosuppression; myocardial disease
Determination of concurrent processes	Anti-Hu (ANNA-1)	Strong indication of underlying malignancy in patients with encephalomyelitis
Treatment selection	ACPA and rheumatoid factor	Higher possibility of favorable response with rituximab or abatacept in RA patients
Monitoring disease activity	Anti-native DNA, anti-nucleosome Anti-proteinase 3 (c-ANCA pattern)	Titers correlate with lupus nephritis activity Increasing levels (or persistent high titers) indicate probable reactivation of disease

ACPA anti-citrullinated peptide antibodies, SLE systemic lupus erythematosus, DM1 type 1 diabetes mellitus, RA rheumatoid arthritis, HLA-DRB1-SE HLA-DRB1 alleles carrying the shared epitope, MDA-5 melanoma differentiation-associated gene 5, DM dermatomyositis, SRP signal recognition particle, ANNA-1 anti-neuronal nuclear antibody 1, c-ANCA cytoplasm pattern of anti-neutrophil cytoplasm antibody

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease with multifactorial etiology, heterogeneous and pleomorphic clinical manifestations,

and potential to affect several organs and systems. The disease is characterized by variable onset and then differing periods of flare and remission [3]. The prevalence varies according to ethnicity and environmental conditions, occurring in 20 to 150 cases/100,000 inhabitants in

Table 3 Autoantibody frequency in circumscribed and systemic autoimmune diseases

Circumscribed autoimmune diseases		Systemic autoimmune diseases	
Acetylcholine receptor	Myasthenia gravis (80–90)*	Centromere	SSc (12–40)
F-actin	Type 1 autoimmune Hepatitis (60–80)	dsDNA	SLE (30–65)
Adrenal cortex	Autoimmune polyglandular syndrome (85–100)	Fibrillarin	SSc (3–10)
Aquaporin 4	Neuromyelitis optica (85–90)	Jo-1 (His tRNA synthetase)	ASS (30)
Glutamic acid decarboxylase	Type 1 diabetes mellitus (80) Stiff-person syndrome (> 90)	Nucleosome (chromatin)	SLE (50–75)
Glomerular basement membrane	Goodpasture's syndrome (> 95)	Mi-2	DM (10–30)
Insulin	Type 1 diabetes mellitus (> 90)	Ribosomal P	SLE (10–20)
Islet cell	Type 1 diabetes mellitus (85)	SS-A/Ro60	SjS (30–60) SLE (25–30)
LKM-1	Type 2 autoimmune Hepatitis (80%)	SS-B/La	SjS (10–40) SLE (5–15)
Mitochondria	Primary biliary cholangitis (85)	Scl-70 (topo I)	SSc (18–30)
Tissue transglutaminase	Celiac disease (90)	Sm	SLE (5–15)
		To/Th	SSc (4–10)

SSc systemic sclerosis, dsDNA double-stranded DNA, SLE systemic lupus erythematosus, ASS anti-synthetase syndrome, DM dermatomyositis, SjS Sjögren's syndrome

*Percent frequency

the USA [4]. Geographic regions where individuals have high exposure to the sun exhibit higher incidence rates [5]. Reflecting an important hormonal influence, it is more frequent in women (7:1 to 15:1 women/men) and the disease onset occurs between 16 and 55 years of age in 65% of the cases. Although less frequent in males and childhood, SLE is usually more severe in these gender and age groups. Regarding ethnicity, SLE is more prevalent and severe in Afro-descendant and Asian individuals [3, 4].

The interaction between hormonal, environmental, and infectious factors in individuals with an appropriate genetic predisposition is believed to lead to loss of immunological tolerance and the development of the disease. Infectious agents (e.g., Epstein–Barr virus), some medications (procainamide, hydralazine, hydrazide, biologicals, etc.), ultraviolet radiation (sunlight), and hormonal factors (increased estrogen/androgen ratio) are recognized as possible triggers. Immunological system dysregulation is characterized by the presence of multiple autoantibodies directed mainly against nuclear antigens but also a host of cytoplasmic and extracellular antigens [2]. Some autoantibodies directed against specific cell-surface antigens participate in the pathogenesis of the disease, leading to cytopenia and neuropsychiatric impairment. Tissue damage results from the deposition of immune complexes, activation of the complement system, and consequent inflammatory processes. In addition, SLE patients have a deficiency in the clearance of circulating autoantigens and immune complexes, favoring the perpetuation of the pathogenic process [3].

Given the complexity and the heterogeneous phenotypic spectrum of the disease, biomarkers that can lead to a more accurate definition of an individual patient pathology are essential for PM regarding diagnosis, treatment, and prognosis. Autoantibodies, in particular, play an important role in

the prediction, diagnosis, classification, and fine phenotype definition of SLE (Table 4).

Antinuclear Antibodies

Antinuclear antibody (ANA) screening by indirect immunofluorescence on HEp-2 cells (HEp-2 IFA) is positive in up to 98% of SLE patients with active disease [6]. A positive HEp-2 IFA test has been included as a required criterion in the most recent ACR/EULAR classification criteria for SLE [6]. However, HEp-2 IFA can also be positive in other immune-mediated rheumatic diseases, such as systemic sclerosis (up to 95%), mixed connective tissue disease (100%), Sjögren's syndrome (68–96%), and less so in immune-mediated myopathies (40–80%). In addition, as a consequence of the increased sensitivity of the method, 12.6 to 22.6% of healthy individuals may also have a positive HEp-2 IFA test [7, 8]. Interestingly, it has been reported recently that the frequency of positive HEp-2 IFA tests, but not of autoimmune diseases, has been increasing along the decades, suggesting a distinction between autoimmunity and autoimmune disease [9]. In this context, it should be noted that the immunofluorescence pattern in the HEp-2 IFA test holds relevant associations to the autoantibodies present in the sample and that several of the SLE-specific autoantibodies hold distinctive HEp-2 IFA pattern associations [10, 11]. The most relevant HEp-2 IFA patterns have been classified with alpha-numeric codes by the International Consensus on ANA Patterns (ICAP) initiative (www.anapatterns.org) [12]. Therefore, the interpretation of the HEp-2 IFA test in the context of SLE should be personalized as some patterns (e.g., DFS-like pattern/AC-2; centromere pattern/AC-3, nucleolar patterns/AC8-AC-10, NuMA-like pattern/AC-26; Scl-70-like pattern/AC-29) present no relevant relationship with SLE

Table 4 Autoantibodies in systemic lupus erythematosus

Autoantibody	Frequency (%)	Relevance and specific associations
dsDNA	40–70	Correlates with disease activity, especially renal (proliferative glomerulonephritis) and CNS High specificity; severe multi-system SLE
Nucleosomes	70–80	Correlates with disease activity High specificity
Sm	10–30	High specificity for SLE; higher frequency in African ethnicity; associated with CNS and kidney involvement, skin manifestations
U1-RNP	30–40	Correlates with Raynaud's phenomenon, scleroderma-like nailfold capillaroscopic abnormalities
SS-A/Ro60	30–40	Sjögren's syndrome, subacute cutaneous lupus and neonatal SLE
SS-B/La	10–15	Sjögren's syndrome and neonatal SLE
Ribosomal P	10–47	Psychosis, lupus hepatitis, membranous glomerulonephritis
Histone	60–95	SLE, drug-induced SLE
Phospholipids*	20–40	Antiphospholipid syndrome, neurological manifestations, APS nephropathy, thrombocytopenia

CNS central nervous system, SLE systemic lupus erythematosus, APS antiphospholipid syndrome

*Anti-phospholipid antibodies is a general term that comprises antibodies to cardiolipin and other phospholipids, the lupus anticoagulant test and antibodies to antigens that are not phospholipids, e.g., β 2 glycoprotein I (β 2GPI) and phosphatidylserine/prothrombin (PS/PT)

whereas some others (e.g., homogeneous nuclear/AC-1; coarse speckled nuclear/AC-5) are frequently observed in SLE patients [11]. These considerations are particularly relevant, as a positive ANA test is an entrance item in the ACR/EULAR SLE classification criteria [6]. Table 5 illustrates some relevant patterns occurring in SLE and other systemic autoimmune diseases.

In addition to contributing to diagnosis and classification, the HEp-2 IFA test presents additional opportunities for PM. Recent data suggest that SLE patients with positive HEp-2 IFA and/or anti-dsDNA respond better to some biological DMARD in comparison with seronegative patients [6, 13, 14]. Moreover, the HEp-2 IFA titer and pattern can change according to the disease activity status. In a cross-sectional and prospective study, Prado et al. showed that high titer HEp-2 IFA and the homogeneous nuclear pattern (AC-1) were associated with higher SLE disease activity index (SLEDAI) whereas lower titer HEp-2 IFA and fine speckled nuclear pattern (AC-4) were associated with lower SLEDAI [15]. In addition, SLE patients with a negative ANA HEp-2 IFA tend to be older and Caucasian [16].

Autoantibodies to SS-A/Ro, SS-B/La, U1-RNP, and Sm

Antibodies such as anti-U1-RNP, anti-SS-A/Ro60, and anti-SS-B/La are frequently found in patients with SLE, but they are not specific for this disease [17]. Anti-SS-A/

Ro60 antibodies are present in 30–40% of SLE patients and 60–90% of patients with subacute cutaneous lupus. Both anti-SS-A/Ro60 and anti-SS-B/La are associated with sicca syndrome in SLE patients [17] and are also associated with the risk of neonatal lupus (NLE). Isolated anti-SS-B/La rarely imposes risk, but, when associated with anti-SS-A/Ro60, the risk of NLE increases. Among babies born to mothers carrying anti-SS-A/Ro60 with or without anti-SS-B/La, approximately 10% develop skin rash, 20% transient cytopenia, and 30% mild transient transaminitis [18]. These complications are short-lived and spontaneously resolve within a few months when the maternal antibodies disappear from the child's circulation [19]. The exception is the congenital complete atrioventricular block, which, although rarer (1–2%), is irreversible. If previous maternal history of a fetus with complete atrioventricular block is present, the chance of recurrence increases to 13–18% [20]. In adult patients, anti-SS-A/Ro60 is associated with QTc interval prolongation [21] and an increased risk of developing complex ventricular arrhythmias [22].

Anti-U1-RNP and anti-Sm antibodies react with antigens in small nuclear ribonucleoprotein macromolecular complexes that function in the splicing of precursor messenger RNA [23]. Anti-Sm and anti-U1-RNP are more prevalent in African American and Afro-Caribbean as compared to Caucasian SLE patients. Anti-U1-RNP presents moderate sensitivity and is not specific for SLE, as it is also observed in SSC

Table 5 HEp-2 IFA patterns, associated autoantibodies, and most frequent clinical associations

HEp-2 IFA patterns	Autoantibodies	Most frequent clinical associations
Nuclear patterns		
Dense fine speckled (AC-2)	LEDGF/p75	Individuals without objective evidence of systemic autoimmune disease. Rarely found in rheumatic immune-mediated disease
Fine speckled (AC-4)	SS-A/Ro60	SLE, subacute cutaneous lupus, neonatal lupus, primary SjS, systemic sclerosis, polymyositis, PBC
Homogeneous (AC-1)	SS-B/La	SLE, neonatal lupus, primary SjS
	dsDNA	SLE; occasionally in PBC patients
	Nucleosome	SLE
Large/coarse speckled (AC-5)	Histone	Procainamide-induced lupus, SLE, rheumatoid arthritis, juvenile idiopathic arthritis, Feltz syndrome, and AIH
	Sm	SLE
Centromere (AC-3)	U1RNP	SLE, mixed connective tissue disease, systemic sclerosis
	CENP-A, CENP-B	Systemic sclerosis, PBC, SjS
Cytoplasmic patterns		
Dense fine speckled (AC-19)	PL7/PL12	Anti-synthetase syndrome; polymyositis
	Ribosomal P	SLE; occasionally in AIH
Fine speckled (AC-20)	Jo1	Anti-synthetase syndrome, polymyositis, dermatomyositis
Reticular dotted (AC-21)	Mitochondrial pyruvate dehydrogenase	PBC, systemic sclerosis

Adapted from Dellavance et al. [10] and Damoiseaux et al. [11]

SLE systemic lupus erythematosus, SjS Sjögren's syndrome, PBC primary biliary cholangitis, AIH autoimmune hepatitis

and MCTD [17]. SLE patients with anti-U1-RNP antibodies have increased frequency of scleroderma-like nail fold capillaroscopic abnormalities and Raynaud's phenomenon [24]. Anti-Sm antibodies are present in 12–30% of SLE patients with a specificity of 96 to 98%. Anti-Sm antibodies have been associated with lupus nephritis, especially when associated with anti-dsDNA antibodies, neuropsychiatric lupus, serositis, pulmonary fibrosis, and peripheral neuropathy [25, 26]; however, they are not associated with disease activity [17].

Anti-Double Stranded DNA (dsDNA) Antibodies

Anti-dsDNA antibodies are specific biomarkers for SLE and constitute part of the classification criteria [6]. This biomarker is widely used in daily clinical practice for diagnosis and disease monitoring as it is rarely seen in conditions other than SLE and the serum levels correlate with disease activity, especially nephritis activity. Despite its high specificity for SLE, the sensitivity is only 50–60% [17]. In clinically asymptomatic SLE patients, rising anti-dsDNA titers herald imminent risk for disease flare, especially when accompanied by decreasing serum complement concentrations [27]. Controlled studies in clinically stable SLE patients with rising anti-dsDNA titers showed that patients who receive glucocorticoids developed fewer severe flares compared to those who did not [28]. However, it is not recommended to treat clinically asymptomatic SLE patients with stable and persistent serological activity due to the risk of adverse effects of glucocorticoids and immunosuppressant therapy [27].

Anti-Nucleosome Antibodies

The nucleosome is a chromatin unit formed by dsDNA coiled around a histone octamer. Anti-nucleosome (NCS) antibodies have high sensitivity (48–100%) and specificity (90–99%) for SLE, showing association with disease activity, especially active nephritis. Anti-NCS antibodies frequently precede the appearance of anti-dsDNA antibodies and, when both coexist, anti-NCS and anti-dsDNA serum levels show a good correlation. Anti-NCS serum levels were reported to correlate with the histological activity index of lupus nephritis [29]. These autoantibodies are especially useful in lupus nephritis patients that do not present anti-dsDNA and anti-Sm antibodies [17]. Patients with clinically quiescent SLE and high anti-NCS levels have more frequent flares than their negative counterparts and should be closely monitored for early detection and timely treatment of flares [30]. The coexistence of anti-nucleosome, anti-dsDNA, and anti-histone antibodies is associated with severe kidney involvement [31] and an increased risk of renal failure requiring transplantation [32].

Anti-C1q

Anti-C1q antibodies are not specific for SLE because they are also found in patients with systemic sclerosis, Sjögren's syndrome, rheumatoid arthritis, hypocomplementemic urticarial vasculitis, antiphospholipid syndrome (APS), and dermatomyositis. However, anti-C1q is far more frequent in SLE than in other rheumatic diseases (OR 2.7, 95% CI 1.8–4, $p < 0.001$) [33]. In addition, anti-C1q is more frequent in lupus nephritis, showing a good correlation with nephritis activity [33–35]. In a multicentric cohort, anti-C1q in combination with anti-dsDNA and low complement presented the strongest serological association with renal involvement (OR 14.9; 95% CI 5.8–38.4) [30]. Concomitant anti-dsDNA and anti-C1q positivity confers a predictive value of 67% for active lupus nephritis while when both autoantibodies are absent the negative predictive value for active lupus nephritis is 74% [36]. In proliferative lupus nephritis, anti-C1q antibodies show a sensitivity of 80.5% and a specificity of 71% for the diagnosis of renal flares [37].

Anti-Ribosomal P Protein

The frequency of anti-ribosomal P protein (anti-ribosomal P) antibodies in SLE patients varies from 10 to 47% and this variability is known to be dependent on the immunoassay employed, ethnicity or regional differences, cohorts studied, and the age of disease onset. Anti-ribosomal P has specificity for SLE of 96.1 to 98.4% [38, 39]. In addition, anti-ribosomal P shows some peculiar phenotypic associations in SLE. Since the first report of an association between anti-ribosomal P and psychosis [40], the association of this antibody in neuropsychiatric SLE (NPSLE) is the subject of several studies. A recent meta-analysis confirmed the association between anti-ribosomal P and neuropsychiatric manifestations (OR 1.95; 95% CI 1.52–2.50) with considerable heterogeneity of manifestations [38]. When NPSLE was stratified by specific NPSLE features, central nervous system involvement, depression, and psychosis remained associated with anti-ribosomal P. In particular, the association of anti-ribosomal P was stronger with psychosis (OR 3.08; 95% CI 1.94–4.87) and depression (OR 3.03; 95% CI 1.32–6.95).

Anti-ribosomal P antibodies also show phenotypic associations with lupus nephritis, as the frequency of anti-ribosomal P antibodies was shown to be higher in SLE patients with class V lupus nephritis than in patients with other classes of glomerulonephritis (72% versus 28%; $p = 0.005$) [41]. In addition, a better long-term renal outcome was observed in SLE patients with positive anti-ribosomal P antibody and no anti-dsDNA during nephritis flares [42]. Lupus hepatitis also presents a strong association with anti-ribosomal P (OR 8.44; 95% CI 3.16–22.53), which has been reported also in 9.7% of

autoimmune hepatitis patients without clinical or laboratory evidence of SLE [43].

Therefore, anti-ribosomal P antibodies provide useful information for PM and should be used more often in clinical practice. When other SLE-specific autoantibodies are lacking, anti-ribosomal P may represent a valuable biomarker for diagnosis. In the presence of other autoantibodies, anti-ribosomal P may add valuable information for fine-tuning the disease phenotype. Of interest, a clue for the presence of anti-ribosomal P antibodies is the dense fine speckled cytoplasmic pattern (AC-19) on the HEp-2 IFA test, especially if associated with weak nucleolar staining [10, 11]. Nonetheless, it should be noted that up to 40% of samples with anti-ribosomal P antibodies yield no relevant HEp-2 IFA reactivity [12].

Antiphospholipid Antibodies

Antiphospholipid antibodies (aPL) (lupus anticoagulant; anti-cardiolipin; anti- β 2 glycoprotein-1) can be found in 20 to 40% of SLE patients and 10 to 20% of these will present manifestations of the antiphospholipid syndrome (APS), including vascular thrombosis (arterial or venous) and gestational morbidity. Apart from the standard APS manifestations, aPL are associated with a worse prognosis in SLE as they are also associated with some neuropsychiatric SLE manifestations (seizures, cerebrovascular disease, cognitive deficit, and myelitis), renal involvement (APS nephropathy) [44], and thrombocytopenia [45]. Thus, given their frequency and connection with potential morbidity, all SLE patients should be screened for these antibodies [17].

Complement System Interface with Autoantibodies

Complement system assessment (C3, C4, CH50) is useful for monitoring lupus disease activity [17]. In more than 50% of cases, there is evidence of complement consumption in the active phase of the disease, frequently associated with an increase in anti-dsDNA titers. Furthermore, deficiency of early components of the classical complement pathway is associated with an increased risk of SLE and related syndromes. The combination of normal C3 levels and decreased hemolytic complement (CH50) should raise the possibility of genetic complement deficiency and determination of C1q, C2, and C4 serum levels is recommended [17]. The autoantibody profile presents some peculiarities in SLE associated with inherited complement deficiency. C1q deficiency is extremely rare and is strongly associated with SLE and recurrent infections. C1q-deficient SLE presents as early-onset and severe disease with frequent skin and kidney involvement, high frequency of anti-SS-A/Ro60, and positive HEp-2 IFA, but rarely have anti-dsDNA antibodies [46]. C2 deficiency is

the most frequent inherited complement deficiency and up to 30% of the C2-deficient patients develop SLE or lupus-like disease. C2-deficient SLE patients tend to present a milder disease (predominately articular and cutaneous manifestations) and have a high frequency of anti-SS-A/Ro60 antibodies [47]. Complete C4 deficiency is very rare, but 75% of such individuals develop SLE. Low gene copy number for C4 is not so rare and individuals with low copy numbers for C4A are at higher risk of SLE. C4-deficient SLE patients have a high frequency of nephritis and anti-SS-A/Ro60 antibodies, but a low frequency of anti-dsDNA antibodies [48, 49].

Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) was first described in 1983 as a condition characterized by thrombosis and recurrent miscarriage possibly associated with the presence of antiphospholipid (aPL) antibodies [50]. APS-associated autoantibodies recognize predominantly protein-phospholipid complexes. In the early 1990s, β 2-glycoprotein I (β 2GPI) was identified as an essential component in the so-called anticardiolipin (aCL) antibody assay and as a genuine target by itself of APS autoantibodies [51]. This finding allowed discrimination between β 2GPI-dependent aCL, which correlates with thrombosis and fetal loss, and β 2GPI-independent aCL, which is non-specific [52].

APS is a systemic autoimmune disease, with thrombotic and inflammatory mechanisms orchestrated by aPL antibodies, and presents with a variety of clinical manifestations, including venous/arterial thrombosis and pregnancy morbidity as well as "non-classical" manifestations, such as thrombocytopenia, nephropathy, seizures, dementia, valvular heart disease, and others. APS can be classified as primary or secondary when it is associated with another autoimmune disease, most frequently SLE [53]. The 2006 Sydney APS classification criteria involve at least one clinical and one laboratory criterion (Table 6) [53]. An updated set of classification APS criteria is under consideration [54].

The aPL antibody profile is an important factor in determining the risk of thrombotic and obstetric events. Interestingly, the relationship between the presence of aPL antibodies and thrombosis is not an "all or nothing" phenomenon and is related to the type, titer, and persistence of the antibody, number of different antibodies, the coexistence of other thrombosis risk factors, and the presence of associated autoimmune diseases [55]. In addition to the aPL antibodies belonging to the APS criteria, other antibodies have been implicated in APS such as IgA aCL, IgA anti- β 2GPI, anti-phosphatidylserine/prothrombin (aPS/

Table 6 Classification criteria for antiphospholipid syndrome (Sydney, 2006)***Clinical criteria****Vascular thrombosis**

– One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by objective validated criteria. For histopathological confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall

Pregnancy morbidity

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus
- One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia or severe pre-eclampsia defined according to standard definitions or recognized features of placental insufficiency
- Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomical or hormonal abnormalities and paternal and maternal chromosomal causes excluded

Laboratory criteria

- Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart
- Anticardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (> 40 GPL or MPL or > 99th percentile), on two or more occasions, at least 12 weeks apart
- Anti- β 2-glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma (in titer > 99th percentile), present on two or more occasions, at least 12 weeks apart

Adapted from Miyakis et al. [53]

*At least one clinical and one laboratory criteria are necessary for classification

PT) complex, anti-domain I β 2GPI, anti-prothrombin, anti-vimentin, anti-annexin, anti-phosphatidylserine, and anti-phosphatidylethanolamine [56].

Lupus Anticoagulant (LAC) Antibody

In 1952, Conley and Hartmann were the first to identify a circulating factor that prolonged blood-clotting time in two SLE patients; aptly named lupus anticoagulant (LAC). In a retrospective analysis, it is noteworthy that these two patients also had a false-positive test for syphilis, later recognized as a clue for APS. In 1963, it was noticed that LAC was unexpectedly associated with *in vivo* thrombotic manifestations and eventually shown to be associated with pregnancy morbidity [57]. In APS patients, LAC is the autoantibody most frequently associated with thrombotic and obstetric events and its presence, *per se*, classifies patients as at high risk for thrombotic events and pregnancy morbidity. A meta-analysis with LAC-positive patients found the odds ratio (OR) to be 6.14 (95% CI 2.7–13.8) for venous thrombosis and 3.6 (95% CI 2.7–13.8) for arterial thrombosis [58].

The methodology of LAC detection is based on a three-step procedure including a screening step, a mixing step, and a confirmation step. Strict adherence to the method and appropriate pre-analytical precautions in obtaining and handling the blood sample are essential for reliable results. Of clinical importance and a limitation of the assay, patients must not be on anticoagulation therapy as this and other factors such as C-reactive protein can be associated with false-positive results [59].

Anticardiolipin Antibodies (aCL)

aCL antibodies are directed against the complex of β 2GPI and cardiolipin, a phospholipid found almost exclusively in the mitochondrial membrane and cell walls of bacteria. aCL is associated with an increased risk of thrombosis when present in moderate or high titer (> 40 GPL/MPL or > 99th percentile, measured by a standardized ELISA). A meta-analysis found that the presence of aCL has an OR of 1.46 (95% CI 1.06–2.03) for venous thrombosis and 2.65 (95% CI 1.75–4.00) for arterial thrombosis [58].

IgG and IgM are the predominant aCL isotypes. The frequency of aCL IgA antibodies in APS is variable and their clinical significance is still uncertain. Although some studies suggest a pathophysiological role for IgA aCL, its presence does not seem to lead to clinical manifestations in a significant number of patients. Furthermore, the prevalence of aCL IgA antibodies is very low in APS patients lacking IgG and IgM aCL, which restricts substantially their diagnostic value [60]. aPL antibodies must always be interpreted taking into account the clinical context, as aCL and anti- β 2GPI antibodies, especially IgM isotype, can also be found as epiphenomena in infectious diseases without correlation with thrombotic events.

Anti-Beta 2-Glycoprotein I (β 2GPI) Antibodies

Anti- β 2GPI antibodies target the multifunctional plasma protein Beta-2-Glycoprotein I, which undergoes a conformational change in its structure upon binding to cardiolipin.

The β 2GPI/cardiolipin complex increases the expression of tissue factors and leads to activation of endothelial cells, monocytes, neutrophils, fibroblasts, and trophoblasts, which results in a pro-coagulant state. Anti- β 2GPI antibodies of IgG and/or IgM isotypes at titer greater than the 99th percentile, measured by a standardized ELISA, are clinically relevant [53].

Unlike aCL IgA antibodies, IgA anti- β 2GPI antibodies are reported to be related to thrombotic phenomena. A retrospective study in 2013 suggested that IgA anti- β 2GPI may be useful in identifying patients with APS negative for IgG and IgM anti- β 2GPI [61]. In fact, in three different cohorts, it was observed that a significant portion of patients with clinical manifestations of APS was positive only for IgA anti- β 2GPI [60]. In kidney transplant patients, IgA anti- β 2GPI confers a high risk for early graft loss caused by thrombosis and a high risk of delayed graft dysfunction [62]. However, more studies are necessary to understand the precise pathogenic action of this antibody and to standardize currently available commercial laboratory tests.

Phosphatidylserine/Prothrombin Complex (aPS/PT) Antibodies

A meta-analysis that included more than 7000 patients and controls found that aPS/PT seemed to represent a stronger risk factor for arterial (OR 5.11; 95% CI 4.2–6.3) and venous (OR 1.82; 95% CI 1.44–2.75) thrombosis than antibodies to isolated prothrombin [63]. In addition, a strong association between aPS/PT IgG and LAC has been reported [64]. Despite not being included in the current APS classification criteria, this antibody has been incorporated into the two scores currently available to evaluate thrombosis risk in APS patients: the Antiphospholipid Score (aPL-S) [65] and the Global Antiphospholipid Syndrome Score (GAPSS) [66]. aPS/PT antibodies may be especially useful in cases with high suspicion of APS and negative results for “classic (or criteria) aPL” antibodies or in patients in whom the LAC test is unreliable due to anticoagulation therapy.

Anti-Domain I β 2GPI (anti-DI β 2GPI) Antibody

β 2GPI is a plasma glycoprotein composed of five highly homologous regions called domains I to V, from the N terminus to the C terminus. When in solution, β 2GPI presents a circular conformation, but its structure becomes linear and elongated by binding to phospholipids and the point of attachment with the phospholipid corresponds to a hydrophobic loop surrounded by positively charged amino acids located in domain V [52]. Domain I is hidden in the circular conformation of β 2GPI but becomes exposed upon elongation promoted by binding to phospholipids. Currently, antibodies against β 2GPI are understood as a heterogeneous

family of antibodies, which recognize different domains of the glycoprotein. Some studies suggest that domain 1 anti- β 2GPI (DI β 2GPI) antibodies are the most pathogenic in APS and have a higher association with thrombotic events than antibodies to the intact anti- β 2GPI where antibodies may bind to the other domains [60]. A systematic review that included 1585 patients confirmed a strong association of anti-DI β 2GPI antibody with thrombotic events [67]. Anti-DI β 2GPI antibody is more frequently detected in triple aPL-positive patients, correlates with medium–high titer aPL, and is associated with clinical manifestations such as thrombosis and pregnancy morbidity [68]. It has been shown recently that the combination of anti-DI β 2GPI and IgG/IgM aPS/PT antibodies confers a high positive predictive value for APS diagnosis and high risk for thrombosis [69].

APS Risk Profile Assessment

One of the objectives of PM is to offer the possibility of risk stratification using easily accessible and reproducible biomarkers. The determination of LAC, aCL, and anti- β 2GPI antibodies allows stratifying patients at low and high risk for APS-related events (Table 7). The presence of LAC has been associated with a higher risk for thromboembolic events (OR 4.4; 95% CI 1.5–13.3) when compared to aCL or anti- β 2GPI. On the other hand, triple positivity for these autoantibodies confers the highest risk for thrombotic events (OR 33.3; 95% CI 7.0–157.6) [70] as well as a worse prognosis for obstetric morbidity [71].

Scores that include the presence of aPL antibodies have been developed for assessing the diagnosis of APS and thrombosis risk (aPL-S) [65] and the risk for thrombosis and pregnancy loss, taking into account the aPL profile, traditional cardiovascular risk factors, and the autoimmune antibody profile (Global Anti-Phospholipid Syndrome Score—GAPSS) [66]. GAPSS includes hyperlipidemia (3 points),

Table 7 High-risk and low-risk profiles in APS patients according to anti-phospholipid antibodies

High risk

Presence in two or more occasions at least 12 weeks apart:

- Lupus anticoagulant (measured according to ISTH Guidelines)
- Double positive: lupus anticoagulant + aCL or β 2GPI
- Triple positive: lupus anticoagulant + aCL + β 2GPI
- Persistence high aPL titers

Low risk

Isolated aCL or anti- β 2GPI at low or moderate titers, especially if transient

Adapted from Tektonidou et al. [44]

aCL anticardiolipin antibodies, β 2GPI anti-Beta2-glycoprotein I, aPL antiphospholipid antibodies

arterial hypertension (1 point), aCL IgG/IgM (5 points), LAC (4 points), and aPS/PT IgG/IgM (3 points). Scores of 10, 12, and 15 had a sensitivity of 0.709, 0.578, and 0.378, respectively, and a specificity of 0.793, 0.817, and 0.950, respectively, for thrombotic events. The Adjusted GAPSS (aGAPSS) excludes aPS/PT antibodies from the scoring algorithm, recognizing that these antibodies are currently not included in the APS criteria classification and are not assayed routinely [72].

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a promising clinical scenario where PM plays a rapidly emerging significant role. The prevalence of RA ranges from 0.3 to 1% of the adult population with a bimodal peak of incidence affecting predominantly women between 40 and 70 years of age [73, 74]. Recent studies reveal that during a 10-year course of RA about half of the patients cannot maintain a full-time job, confirming that pain, stiffness, articular edema, and joint destruction significantly limit work efficiency in RA patients [73]. Growing evidence has demonstrated that the discrete mechanisms that operate across the RA pathophysiological continuum are suitable to therapeutic interventions that might abrogate or even prevent RA [75]. A better understanding of the mechanisms and biological interactions addressing genetic factors, environmental contributors, neo-antigen generation, innate and adaptive immune cell responses, autoantibodies, and cytokines has been crucial for the advancement in RA treatment [76]. Individual-specific interventions should be feasible and effective for preventive strategy in high-risk individuals and treatment in patients with established disease.

RA presents a heterogeneous disease phenotype and considerable variability is observed in terms of individual patient progression from pre-clinical stages to established RA, disease severity, and treatment response. Over the natural history of RA, including loss of self-tolerance at pre-clinical stages, different triggers and mechanisms come into play at different time frames depending on the patient. This represents an opportunity to individualize RA management and ultimately put it forward as a genuine application of PM [77]. In that sense, clinical and biological markers might serve as a research and clinical diagnostic and prognostic tools, contributing to therapeutic precision in RA. Such an approach would address the challenge and unmet need of early identification and diagnosis of individuals at high risk or with established early RA, classification of disease severity, and choice of the right pharmacological intervention at the individual level [78]. In other words, PM in RA might allow the clinicians to better diagnose (RA vs. non-RA), to understand the prognosis of the disease (progressor vs. non-progressor; responder vs. non-responder), and to search

for clinical or biological markers to guide target treatment selection in an individual patient with uncontrolled disease.

RA encompasses a temporal disease spectrum in which genetically predisposed individuals under the influence of the appropriate environmental factors evolve through successive stages, with an initial preclinical asymptomatic phase when the only evidence is the presence of RA-associated autoantibodies [79, 80]. Gradually some of these individuals develop non-specific clinical manifestations, including malaise, weight loss, poor appetite, and arthralgia. Eventually, patients will evolve with initial synovitis (early RA) and further progress to full-blown RA [81].

RA etiopathogenesis has been linked to multiple mechanisms involving both innate and adaptive immune responses. Genetically predisposed individuals can recognize and mount an adaptive immune response to post-translational modifications (PTM) of autoantigens. This phenomenon occurs mainly in the mucosa of the lungs and the gut, where smoking and the microbiota, respectively, promote PTM of several self-constituents by diverse biochemical mechanisms including citrullination, acetylation, carbamylation, and malondialdehyde acetaldehyde [82–88]. Once the neo-antigens are presented by dendritic cells and macrophages to T cells, activation of the adaptive immune response in lymphoid tissues takes place leading to the initiation of the autoimmune reactivity characteristic of RA. Activation of B cells follows in the process with autoantibody formation, mainly rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA). Clonal activation and expansion of Th1 and B cells further stimulate stromal cells in the synovial layer (such as fibroblast-like synoviocytes, dendritic cells, and macrophages) that will then produce a range of cytokines and inflammatory factors that perpetuate the inflammatory milieu and ultimately lead to cartilage degradation and osteoclast engagement in subchondral bone destruction [83, 89]. The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) established classification criteria for RA based on the presence of a combination of tender and swollen joints, duration of symptoms, acute-phase reactants, and autoantibodies, including ACPA and RF. Despite different biologic bases and predictive abilities, ACPA and RF carry the same weight in the classification criteria [90], while the marked difference in the predictive ability for ACPA titer (low versus high) is somewhat neglected [91]. A meta-analysis on RF and anti-CCP2 showed a sensitivity of 69% and 67% and a specificity of 85% and 95%, respectively, in the discrimination of RA patients and healthy individuals [92]. Up to 80% of RA patients present detectable autoantibodies [93], the presence of which identifies a subgroup of RA patients that are more homogenous regarding risk factors and clinical disease course (Table 8). However, the field needs further progress toward better disease prediction and prevention, accurate diagnosis, prognosis, and individualized treatment.

Table 8 Autoantibodies in rheumatoid arthritis, analytical performance, and main characteristics

Autoantibody	Sensitivity (%)	Specificity (%)	Characteristics
RF	50–70	50–95	Most well-known RA antibody; associated with erosive disease
ACPA	60–70	95	Higher specificity for RA than RF; associated with erosive disease
Anti-CarP	44	89	Lower sensitivity than RF and ACPA; present in a fraction of RF/ACPA-negative patients; associated with radiographic progression
Anti-PAD4	38	96	Associated with radiographic progression and severe forms of RA

RF rheumatoid factor, RA rheumatoid arthritis, ACPA anti-citrullinated peptide antibody, CarP carbamylated peptides, PAD4 peptidyl arginine deaminase 4

Rheumatoid Factor

RF, an autoantibody directed against the Fc part of aggregated human IgG, was the first autoantibody system to be described in RA. RF has a sensitivity ranging from 60 to 90% and a specificity ranging from 48 to 92%, according to different studies (reviewed in 88). Particularly at low titer, RF occurs in variable frequency in other systemic autoimmune diseases, chronic infections, and cancer. Healthy individuals usually present low-titer and low-affinity poly-reactive RF, with increasing frequency in older individuals. RF may occur as an IgM, IgA, or IgG isotype and the IgM RF is most commonly tested in the clinical laboratory. The coexistence of IgM and IgA RF has higher specificity of the diagnosis of RA, especially at moderate to high titer.

Anti-Citrullinated Peptide Antibodies

Although the definitive antigenic characterization was achieved in 1998, the first reports on this class of autoantibodies date decades before, with the anti-perinuclear factor reported by IFA on oral mucosa cells in 1964 and the anti-keratin antibodies reported by IFA on rat esophagus in 1989 [94]. Citrullination is a process by which the enzyme peptidyldeaminase converts histidine into citrulline in the presence of Ca⁺⁺. Citrullination is particularly evident in inflammation, apoptosis, and keratinization. Different citrullinated peptides can be recognized by ACPA from different patients, including citrullinated self-proteins, such as vimentin, fibrinogen, filaggrin, fibronectin, and α -enolase (reviewed in 90). ACPA occurs in circa 70% of RA patients and has a specificity of 98%. With higher specificity for RA, ACPA were included in the 2010 ACR-EULAR classification criteria for RA [91].

Patients with ACPA and/or RF show a clear association with HLA-DRB1 alleles containing the shared epitope (HLA-DRB1-SE). As discussed ahead, ACPA/RF-positive RA is associated with increased radiographic progression and joint damage, especially in the case of multiple autoantibodies [95–97]. Despite being instrumental in the diagnosis

and classification of RA, there are still significant limitations and challenges in the use of ACPA and RF in clinical practice. There is significant variability in the performance characteristics of the available RF and ACPA immunoassays and that impacts RA classification and further management [91].

Antibodies to Post-Translational Modifications of Autoantigens

Proteins are sequences of amino acids encoded by specific genes; however, a substantial proportion of proteins suffer chemical modifications after translation at the rough endoplasmic reticulum. Frequent post-translational modifications (PTM) are glycosylation, citrullination, methylation, acetylation, and ubiquitination of specific amino acids. PTM has relevant structural and functional consequences, allowing plasticity and functional diversity. Of special interest, PTM can have also immunogenic consequences, as autoantibodies recognizing specifically peptides with a modified amino acid are observed in RA. ACPA were the first autoantibodies against PTM proteins. Other RA-associated autoantibodies against proteins with PTM, such as carbamylation and acetylation, have been identified [88, 98]. These novel autoantibodies have been investigated in many studies aiming to discover and validate novel markers for diagnosis, disease activity assessment, and prognosis in RA. Only a few of these novel markers have come into clinical use.

How Autoantibodies Can Contribute to PM in Rheumatoid Arthritis

Multiple parameters contribute to estimating the potential disease severity and therapeutic requirements of patients with RA, including female gender, the clinical level of disease activity at presentation (joint count, extra-articular manifestations), acute-phase tests (C-reactive protein, erythrocyte sedimentation rate), and early imaging findings [99]. Autoantibodies (RF, ACPA, anti-carbamylated peptide) are among the variables associated with more aggressive disease [86, 100–102]. ACPA and RF have been demonstrated to

be associated also with extra-articular manifestations, cardiovascular disease, and premature mortality in RA [103]. The genetic make-up also contributes to the severity of the disease. HLA-DRB1-SE alleles are associated with more severe disease and extra-articular manifestations, especially when present in both chromosomes [104]. In a more refined phenotypic analysis, it has been demonstrated that the coexistence of ACPA, RF, and HLA-DRB1-SE alleles is associated with the predominance of erosions over joint narrowing in RA patients [105]. Polymorphism in other genes, such as FOXO3, IL2RA, DKK1, GRZB, MMP9, and SPAG16, is associated with more severe disease [106–108]. Finally, the time to the introduction of disease-modifying therapy is an adjustable variable that also can affect the prognosis.

Although RA is a potentially severe and crippling disease, very early treatment has proven successful in avoiding an unfavorable evolution. It is now established that there is a window of opportunity for optimal therapeutic intervention at the earliest stages of the disease [109]. It is relevant that RF and ACPA are detectable in the serum of individuals for several years before the clinical onset of the disease [110, 111]. Two-thirds of individuals ultimately diagnosed with RA were positive for ACPA 6 to 10 years before their diagnosis [112]. Therefore, these autoantibodies have been instrumental in the very early identification of arthralgia patients that are bound to develop RA. The presence of such autoantibodies might enable the identification of people at increased risk of developing RA as well as patients at early stages of the disease, contributing to the timely treatment of patients within the RA period. However, the positive predictive value is moderate (~70%) and about 2–5% of the healthy population have ACPA. In addition, less than 50% of arthralgia patients with ACPA and/or RF develop RA within 1 year [113, 114]. Thus, the post-test probability for ACPA in RA has been estimated at only 50%, although it might be increased with very high titers of ACPA or by combining ACPA with other biomarkers [74]. Autoantibodies against an array of PTM proteins may contribute to increasing the accuracy in spotting very early RA patients. A recent meta-analysis investigating healthy controls, RA first degree relatives, pre-RA individuals, and RA patients indicated that the positivity for RF and/or ACPA yields a specificity and sensitivity of 65–100% and 59–88%, respectively, for the correct identification of RA and pre-RA patients, whereas the triple positivity of RF, ACPA, and anti-carbamylated peptide (CarP) antibody yielded a specificity of 98–100% and a sensitivity of 11–39% [115].

The conjugation of information on the genetic make-up should strengthen the prediction capability for the identification of very early RA, especially when analyzing arthralgia patients. In addition to the HLA-DRB1-SE alleles, over 100

RA susceptibility loci have been identified. Although most of these alleles have a weak association with RA, the cumulative effect of several of them under the appropriate environmental stimuli may result in a strong trend for RA development [74]. Interestingly, some susceptibility alleles (e.g., AFF3, CD28, and TNFAIP3) are associated with ACPA-positive RA whereas others (e.g., PRL and NFIA) occur in ACPA-negative RA [116, 117]. The interaction of genetic background, environmental agents, and autoantibodies can be relevant to the personalized management of individuals at risk for RA development. This is well illustrated by the observation that the association between tobacco exposure and RA development is strongest in ACPA-positive individuals with at least one copy HLA-DRB1-SE allele [103, 118]. It should be noted that not all autoantibodies against PTM show association in terms of relationship to tobacco exposure and HLA-DRB1 gene susceptibility [84]. This information may be relevant when recommending absolute smoking cessation in a particular individual. In addition to genetic and autoantibody parameters, inflammatory biomarkers may also contribute to PM in RA. For example, in conjugation with other biomarkers, calprotectin has been reported to have a role in the early diagnosis of RA [119, 120], as well as in predicting relapse after therapy discontinuation [121].

Finally, autoantibodies can contribute also to the selection of the appropriate therapeutic agent to be used in an individual patient. By identifying patients at risk of more severe disease, RF and ACPA help segregate potential candidates for the use of biological DMARD. In addition, the presence of RF and/or ACPA indicates a higher chance of response to some specific targeted-therapy agents. Thus, a recent pooled analysis from 16 European RA registries found that RA patients with RF and/or ACPA have a higher frequency of sustained remission under rituximab (5.9%; 95% CI 4.7–7.3) and abatacept (1.5%; 95% CI 1.1–1.9) than double-negative RA patients. Seropositive patients also had a slightly better response with tocilizumab compared to seronegative patients and no difference was observed regarding TNF inhibitors [122]. Tofacitinib, an oral Janus kinase (JAK) inhibitor approved for RA treatment, has been also found to be more effective in seropositive patients. Pooled data from five phase-III studies showed that a higher proportion of double-positive (ACPA and RF) RA patients achieved a favorable response to JAK-inhibitors than double-negative patients did [123]. Autoantibodies may also help in the decision of discontinuation of biological DMARD in RA, as observed in a systematic review that indicated that low levels or absent ACPA and RF predict successful discontinuation of therapy [124]. These observations indicate the potential of autoantibodies to help tailor the therapeutic strategy in RA.

Primary Sjögren's Syndrome

Primary Sjögren's syndrome (pSjS) is a systemic inflammatory disease characterized by lymphocytic infiltrates in exocrine glands, mainly salivary and lacrimal glands, with ensuing dryness of affected areas clinically manifested most often as xerostomia and keratoconjunctivitis sicca (sicca syndrome). In addition, there is a wide range of extra glandular manifestations, including interstitial nephritis, small vessel vasculitis with palpable purpura, peripheral neuropathy, lymphadenopathy, and splenomegaly [125]. pSjS affects predominantly middle-aged women with an estimated incidence of 6.92 per 100,000 person-years and prevalence of 60.8 cases per 100,000 inhabitants [126]. The clinical spectrum is heterogeneous, varying from mild benign sicca syndrome to heterogeneous degrees of lymphoid infiltration in different tissues/organs and evolving to lymphomatous lesions (non-Hodgkin lymphoma) in some patients [127].

The pathogenesis of pSjS is characterized by B-cell hyperactivation, which is the basis for the clinical and laboratory manifestations, such as hypergammaglobulinemia, high titer RF, lymph node enlargement, and lymphoid infiltration in several tissues and organs [128]. Ocular and oral mucosal dryness are characteristic phenotypic features in pSjS. However, sicca syndrome (i.e., oral and ocular mucosal dryness) and Sjögren's syndrome are not synonymous, and most elderly adults with sicca syndrome do not have Sjögren's syndrome. In Sjögren's syndrome, there is autoimmune-based lymphocytic infiltrate and inflammation of the lacrimal and salivary glands, resulting in impaired tear and saliva production [125]. In contrast, several distinct mechanisms may lead to non-inflammatory sicca syndrome, including age-related atrophy and drug-related gland dysfunction. The correct identification of the basis for the sicca syndrome is very important since the identification of those patients with an autoimmune basis for their sicca manifestations is the first step in deciding whether therapies directed at the immune system might be beneficial and whether close monitoring for the appearance of lymphoma should be undertaken. In contrast, such therapies and their attendant risks should be avoided in patients with non-autoimmune sicca syndrome. Autoantibodies play a significant role in that matter.

The complexity and heterogeneity of pSjS, with its wide spectrum of glandular and extra glandular features, indicate the need for biomarkers for better discrimination and contribution to PM [129]. Autoantibodies contribute to the early diagnosis and stratification of patients according to the risk for systemic complications and development of lymphoma, thus contributing to individualized surveillance of pertinent complications and custom-made treatment of the patients.

Immunological patterns defined by serology and other biomarkers contribute to predicting the phenotypic expression of the disease already at the time of diagnosis and may guide physicians to design specific personalized management during the follow-up of patients with pSjS [130, 131].

ANA, RF, anti-SS-A/Ro60, and anti-SS-B/La autoantibodies are key serological findings in pSjS. Autoantibodies to the Sjögren-related antigen A (also known as anti-SS-A/Ro60 antibodies) and the Sjögren-related antigen B (also known as anti-SS-B/La antibodies) are central for the pathobiology and diagnosis of this disease. Together with the development of sicca symptoms, systemic involvement, lymphocytic infiltration to exocrine glands, and the increased risk of lymphoma, these autoantibodies are pivotal elements in this disease [129]. As in other autoimmune diseases, the presence of autoantibodies can precede pSjS development for years. In seropositive pSjS patients, at least one autoantibody specificity (ANA, RF, anti-SS-A/Ro60, or anti-SS-B/La) was detected in 81% of these patients up to 20 years (median 4.3–5.1 years) before diagnosis [132].

Antinuclear Antibodies and Rheumatoid Factor

ANA are found in over 80% of patients with pSjS and have been proven very helpful in identifying the disease in patients presenting with sicca features. ANA frequently occur at moderate to high titer and typically present as a fine speckled nuclear pattern (AC-4 according to ICAP nomenclature), which is frequently associated with anti-SS-A/Ro60 and anti-SS-B/La [12]. In addition, some pSjS patients may present a peculiar nuclear and mitotic apparatus pattern (AC-26) characteristic of anti-NuMA antibodies. Anti-NuMA is also observed in SLE and other diseases but occurs at a higher frequency in pSjS [133]. RF is an autoantibody directed against the Fc region of IgG, being present at high titer in 50% of patients with pSjS and bearing association with several clinical, histopathological, and laboratory features [129]. Despite being helpful for diagnostic assessment, ANA and RF are not included in the current classification criteria for pSjS [134], as they are common in a range of autoimmune diseases and present low specificity for pSjS.

Anti-SS-A/Ro and Anti-SS-B/La Antibodies

Anti-SS-A/Ro antibodies are detected in up to 70% of patients with pSjS and have been detected several years before the diagnosis of pSjS [132]. The SS-A/Ro system includes two distinct and independent proteins of 60 and 52 kDa, respectively, although the original description probably referred only to the 60-kDa component [135]. Ro60 is a component of small cytoplasmic ribonucleoprotein complexes (hY-RNA) and binds misfolded noncoding

RNA [136]. Ro52, also known as TRIM21, belongs to the Tripartite Motif Protein (TRIM) family, being involved in protein ubiquitination [137]. Antibodies to Ro52 and Ro60 may coexist in the same patient but frequently occur as independent autoantibodies. It should be noted that anti-Ro52 is best detected in immunoblot/immunodot and ELISA assays because it is not detected by HEp-2 IFA, immunoprecipitation, and/or double immunodiffusion [138]. It has been demonstrated that anti-Ro52 and anti-Ro60 antibodies have different clinical associations; however, some controversy exists probably as a result of different immunoassays used in the studies. Anti-Ro52 antibodies are preferentially detected in specific anti-Ro52 immunoassays and not detected in “general” anti-SS-A/Ro immunoassays [139]. The combination of Ro52 and Ro60 in some immunoassays represents a major confounding factor in the literature. Although most frequently observed in autoimmune patients, the exclusive presence of anti-Ro52 (absence of anti-Ro60) frequently occurs also in non-autoimmune patients. In addition, anti-Ro52 occurs in a wider spectrum of autoimmune patients as compared to anti-Ro60 antibodies [140]. Patients with anti-Ro52 and anti-Ro60 have a higher probability of presenting anti-SS-B/La compared to those with just one anti-SS-A/Ro component. The “triad” (anti-Ro-60, anti-Ro-52, and anti-SS-B/La) is strongly associated with pSjS and indicates more severe pSjS with higher severity of salivary gland lymphocytic infiltration, parotid enlargement, and hypergammaglobulinemia [140, 141]. In addition, anti-Ro52 antibodies have been associated with an increased risk of interstitial lung disease in pSjS patients [142]. In general, the presence of anti-SS-A/Ro (with or without anti-SS-B/La) is associated with more severe disease, characterized by systemic manifestations and a higher frequency of non-Hodgkin lymphoma. In a large series of 548 pSjS patients with documented salivary gland histopathological pSjS-specific alterations, Quartuccio et al. showed that the 342 patients with antibodies to SS-A/Ro and/or SS-B/La had a higher frequency of salivary glandular enlargement, purpura, leukopenia, decreased serum C3 and C4, hypergammaglobulinemia, ANA, RF, serum cryoglobulins and lymphoma as compared to 206 patients without anti-SS-A/Ro and anti-SS-B/La [143].

SS-B/La is a 48 kDa phosphoprotein involved in RNA metabolism and anti-SS-B/La antibodies are detected in up to 50% of patients with pSjS. The presence of anti-SS-B/La, especially when associated with anti-SS-A/Ro, indicates a less benign disease phenotype, with a higher prevalence of lymphoproliferative manifestations and a higher risk of evolution to lymphoma [144]. The presence of anti-SS-B/La antibodies without concomitant anti-SS-A/Ro antibodies is rather infrequent and usually marks less severe disease with fewer clinical and immunological features of the syndrome

and with a relatively low frequency of severe organ-specific involvement [145, 146].

A small percentage of pSjS patients present ACPA and anti-centromere antibodies. Anti-centromere antibodies were associated with older age, more severe salivary gland dysfunction, lower frequency of anti-SS-A/Ro, anti-SS-B/La, and RF, as well as lower serum immunoglobulin. These patients have a higher frequency of Raynaud’s phenomenon, sclerodactyly, and dilated nail fold capillaries [147]. The presence of ACPA in pSjS patients is associated with a higher frequency of arthralgia and progression to RA [148].

Cryoglobulins

Also seen in pSjS patients, cryoglobulins are immunoglobulins that precipitate *in vitro* at temperatures below 37 °C and become soluble after rewarming. Circulating cryoglobulins are present in about 10% of the patients with pSjS and are associated with more severe disease [149]. Since mixed cryoglobulinemia is traditionally regarded as a crossroads between autoimmune disease and cancer [150], closer monitoring should be done in the presence of this immunological marker. Cryoglobulins in pSjS patients are associated with a higher frequency of systemic disease with extra glandular involvement, increased risk of B cell lymphoma, and higher mortality [151, 152]. Lower levels of complement C4, higher levels of RF, and serum monoclonal IgM gammopathy are associated with cryoglobulinemia in pSjS patients [149].

Low Complement Levels

The complement cascade and regulatory proteins are involved in the pathogenesis of the SjS and other autoimmune diseases [153]. Found in 10–25% of patients with pSjS, low levels of complement (C3 and/or C4) have been associated with more severe disease and poor prognosis. A recent multi-ethnic international cohort comprising 10,500 pSjS patients from 22 countries has found a prevalence of low C3 and C4 levels of 13.4% and 14.5%, respectively [130]. Besides cryoglobulin, low C3 and low C4 levels significantly correlated with systemic activity [130]. Like cryoglobulin, hypocomplementemia has been associated with a higher risk for B cell lymphoma progression and death [151]. Type II mixed cryoglobulins with RF activity and hypocomplementemia are the strongest and most validated predictors of lymphoma in pSjS [152, 154].

Monoclonal Gammopathy

Monoclonal gammopathy related to pSjS has been recognized as a key marker of disease prognosis and outcome. Circulating monoclonal immunoglobulins have been found

in up to 22% of patients with pSjS, with monoclonal IgGκ being the most frequent band type [151, 155]. Parotid enlargement, vasculitis, neurological involvement, higher frequency of progression to lymphoma, and lower survival have all been associated with monoclonal gammopathy in pSjS patients [155].

Systemic Sclerosis

Systemic sclerosis (SSc) is a chronic autoimmune rheumatic disease characterized by a triad of abnormalities including vasculopathy, fibrosis of the skin and internal organs, and immune dysregulation [156]. The reported incidence and prevalence of SSc vary widely depending on geographic location and methods used in each study. The prevalence of SSc varies from 7 to 489 cases per million and the annual incidence from 0.6 to 122 cases per million inhabitants [157]. SSc is more frequent in women with a female to male ratio of 4:1 to 6:1 and a peak incidence between ages of 45 and 64 years.

The clinical manifestations of the disease are highly heterogeneous, although a common feature heralding the onset of the disease is Raynaud's phenomenon. Once the diagnosis has been made, classification into disease subsets, as well as stratification of risk of future organ involvement and other prognostic features are extremely important in clinical practice [158]. Patients with SSc are classified based on the extent of skin involvement: (1) diffuse cutaneous SSc (dcSSc) with skin involvement proximal to the elbows and knees and/or truncal involvement; (2) limited cutaneous SSc (lcSSc) with restricted involvement affecting the hands and limbs below the elbows or knees, with or without face and neck involvement. In general, these two subsets have distinct

clinical courses as well as organ involvement and specific serum autoantibody profiles, with a more severe prognosis in patients with dcSSc [158, 159]. A small number of patients have clinical features of SSc but no evidence of cutaneous involvement (SSc sine scleroderma).

A hallmark of SSc is the presence of circulating autoantibodies reactive with various cellular components in over 95% of the patients. Anti-topoisomerase I, anti-centromere (ACA), and RNA polymerase III (RNAP) represent the three most frequently detected autoantibodies in SSc and are considered highly specific for this disease [160]. Other SSc-specific autoantibodies include anti-U3-RNP/fibrillarin, anti-Th/To, anti-RNA-polymerase I and II, and anti-U11/U12 RNP [161]. Some autoantibodies, including anti-PM/Scl, anti-Ku, anti-U1-RNP, anti-SS-A/Ro60, anti-Ro52/TRIM21, and anti-NOR 90, reported in other autoimmune diseases, are also found in SSc patients [160]. The most frequent antibodies in SSc and their clinical association are depicted in Table 9. As autoantibodies may precede the onset of symptoms, they are helpful for the early diagnosis of the disease [162]. In addition to contributing to the diagnosis, autoantibodies are associated with distinctive clinical manifestations and prognostic profiles in SSc. Therefore, they are attractive biomarkers for SSc and might help identify and predict the clinical outcome of SSc patients [163].

Anti-Topoisomerase I

Anti-topoisomerase I (anti-Topo I or anti-Scl-70) was originally isolated as an antibody that reacted with a 70 k-Da protein in immunoblot, in retrospect realized to be a degradation product of the full-length protein that was later identified as the 100-kDa DNA topoisomerase I, an enzyme that catalyzes relaxation of supercoiled double-stranded DNA [159]. Anti-topo I antibodies are detected in 17–41% of patients and are

Table 9 Phenotypic associations of autoantibodies in systemic sclerosis

Autoantibody target	Disease phenotype and associations	Sensitivity/specificity (%)
Centromere (CENP-A/B)	lcSSc, telangiectasia, calcinosis, PAH	20–42/93
DNA topoisomerase I (Scl-70)	dcSSc, severe and extensive skin involvement; digital ulcers, ILD, cardiac involvement, malignancy	17–41/90–100
RNA polymerase III (RNAP)	dcSSc, renal crisis, malignancy	5–25/97
Fibrillarin (U3-RNP)	dcSSc, cardiomyopathy, severe small bowel involvement, PAH, ILD, myopathy	4–10/97
Th/To	lcSSc, puffy fingers, small bowel disease, PAH, ILD	1–13/97–98
U1-RNP	lcSSc, puffy fingers, Raynaud's phenomenon, PAH, overlap syndrome	6–7%/NA
U11/U12-RNP (RNP-C3)	ILD	1–3/NA
PM/Scl	Myositis, arthritis, ILD, mechanic's hands	3–13/88
Ku	Arthritis, myositis	2–4/96
NOR-90 (hUBF)	lcSSc, ILD	4–6/96

lcSSc limited systemic sclerosis, dcSSc diffuse systemic sclerosis, ILD interstitial lung disease, PAH pulmonary arterial hypertension, NA not available

highly specific for SSc [159–162]. Anti-topo I is typically associated with dcSSc, although it can be found also in some cases of lcSSc [163]. Anti-topo I antibodies are associated with a higher risk of severe interstitial lung disease (ILD) [162], presence of digital ulcers, cardiomyopathy, high skin score [164], and a more severe phenotype with an increased risk of mortality [159, 160, 162, 165]. Finally, anti-topo I antibodies have been associated with malignancies in some studies [166]. Thus, all SSc patients with anti-topo I should be screened and closely monitored for the presence of ILD and cardiac involvement with high-resolution chest CT (HRCT), pulmonary function testing, electrocardiogram, and transthoracic echocardiogram, especially in the first five years of disease [167].

Anti-Centromere (ACA)

The main targets of ACA are centromere proteins (CENP) CENP-A, CENP-B, and CENP-C, although CENP-B, an 80-kDa kinetochore protein, seems to be the primary autoantigenic target with several reactive epitopes recognized by ACA in SSc [168]. ACA were first described when HEp-2 cells came into use as the substrate of choice for ANA testing because they produce a characteristic centromere staining pattern [160]. The frequency of ACA in SSc has been reported to be 20–42% in many ethnic groups [161, 169].

These antibodies are associated with lcSSc, long-standing Raynaud's phenomenon, presence of calcinosis, and a higher risk of developing pulmonary arterial hypertension (PAH) [169]. When found in patients with Raynaud's phenomenon, ACA indicates a high probability of progression to SSc [170]. Survival rates of patients with ACA are better than those of patients with most of the other SSc-associated antibodies including anti-topo I [165]. The presence of ACA is not exclusive of SSc and has been detected in patients with primary biliary cholangitis, pSjS and even in SLE [171].

Anti-RNA Polymerase III (RNAP III)

Anti-RNAP III antibodies target the 115-kDa and 138-kDa components of RNA polymerase III and are detected in 5 to 25% of SSc patients with high variability among different ethnic-geographic groups [159, 160]. As anti-RNAP III antibodies frequently coexist with antibodies to RNAP I and RNAP II, the HEp-2 IFA pattern may show as fine speckled nucleoplasmic staining with additional coarse speckles and speckled nucleolar pattern [11]. Anti-RNAP III is highly specific for SSc, especially the dcSSc subset, and indicates a high risk of severe, rapidly progressing cutaneous thickening and higher risk of renal crisis [172, 173]. Scleroderma renal crisis has been reported in RNAP III-positive patients with SSc sine scleroderma [174]. It has also been shown that patients with anti-RNAP III antibodies present a higher risk

of development of malignancy, especially within the first years of disease onset [166, 173]. Anti-RNAP III has also been associated with a higher risk for gastric antral vascular ectasia (GAVE) [173, 175].

Of special interest for SSc, anti-RNAP III antibody-positive patients should have regular screening for age-appropriate cancer and be alert to the increased risk of renal crisis. Appropriate monitoring of blood pressure should be performed for early detection of renal crisis since the prompt initiation of angiotensin-converting enzyme inhibitor can be life-saving [160, 167].

Anti-U3-RNP/Fibrillarin

Anti-U3-RNP/fibrillarin antibodies target the nucleolar U3-RNP complex, involved in pre-rRNA processing. Anti-fibrillarin is detected in 4–10% of SSc patients and is more frequent in African-Americans [176, 177]. They are more frequent in patients with dcSSc and are associated with multi-organ involvement [159, 160], increased risk of cardiac involvement, renal crisis, ILD, PAH, and small bowel and muscle involvement. In a study of an African-American SSc cohort, anti-fibrillarin was associated with younger age of onset, higher frequency of digital ulcers, pericarditis, and severe lower gastrointestinal involvement, but less severe ILD and no change in survival [176]. Altogether, anti-U3-RNP/fibrillarin antibodies indicate a worse prognosis in SSc patients [159, 177]. A limitation of detecting anti-fibrillarin is the lack of a high-sensitivity and -specificity immunoassay in the clinical laboratory.

Anti-Th/To

Anti-Th/To antibodies target proteins 18–120 kDa associated with Th/7–2 and To/8–2 RNA. Anti-Th/To is relatively specific for SSc and occurs in 1–13% of patients, especially in those with lcSSc [159–161]. Anti-Th/To is associated with a higher frequency of ILD, PAH, and scleroderma renal crisis. The increased frequency of organ involvement results in reduced survival among these patients [178]. Patients with idiopathic ILD and a homogeneous nucleolar HEp-2 IFA pattern (AC-8) frequently have anti-Th/To antibodies and may progress to full-blown SSc or systemic sclerosis *sine scleroderma* [179].

Other Antibodies Associated with SSc

Other antibodies found in patients with SSc include anti-PM/Scl, anti-U1-RNP, anti-Ku, anti-U11/U12 RNP, anti-Ro52, and anti-NOR-90. Anti-Ro52 occurs in up to 20% of SSc patients and is reported to be associated with ILD and overlap syndrome [180]. Anti-U11/U12 RNP is highly specific for SSc, but occurs in only 1–3% of patients with

this disease, being associated with severe ILD and severe pulmonary involvement [181]. Anti-PM/Scl antibodies are detected in 3–13% of SSc patients [236] and are more frequent in patients with lcSSc as well as in overlap syndrome with myositis, joint involvement, and calcinosis. Anti-PM/Scl antibodies may also be detected in the serum of patients with polymyositis, dermatomyositis, and undifferentiated connective tissue disease [182, 183]. Severe organ involvement is rare, and therefore anti-PM/Scl antibodies indicate a favorable prognosis.

Anti-U1-RNP are detected in 6–7% of patients with SSc. Anti-U1 RNP was first characterized as an antibody associated with mixed connective tissue disease (MCTD), but it can be also detected in patients with pure SSc as well as in other autoimmune rheumatic diseases, such as SLE [159–161]. In SSc, anti-U1-RNP is associated with limited skin involvement, Raynaud's phenomenon, arthritis, myositis, and overlap syndrome. The association with PAH is observed in some cohorts. The general prognosis tends to be favorable.

Anti-Ku is detected in 2–4% of SSc patients [159–161] and has been associated with lcSSc, presence of myositis, ILD, arthritis, and less vascular involvement [184]. This autoantibody has also been described in patients with SLE, dermatomyositis (DM), and polymyositis (PM). Anti-NOR-90 antibodies can be detected in 4–6% of patients with SSc but are also present in other autoimmune diseases such as RA, SLE, and Sjögren's syndrome [185] as well as in sera of some patients with malignancy [163]. When it is the only autoantibody in the sample, anti-NOR-90 yields a characteristic HEp-2 IFA pattern (AC-10) characterized by nucleolar speckled pattern and up to 10 discrete bright dots at the metaphase plate [11]. Although large-scale studies are not available, anti-NOR 90 antibodies may be considered a marker of limited SSc and mild involvement of internal organs.

Idiopathic Inflammatory Myopathies

Idiopathic inflammatory myopathies (IIM) are a heterogeneous and rare group of autoimmune disorders that affects the skeletal muscles and multiple organs. Historically, IIM was classified into two main subgroups: polymyositis and dermatomyositis (DM) [186, 187]. The progressive discovery of myositis-specific antibodies (MSA) and the refinement of specific clinical and histopathological features have gradually improved and expanded the classification into a more comprehensive and personalized framework. Although not established as a formal classification system for inflammatory myopathies, distinct subsets of IIM have been recognized, including PM, adult DM, juvenile DM, amyopathic DM, sporadic inclusion body myositis (sIBM), immune-mediated necrotizing myopathy (IMNM), and

anti-synthetase syndrome (ASS). In view of the current classification system, PM has now largely been considered a diagnosis of exclusion [188, 189].

The annual incidence of IIM ranges from 0.58 to 19 per million and the prevalence from 2.4 to 33.8 per 100,000 inhabitants [190]. It should be noted, though, that epidemiological data are quite variable since IIM are rare and diagnostic and classification criteria have changed significantly during the last decades. In general, IIM are most common in females with a female/male ratio of 2:1 with a peak prevalence between 45 and 60 years of age.

As in other autoimmune rheumatic diseases, autoantibodies are useful biomarkers for the diagnosis and classification of IIM. Autoantibodies, detected in up to 60% of IIM patients, have been classified into myositis-specific autoantibodies (MSA) and myositis-associated autoantibodies (MAA) [191]. Classic MSA include anti-Jo-1 (histidyl transfer RNA synthetase) and antibodies to other aminoacyl transfer RNA synthetases (ARS), anti-Mi-2, and anti-signal recognition particle (SRP) antibodies (Fig. 1). The most frequent MAA include anti-PM/Scl, anti-Ku, anti-U1-RNP, and anti-Ro52, which are found also in other conditions including SSc, SLE, and overlap syndromes [192].

In recent years, major advances have been made in the characterization and the discovery of new autoantibodies in IIM. As such, a major shift has been made including the recognition of autoantibodies specifically associated with sIBM, distinct subsets of DM, and a higher risk of cancer or ILD. The detection of MSA and MAA has become a key feature for early diagnosis and classification of IIM, contributing decisively to the definition of clinically distinguishable IIM subsets, stratification of risk of organ involvement, and to a more personalized approach [191, 192]. The most noteworthy autoantibodies identified to date and their clinical associations, summarized in Table 10, are discussed as follows.

Anti-ARS

Autoantibodies against ARS represent the most common MSA and can be detected in 22–35% of IIM patients [191–193]. These autoantibodies target a family of cytoplasmic amino acid-charging enzymes, named aminoacyl transfer RNA (tRNA) synthetases (ARS). At least eight anti-ARS have been identified including anti-Jo-1 (anti-histidyl-tRNA synthetase), anti-PL7 (anti-threonyl-tRNA synthetase), anti-PL12 (anti-alanyl-tRNA synthetase), anti-EJ (anti-glycyl tRNA synthetase), anti-OJ (anti-isoleucyl-tRNA synthetase), anti-Ha (anti-tyrosyl-tRNA synthetase), anti-KS (anti-asparaginyl-tRNA synthetase), and anti-Zo (anti-phenylalanyl-tRNA synthetase) antibodies (Fig. 1). Anti-Jo-1 antibodies are the most common, occurring in approximately 20% of adult patients with IIM, and the other anti-ARS are collectively found in 1–6% of patients [192, 194].

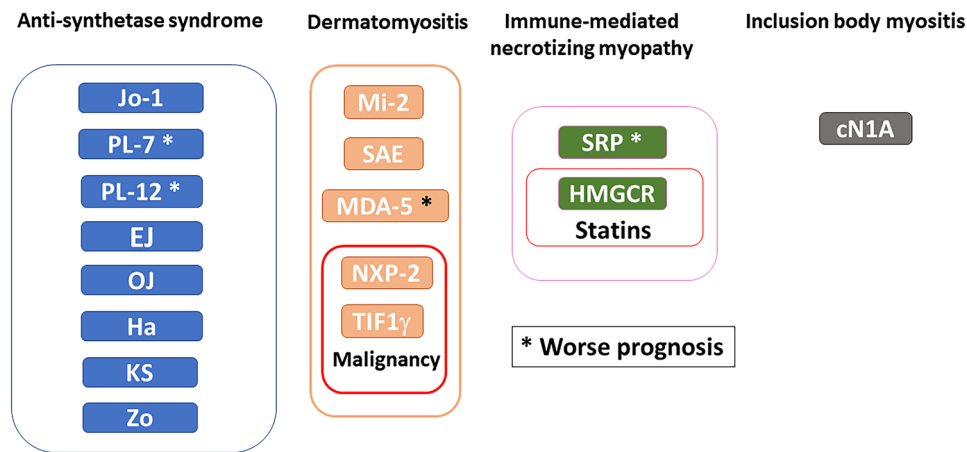


Fig. 1 Myositis-specific autoantibodies. Jo-1: histidyl transfer RNA synthetase; PL7: threonyl-tRNA synthetase; PL12: antalanlyl-tRNA synthetase; EJ: glycyl tRNA synthetase; OJ: isoleucyl-tRNA synthetase; Ha: tyrosyl-tRNA synthetase; KS: anti-asparagyl-tRNA synthetase; Zo: phenylalanyl-tRNA synthetase; Mi-2: complex nucleosome remodeling histone deacetylase; TIF1 γ : transcriptional intermediary factor 1; NXP-

2: nuclear matrix protein 2; MDA-5: melanoma differentiation-associated protein 5; SAE: small ubiquitin-like modifier activating enzyme; SRP: signal recognition particle; HMGCR: 3-hydroxy-3-methylglutaryl CoA reductase; cN1A: cytosolic 5'-nucle-otidase 1A

Anti-ARS autoantibodies are associated with the anti-synthetase syndrome, which is characterized by a spectrum of typical clinical manifestations including myositis, ILD, arthritis, fever, Raynaud's phenomenon, and mechanic's hands. However, anti-ARS antibodies seem to be associated with heterogeneous disease expression and severity, and not every patient with an anti-ARS autoantibody has every feature of the anti-synthetase syndrome. For example, muscle involvement and arthritis are more common in patients with anti-Jo-1 compared to non-Jo-1 patients [195], whereas the

presence of anti-PL7 or anti-PL12 antibodies is associated with a higher rate of ILD and a higher risk of mortality [195, 196]. Anti-ARS autoantibodies are rare in DM, JDM, and other autoimmune rheumatic diseases.

Dermatomyositis-Specific Autoantibodies

To date, five dermatomyositis-specific autoantibodies are identified: anti-complex nucleosome remodeling histone deacetylase (Mi-2), anti-transcriptional intermediary factor

Table 10 Phenotype associations of autoantibodies in autoimmune inflammatory myopathies

Autoantibody target	Disease phenotype and associations	Frequency
Aminoacyl tRNA synthetases *	Anti-synthetase syndrome: myositis, ILD, arthritis, mechanic's hands, and Raynaud's phenomenon	22–35%
Mi-2	DM with typical skin lesions, myositis, good response to therapy, good prognosis	Up to 20% in adults Up to 10% in JDM
MDA-5	CADM, ILD, DM typical skin rashes, poor prognosis	10–15
TIF-1 γ	Severe DM, malignancy, DM typical skin rashes	7% in adults
NXP-2	Malignancy, severe muscle involvement and skin disease, calcinosis	2–17% in adults 15–22% in JDM
SAE	DM typical skin rashes Later muscle involvement	1.5–8%
SRP	IMNM, severe muscle weakness, very high CK levels, myocarditis, poor response to therapy and severe prognosis	2–5%
HMGCR	IMNM, very high CK levels, association with statin use	6–34%
Anti-cN1A	Inclusion body myositis, insidious onset of muscle weakness	30–50% in IBM

DM dermatomyositis, CADM clinically amyopathic dermatomyositis, ILD interstitial lung disease, CK creatinine kinase, IMNM immune-mediated necrotizing myopathy

*Jo-1 (histidyl-tRNA synthetase), PL-7 (threonyl-tRNA synthetase), PL-12 (alanlyl-tRNA synthetase), OJ (isoleucyl-tRNA synthetase), EJ (glycyl-tRNA synthetase), KS (asparaglyl-tRNA synthetase)

1 (TIF1) γ , anti-nuclear matrix protein 2 (NXP-2), anti-melanoma differentiation-associated protein 5 (MDA5), and anti-small ubiquitin-like modifier activating enzyme (SAE) [189].

Anti-Mi-2, the first specific autoantibody described in DM, is the most common antibody associated with DM, occurring in up to 20% of adult patients and up to 10% of JDM. Anti-Mi-2 is associated with classic features of DM including Gottron papules/sign, heliotrope rash, nail fold erythema, and violaceous rash including the V-sign and Holster sign. These patients tend to present with severe myositis, but they typically respond well to steroid therapy and have a good prognosis and a decreased risk of cancer compared with DM patients who are anti-Mi-2-negative [192, 196, 197].

Anti-TIF1 γ was first described as a macromolecular complex of 155/140-kDa proteins and was subsequently identified as the main autoantibody target of the TIF1 (α , β , γ subunits) family of proteins [198]. Anti-TIF1 γ antibodies are strongly associated with malignancies in adult DM patients. In clinical practice, given the high association of anti-TIF1 γ with malignancy, this antibody is considered a key biomarker for cancer in the setting of DM [191, 192]. Anti-TIF1 γ is also associated with typical skin rash, photosensitivity, and low frequency of Raynaud's phenomenon and ILD. Interestingly, these antibodies might also be detected in patients with JDM, but the association with cancer does not apply to children and adolescents [191].

Anti-NXP-2 is an autoantibody first described in a cohort of JDM and juvenile polymyositis patients. Of interest, anti-NXP-2 antibody is characteristically associated with a HEp-2 IFA pattern of multiple discrete nuclear dots (AC-6) [199]. When detected in adult DM, it is strongly associated with malignancy. Anti-NXP-2 is more frequent in JDM with a frequency of 15 to 22% and is associated with a higher risk of developing calcinosis and severe muscle involvement [195, 196].

The anti-MDA-5 antibody, originally named anti-CADM-140 antibody, binds to a 140-kDa cytoplasmic protein and was first described in Asian cohorts as strongly associated with clinically amyopathic DM (CADM) [192, 197]. In addition, anti-MDA-5 antibodies are associated with rapidly progressive ILD and a high risk of mortality [200]. Patients with anti-MDA-5 antibodies might also have atypical skin lesions with cutaneous ulcerations, characteristic palmar violaceous macules/papules, hand swelling, and arthritis [201].

Antibodies to SAE were first identified in 2007 by Betteridge et al. in 11 DM patients [202]. Since then, anti-SAE has been described in several adult DM cohorts with varying frequencies of 1.5–8%. Patients tend to present with extensive skin rash, followed by muscle weakness and systemic manifestations including gastro-intestinal involvement with dysphagia [202].

Autoantibodies Associated with Immune-Mediated Necrotizing Myositis (IMNM)

IMNM is a subtype of myositis characterized by distinct clinical and histopathological features and strong association with antibodies to SRP and to 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR). Patients with IMNM typically present with acute severe muscle weakness, with very high creatine kinase levels, as well as resistance to conventional immunosuppressive therapy. Characteristically, muscle biopsy histopathology shows marked myofibril necrosis with minimal or no inflammatory infiltrate [203].

Anti-SRP antibodies are reported in approximately 5% of all IIM patients and are associated with severe necrotizing myositis, intense muscle weakness, dysphagia, cardiac involvement, and ILD. Anti-HMGCR autoantibodies are associated with IMNM and with the use of statins, although not all patients with these autoantibodies have a history of exposure to prescribed statins. The relationship of anti-HMGCR antibodies and statins is intriguing as HMGCR is the pharmacologic target of statins [203]. Patients with anti-HMGCR-associated myopathy present with weakness, myalgia, and high CK levels, although not always as dramatic as seen with SRP myopathy. Statin-naïve IMNM patients with anti-HMGCR antibodies were reported to be younger, with higher CK levels, and less responsive to treatment as compared to those that received statins.

Anti-cN1A Antibody and Sporadic Inclusion Body Myositis

Sporadic inclusion body myositis (sIBM) is a distinct form of IIM characterized by insidious onset of muscle weakness, an asymmetric pattern of muscle involvement, typical involvement of long finger flexors, quadriceps, and tibialis anterior, but only mild elevation of CK. Dysphagia is common and may present as the initial symptom [188]. The therapeutic response to immunosuppression is poor. In contrast to other inflammatory myopathies, until recently myositis-specific autoantibodies had not been found in sIBM patients. Autoantibodies directed against major epitopes of cN-1A (also known as Mup-44, NT5c1A) are found in 30–50% of patients with sIBM, which has helped distinguish sIBM patients within the context of inflammatory myopathy [204]. Anti-cN1A has moderate sensitivity and high specificity for sIBM. The presence of anti-cN1A antibodies may be associated with muscle weakness but were not associated with a specific HEp-2 IFA staining pattern, hence screening using HEp-2 substrates is unlikely to be a useful predictor for the presence of these autoantibodies [205]. Anti-cN-1A has been observed also in other autoimmune diseases, particularly Sjögren's syndrome and SLE [205, 206]. There are reports of anti-cN1A in JDM [205], but this is at variance

with another recent study [207]. Hence, standardization and harmonization of anti-cN1A assays are very important for future clinical use.

Autoimmune Liver Diseases

Autoantibodies are important biomarkers for the investigation of hepatitis and cholestasis of unknown origin and are part of the criteria in the diagnostic scoring system of autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) (Fig. 2) [208–210]. Herein, we address the clinical associations of autoantibodies in autoimmune liver diseases and their contribution to PM.

Autoimmune Hepatitis

AIH is a chronic inflammatory liver disease with unknown underlying etiology. Distinctive findings are the presence of autoantibodies, high serum levels of immunoglobulin G (IgG) and gamma globulin, and plasma cell hepatic infiltrates [211]. Most autoantibodies in AIH are not disease specific and should be considered along with other clinical, laboratory, and histological elements. Moreover, autoantibodies are lacking in 10–15% of AIH patients, although some of these “seronegative” patients may develop autoantibodies later in the disease course [212]. In addition to pointing to an autoimmune basis and contributing to the diagnosis, autoantibodies help in the differentiation of the two types of AIH (Table 11; Fig. 2). Type I AIH affects mainly adults and is the most prevalent form of AIH, whereas type 2 AIH occurs at a younger age, frequently in children, and has a more aggressive course [213]. Antinuclear antibody (ANA), anti-smooth muscle antibodies (SMA), and anti-F-actin antibodies are typically associated with type 1 AIH [208], whereas antibodies to LKM-1 (liver/kidney microsome 1)

and LC-1 (liver/cytosol 1) occur almost exclusively in type 2 AIH [214, 215].

SMA occur in circa 50% of type 1 AIH patients and can be the only autoantibody present [211, 212]; however, SMA, especially at low titer, may occur in other diseases, including non-alcoholic fatty liver disease (NAFLD) and other liver diseases [216]. In the stomach/kidney/liver indirect immunofluorescence assay (SKL-IFA), SMA reacts with microfilaments in several locations, including transversal fibers in gastric mucosa, *muscularis mucosae*, muscular layer of arteries, glomerulus, and renal tubules. Higher specificity of SMA for AIH occurs with the reactivity to the vascular, glomerular, and tubular components (VGT pattern) [217]. Filamentous actin (F-actin) is the main target of autoantibodies in samples that have the VGT pattern and anti-F-actin antibodies are rather specific for AIH. Anti-F-actin antibodies are determined preferentially by IFA on fibroblasts treated to display the filamentous form of actin. The preservation of filamentous F-actin in ELISA tests may be an issue, resulting in lower specificity for AIH [218, 219].

Anti-neutrophil cytoplasmic antibodies (ANCA) occur in a series of diseases and the immunofluorescence pattern helps trim down the spectrum of associated diseases. The cANCA pattern is associated with anti-proteinase 3 (PR3) antibodies and occurs predominantly in granulomatosis with polyangiitis. The pANCA pattern is associated with anti-myeloperoxidase (MPO) antibodies and occurs in several conditions, including microscopic polyangiitis, rapidly progressive glomerulonephritis with crescents, and small-vessel vasculitis. Of interest to liver autoimmunity, the atypical pANCA pattern is most frequently associated with type 1 AIH, progressive sclerosing cholangitis (PSC), and ulcerative colitis, and the identity of the target autoantigens is a matter of debate [217, 220].

Anti-LKM-1 antibodies target cytochrome P450 CYP 2D6 and are relatively specific for type 2 AIH, although they

Fig. 2 Autoantibodies associated with autoimmune hepatitis and primary biliary cirrhosis.

AIH: autoimmune hepatitis; SMA: smooth muscle antibody; LKM: liver kidney microsome; SLA/LP: soluble liver antigen/liver-pancreas; ASGRP: asyalo glycoprotein receptor protein; LC: liver cytosol; ANA: antinuclear antibody; TRIM21: tripartite motif family of proteins; ANCA: anti-neutrophil cytoplasm antibody; AMA: anti-mitochondria antibody; gp210: glycoprotein 210; KLHL12: kelch-like 12; HK-1: hexokinase 1

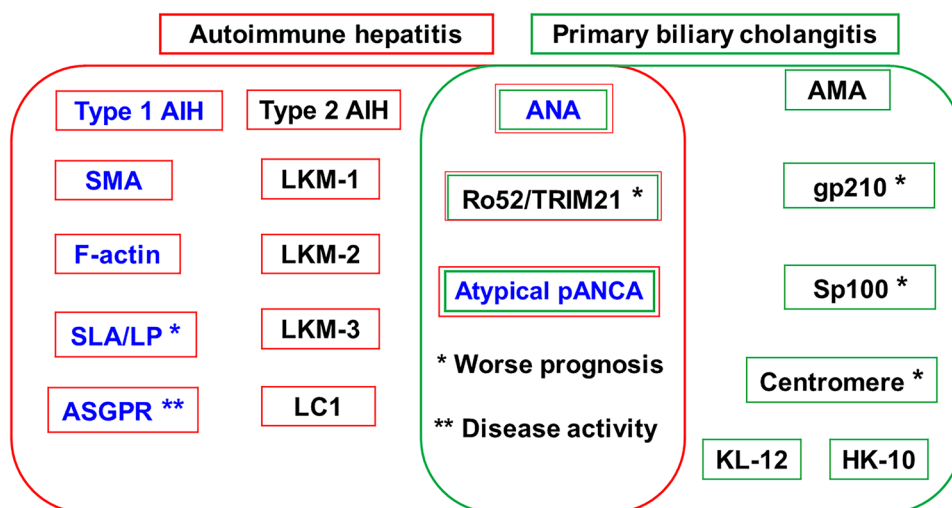


Table 11 Phenotypic associations of autoantibodies in autoimmune liver diseases

Autoantibody target	Liver disease phenotype and remarks
ANA (HEp-2 IFA)	Type 1 AIH; homogeneous or fine speckled nuclear pattern
SMA	Type 1 AIH; V-G-T pattern on SKL-IFA is more specific for AIH
F-actin	Type 1 AIH; more specific than SMA
Atypical pANCA	Type 1 AIH, PSC, UC, PBC
LKM-1	Type 2 AIH
LC1	Type 2 AIH
SLA/LP	AIH; highly specific; more severe disease
ASGPR	AIH; correlates with disease activity
Ro52/TRIM21	AIH; indicates more severe disease
AMA	PBC; also in transient liver inflammation
gp210	PBC; highly specific; indicates more severe disease
Sp100	PBC; indicates more severe disease; occasionally in AIH and other diseases
Centromere	PBC; associated with aggressive disease and higher frequency of pulmonary hypertension
Double-stranded DNA	PBC; PBC/AIH overlap syndrome

ANA antinuclear antibodies, *HEp-2 IFA* indirect immunofluorescence assay on HEp-2 cells, *AIH* autoimmune hepatitis, *SMA* smooth muscle antibody, *pANCA* peripheral anti-neutrophil cytoplasm antibody, *PSC* primary sclerosing cholangitis, *UC* ulcerative colitis, *PBC* primary biliary cholangitis, *LKM-1* liver kidney microsome type 1, *LC1* liver cytosol 1, *SLA/LP* soluble liver antigen/liver pancreas, *ASGPR* asialoglycoprotein receptor, *Ro52* 52-kDa component of the SS-A/Ro system, *TRIM21* tripartite motif family of proteins, *AMA* anti-mitochondria antibody

occur in occasional patients with hepatitis C (HCV), PBC, and PSC [221–223]. Anti-LC1 antibodies target the enzyme formiminotransferase cyclodeaminase and occur in ~25% of patients with type 2 AIH, most frequently in association with anti-LKM-1 antibodies [224, 225]. Although highly suggestive of AIH, anti-LC1 may occur in occasional HCV patients [225]. On the other hand, anti-SLA/LP is highly specific for type 1 AIH and is associated with a more severe disease course [226, 227]. Anti-SLA/LP recognizes the cytosolic protein *O*-phosphoserine-tRNA:selenocysteine-tRNA synthase and is not amenable to detection by IFA on HEp-2 cells or rodent tissue cryosections [220]. Anti-SLA/LP adds value in the laboratory workup of AIH because it can occur as the only autoantibody and has prognostic implications. It is present in no more than 20% of the patients and indicates a more severe disease, especially when associated with anti-Ro52 antibodies [228]. Anti-Ro52 antibodies are observed in ~30% of AIH patients and have been associated with more severe disease with higher probability of cirrhosis, hepatic failure, and liver transplantation [228]. In addition to AIH, anti-Ro52 occurs in several autoimmune rheumatic diseases, including SLE, pSjS, SSc, and immune-mediated inflammatory myopathies. Antibodies against the hepatocyte membrane asialoglycoprotein receptor (ASGPR) occur in 25–80% of patients with AIH and seem to be associated with histologic disease activity, as ASGPR titer and frequency drop with disease remission. ASGPR is observed at low titer in other liver diseases, including viral hepatitis and PBC

[229]. Therefore, moderate to high titers anti-ASGPR are more reliable as a biomarker for AIH.

The HEp-2 IFA test is very informative in the investigation of patients with autoimmune liver diseases, as a homogeneous or fine speckled nuclear pattern is present in 50–80% of type 1 AIH patients [230]. However, it should be noted that a positive HEp-2 IFA test occurs in 13–22% of the general population [8, 231, 232] as well as in patients with non-autoimmune diseases including non-alcoholic fatty liver disease (NAFLD) [233]. Interestingly, several of the liver autoantibodies produce distinctive HEp-2 IFA patterns (Fig. 3) and the recognition of such patterns should guide disease-specific immunoassays for definitive autoantibody identification. A very distinctive cytoplasmic HEp-2 IFA pattern, observed in HCV patients treated with interferon-alpha and ribavirin, is characterized by a cytoplasmic discrete “rods and rings” IFA pattern (AC-23) (Fig. 3). The target antigen is inosine-5'-monophosphate dehydrogenase 2 (IMPDH2) and the cognate autoantibodies appear a few months after initiation of ribavirin therapy [234]. Of interest for PM, Carcamo et al. found that higher titer anti-IMPDH2 antibodies indicate a poor response to therapy [235]. However, this has not been generally observed in other studies [234, 236].

It should be noted that several liver autoantibodies do not yield relevant IFA reactivity on HEp-2 cells, as occurs with antibodies to LKM-1, LC1, ASGPR, SLA/LP, and atypical pANCA. In contrast, the SKL-IFA provides useful

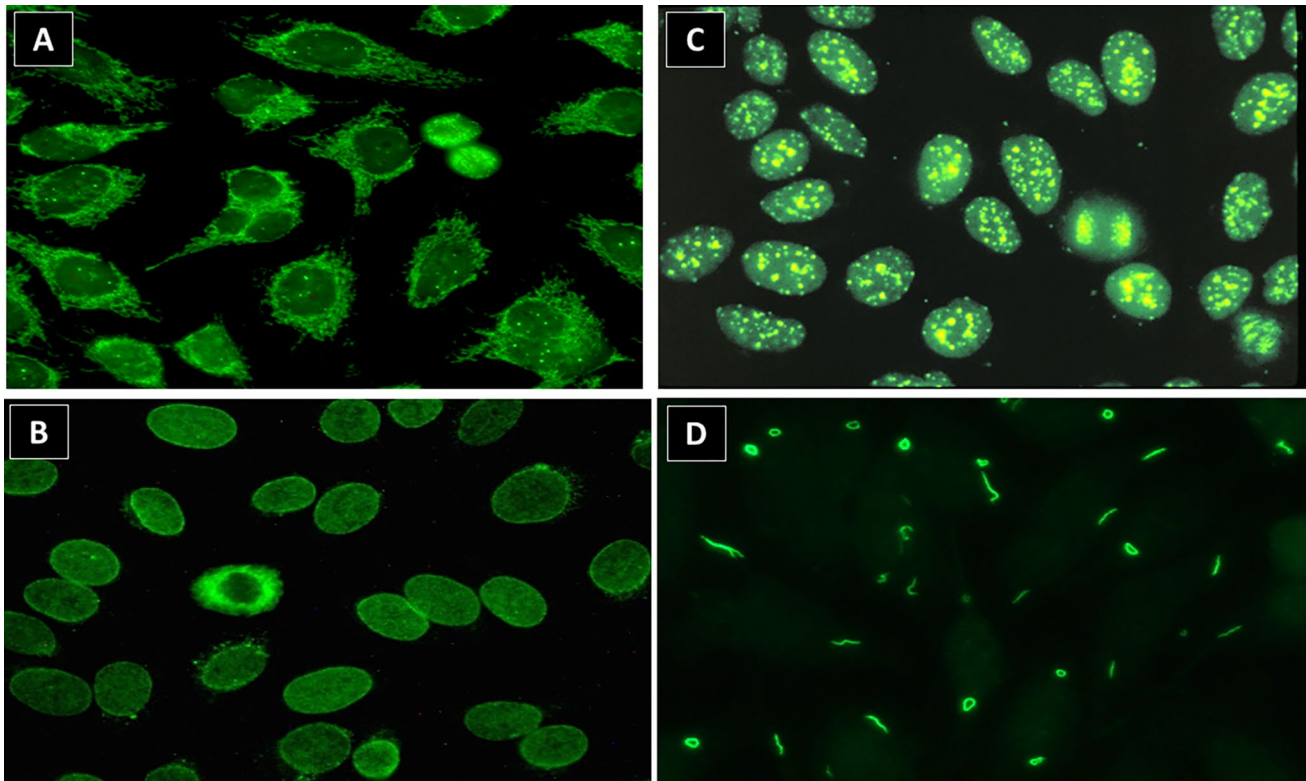


Fig. 3 HEp-2 indirect immunofluorescence assay (HEp-2 IFA) patterns associated with autoimmune liver diseases. **a** Multiple discrete nuclear dots pattern (AC-6) associated with anti-sp100 antibodies and cytoplasmic reticular mitochondria-like pattern (AC-21). **b** Punctate nuclear envelope pattern (AC-11), associated with anti-gp210 anti-

bodies. **c** Centromere pattern (AC-3), associated with antibodies to centromere protein A (CENP-A), CENP-B, and CENP-C. **d** Cytoplasmic rods and rings pattern (AC-23), associated with anti-inosine monophosphate dehydrogenase 2 (IMPDH-2) antibodies

information regarding antibodies to LKM-1, LC1, SMA, and mitochondria (AMA) (Fig. 4). Once the pattern associated with one of these autoantibodies is identified in testing a given sample, the suggested autoantibody specificity can be confirmed using antibody-specific tests based on ELISA, chemiluminescence immunoassay (CLIA), western blot, line blot, or other solid-phase immunoassays. Again, some liver autoantibodies yield no reactivity in SKL-IFA or HEp-2 IFA, such as anti-SLA/LP and anti-ASGPR.

The considerable heterogeneity in the prognosis of AIH is of relevance for PM. While most patients have good long-term survival upon early diagnosis and treatment, some have a severe course culminating in liver failure and the need for liver transplantation [237]. The linkage of autoantibodies to information about other relevant parameters adds value to the definition of disease phenotype, thus contributing to the goals of PM. In the context of AIH, a more severe prognosis is heralded by the presence of anti-SLA/LP antibodies, disease onset before 18 years, and histologic evidence of fibrosis at diagnosis [227, 238, 239]. In contrast, the presence of the HLA-DRB1*0401 allele speaks for a better prognosis [227, 240]. The presence of AMA in the context of AIH

indicates the possibility of an AIH/PBC overlap syndrome. Such patients should be monitored for the development of increased serum alkaline phosphatase and the need for specific treatment with ursodeoxycholic acid [241, 242].

Primary Biliary Cholangitis

PBC is a chronic non-suppurative granulomatous inflammation affecting the small intrahepatic bile ducts [209]. PBC predominates in women and has a very insidious onset and course, being frequently diagnosed only at late stages when cirrhosis is established. Chronic pruritus and malaise are hints that should prompt investigation of PBC. Elevated serum canalicular liver enzymes (mainly alkaline phosphatase and gamma-glutamyl transferase) indicate the possibility of biliary tract involvement, whereas elevated IgM and cholesterol bring PBC to attention.

Over 98% of the patients have autoantibodies, most commonly directed to mitochondrial targets (AMA). AMA bind a series of enzymes in the outer mitochondria membrane with E2 pyruvate dehydrogenase complex (PDC-E2) being the main one. The presence of AMA is highly specific for

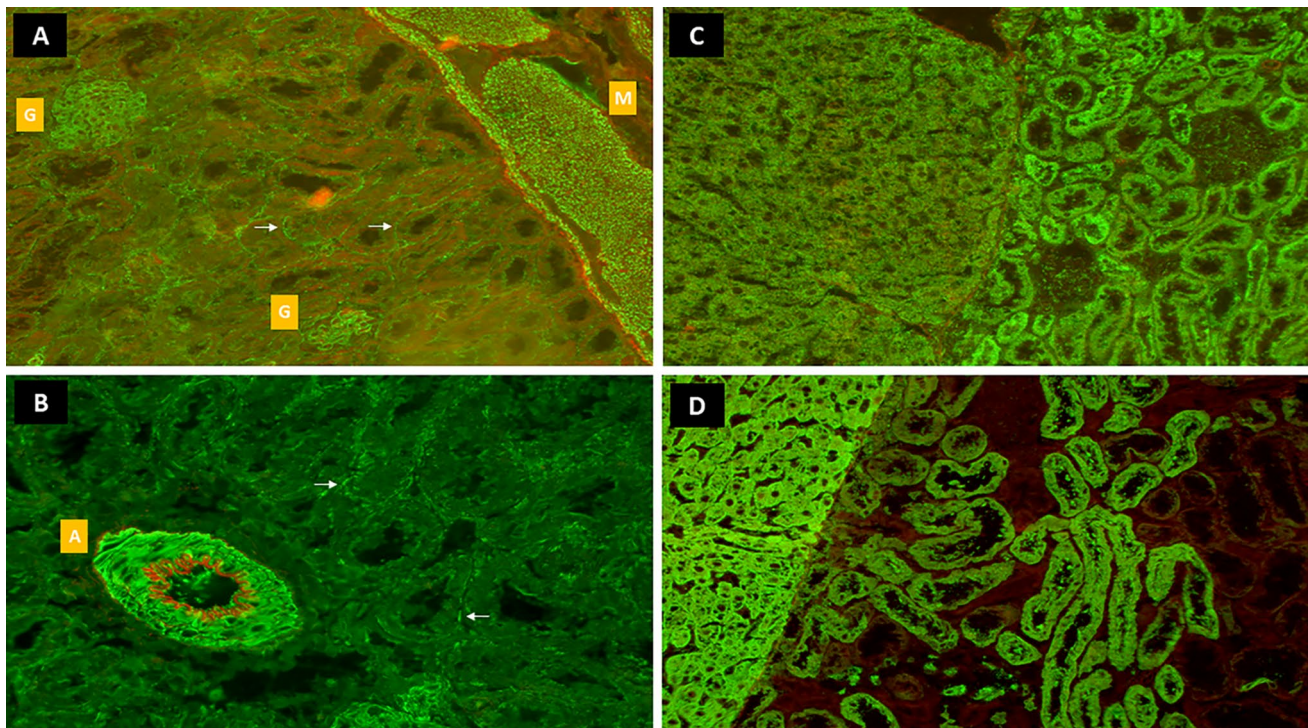


Fig. 4 Immunofluorescence patterns observed in the indirect immunofluorescence assay on rodent tissue cryosections. **a** Smooth muscle antibodies staining the muscularis mucosae and muscle layer (M) in the gastric mucosa, glomeruli (G), and extracellular fibrils around renal tubules (white arrows). **b** Smooth muscle antibodies staining the muscle layer of an arteriole (A) and extracellular fibrils around

renal tubules (white arrows). **c** Anti-mitochondria antibodies cause a cytoplasmic coarse speckled staining of hepatocytes (left) and kidney tubular cells (right). **d** Anti-LKM-1 antibodies cause a strong and dense fine speckled staining of the cytoplasm of hepatocytes (left) and proximal tubule cells (right); distal tubules are weakly or not stained at all (counterstained in red)

PBC and is one of the three criteria for the diagnosis of the disease [210]. It should be noted, however, that some patients with acute liver injury may have transient AMA [243]. In a chronic cholestatic patient with no evidence of another underlying disease, the presence of sustained high alkaline phosphatase levels and AMA (or other PBC-associated autoantibodies) is accepted for the diagnosis of PBC [209]. In patients with established PBC, anti-PDC-E2 antibodies of the IgA class or IgG3 subclass indicate a more aggressive disease [244]. Other autoantibodies specifically associated with PBC are anti-gp210 and anti-sp100. In addition, circa 30% of the patients have anti-centromere antibodies, which are more commonly associated with systemic sclerosis.

The incidental finding of a cytoplasmic mitochondria-like pattern (AC-21) in the HEp-2 IFA test is suggestive of AMA and, therefore, often leads to reflex AMA-specific tests and when these are positive a diagnostic dilemma ensues. If serum alkaline phosphatase levels are normal and there is no evidence of cholestasis or other PBC-related features, no specific treatment is warranted. However, these patients should have follow-up alkaline phosphatase levels

monitored and eventual elevation should prompt further investigation with imaging studies, e.g., acoustic radiation force impulse imaging (ARFI) or magnetic resonance, which may culminate in indication for liver biopsy. This approach may contribute to the prognosis as it has been demonstrated that early treatment with ursodeoxycholic acid is crucial for optimal outcome [245].

Up to 50% of the AMA-negative PBC patients have other autoantibodies detected in the HEp-2 IFA test (Table 11; Fig. 2), yielding characteristic patterns, such as the multiple discrete nuclear dots pattern (AC-6), associated with anti-sp100 and anti-PML, the punctate nuclear envelope pattern (AC-12), associated with anti-gp210, and the centromere pattern (AC-3), associated with anti-CENP-B/A. Anti-sp100 occurs in circa 30% of PBC patients and may occasionally occur in other systemic autoimmune diseases and in AIH [246]. Anti-PML antibodies occur in approximately 20% of PBC patients and frequently coexist with anti-sp100 antibodies [247]. The co-occurrence of anti-sp100 and anti-PML antibodies has been associated to a more rapid and severe progression of PBC [248]. Anti-gp210 antibodies occur in 20% of all PBC patients and in 30–50% of AMA-negative

PBC. The coexistence of AMA frequently obscures the punctate nuclear envelope pattern (AC-12) associated with anti-gp210 antibodies, which can be resolved by using gp210-specific immunoassays. The presence of anti-gp210 has been associated with more aggressive disease [249, 250] and higher incidence of hepatocellular carcinoma [251]. In addition, anti-gp210 is associated with higher frequency of PBC/AIH overlap syndrome. Anti-centromere antibodies are observed in up to 30% of PBC patients and have been associated to increased frequency of portal hypertension [250] and more aggressive disease [249, 252]. Other autoantibodies that may add key evidence for PM include antibodies to kelch-like 12 (KLHL12) and hexokinase 1 (HK-1), which were recently identified as new biomarkers for PBC and notably identified patients who are negative for conventional autoantibodies described above [253].

The PBC/AIH overlap syndrome occurs in circa 10% of PBC patients [254], who tend to present worse prognosis, with lower 5-year survival rate and higher frequency of esophageal varices, gastrointestinal bleeding and liver transplantation [255] as well as poorer response to treatment with ursodeoxycholic acid [256]. Therefore, non-invasive parameters for the identification of PBC/AIH overlap syndrome can help the design of personalized management of such patients. Different authors have shown that PBC/AIH overlap syndrome is particularly associated with antibodies to gp210 [249, 257] and to double-stranded DNA (dsDNA) [257, 258]. Autoantibodies to the tumor suppressor p53 were found to be highly associated with PBC/AIH overlap syndrome in Japanese patients (344), but this was not confirmed in a Canadian cohort [259]. Of interest, the combination of antibodies to dsDNA and elevated serum levels of IgG and alanine aminotransferase (ALT) is highly predictive of PBC/AIH overlap syndrome [257].

The Opportunity of Fine-Tuning the Diagnostic Scoring Systems of AIH and PBC Aiming at Improvement in PM in Liver Autoimmune Diseases

Specific autoantibodies play different roles in the scoring systems of AIH and PCB. Thus, for the diagnosis of AIH, the presence of anti-SMA, anti-LKM-1, and ANA scores one to three positive points each according to the titer (1/40, 1/80, and > 1/80, respectively), whereas the presence of AMA scores one negative point [207]. In contrast, for the diagnosis of PBC, the presence of AMA is one of the diagnostic criteria [208]. Since the antinuclear antibody (ANA) test demonstrates autoantibodies against antigens in the nucleus, cytoplasm, and mitotic apparatus, this test has been recently renamed as indirect immunofluorescence assay on HEp-2 cells (HEp-2 IFA) [11]. The HEp-2 IFA test provides useful elements for the scoring system of AIH and PBC, such as

the demonstration of ANA that integrates the scoring system for AIH as well as several nuclear (AC-3, AC-6, AC-11) and cytoplasmic (AC-21) patterns associated with autoantibody systems relevant to the diagnosis of PBC [257]. The presence of a positive HEp-2 IFA test at titer > 1/80 scores three positive points in favor of AIH. It should be noted, though, that the recommended criteria do not specify what pattern in the HEp-2 IFA test is relevant for the diagnosis of AIH. However, each pattern is associated with a specific set of autoantibodies and some IFA patterns may have confounding significance for the diagnosis of autoimmune hepatic diseases. For example, the cytoplasmic reticular-dotted pattern, classified as AC-21 by ICAP [11, 12], is strongly associated with AMA, thus representing a positive point for PBC diagnosis and a negative point for AIH diagnosis. In contrast, the homogeneous (AC-1) and the fine speckled (AC-4) nuclear patterns are regularly found in 50–80% of AIH patients. Moreover, the cytoplasmic fibrillary linear pattern (AC-15) is associated with SMA and anti-F actin antibodies that are relevant for the diagnosis of AIH. Therefore, the diagnostic recommendations for AIH and PBC should detail the pattern in the HEp-2 IFA test, as this would add value to the interpretation of this autoantibody test in the diagnosis and ultimately in the personalized approach to the patients.

Autoimmune Neurologic Diseases

Over the last 15 years, the field of neuroimmunology has experienced a remarkable development, mostly driven by the discovery of several autoantibodies against an array of neuronal surface and intracellular antigens, as well as glial and synaptic antigens. These autoantibodies were shown to be associated with a variety of clinical syndromes involving several regions of the central nervous system [260]. The utilization of tissue-based array (TBA) and cell-based array (CBA) platforms was instrumental to the discovery and now the routine detection of such autoantibodies. Each of the neurologic autoantibodies is associated with a peculiar spectrum of clinical manifestations (Table 12). Accordingly, the detection of neuronal autoantibodies helps in the differential diagnosis of several neurologic syndromes, and establishment of prognosis and therapeutic decisions, thus contributing substantially to the advancement of PM in neurology. Herein, we address the neuromyelitis optica spectrum disorders (NMOSD), the myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), autoimmune encephalitis (AIE), paraneoplastic encephalitis, and other neurologic conditions specifically associated with some autoantibodies.

NMOSD comprehends a group of demyelinating conditions characterized by episodes of myelitis, optic neuritis, and other neurological manifestations that mimic multiple sclerosis (MS) [261]. Approximately 80% of the NMOSD

Table 12 Clinical phenotypes and autoantibody associations in neurologic autoimmune diseases

<i>Disease phenotype/clinical manifestations</i>	<i>Autoantibody target</i>
NMOSD, optic neuritis, myelitis, area postrema syndrome	AQP4, MOG
ADEM	MOG
Encephalitis and orofacial dyskinesia	NMDAR, neurexin
Morvan syndrome	CASPR2
Encephalitis, fasciobrachial dystonic seizures	LGII
Peripheral hyperexcitability	GABA _A R, GlycR, GAD, CASPR2, DPPX
Limbic encephalitis	GABA _B R, GABA _A R, CASPR2, GAD, LGII, AMPA, NMDAR
Ataxia	mGluR1, GABA _B R, CASPR2, GAD, IgLON5
Refractory status epilepticus	NMDAR, LGII, GABA _A R, GABA _B R, GAD, AMPA
Encephalitis with parasomnia and brainstem dysfunction	IgLON5, GlycR
Encephalitis with myoclonus, hyperplexia, diarrhea	DPPX
Stiff-person syndrome (SPS)	GAD, GlycR, DPPX, GABA _A R, IgLON5
PERM	GAD, GlycR DPPX

NMOSD neuromyelitis optica spectrum disorder, *AQP4* aquaporin 4, *MOG* myelin oligodendrocyte protein, *ADEM* acute demyelinating encephalomyelitis, *NMDAR* *N*-methyl-D-aspartate receptor, *anti-CASPR2* contactin-associated protein-like 2, *LGII* leucine-rich glioma-inactivated-1, *GABA-AR* gamma-aminobutyric acid A receptor, *GAD* glutamic acid decarboxylase, *DPPX* dipeptidyl peptidase-like protein 6, *GABA-BR* gamma-aminobutyric acid B receptor, *AMPA* anti- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *mGluR1* metabotropic glutamate receptor 1, *IgLON5* IgLON family member 5, *GlycR* glycine receptor, *PERM* progressive encephalomyelitis with rigidity and myoclonus

patients have antibodies against the water channel astrocyte protein aquaporin 4 (AQP4), detected by IFA on aquaporin 4–transfected cells (CBA) and/or IFA on rat cerebellum, contributing substantially to the differentiation of NMOSD from MS [262]. Anti-AQP4 discovery led to the recognition of limited forms of the disease (i.e., recurrent transverse myelitis without optic neuritis) as well as symptoms beyond the spectrum associated with optic nerve and spinal cord involvement, such as the area postrema syndrome characterized by intractable nausea or hiccups [263]. Consequently, anti-AQP4 allowed the expansion of the scope of neuromyelitis optica eventually consolidated under the term NMOSD. Recent studies showed that NMOSD may coexist with other autoimmune conditions such as SLE and pSjS [261, 263]. Anti-AQP4 is best tested in peripheral blood as cerebrospinal fluid (CSF) testing is less sensitive. CBA for anti-AQP4 yields sensitivity of 75 to 80% and specificity greater than 99% for the diagnosis of NMOSD [263]. Anti-AQP4 activates complement and plays a role in promoting the cascade of immune-mediated inflammation. These findings suggest that some AQP4-seronegative patients with clinical and neuroimaging features of NMOSD have a different underlying pathogenesis requiring differentiated therapeutic approach [261]. Identification of anti-AQP4 helps personalizing treatment, as eculizumab, a C5 complement inhibitor, was shown to decrease relapse rates by 94% in seropositive NMOSD patients [264].

Myelin oligodendrocyte glycoprotein (MOG) is important in the myelination of nerves in the central nervous system (CNS). Anti-MOG antibodies have been known for

many decades and the initial studies suggested it was a biomarker of MS, but these results were hampered by older-generation immunoassays (ELISA, Western blot) that did not use MOG in its native conformation [265]. With the availability of CBA exposing native MOG at the cell surface, it was demonstrated that anti-MOG is a specific biomarker of a spectrum of CNS inflammatory disorders, often called MOG antibody-associated disease (MOGAD) [261, 266], composed of a constellation of clinical presentations, including myelitis, optic neuritis (often bilateral), NMOSD, acute demyelinating encephalomyelitis (ADEM), and more recently, encephalitis [267, 268]. MOGAD appears to have a particular predilection for children and young adults, but individuals at any age are at risk [269]. The clinical course may be monophasic or relapsing, and higher titers and persistence of anti-MOG over time predict a higher risk of relapse in children and adults [269, 270]. This feature is particularly helpful in the follow-up and personalized therapy of patients.

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) located in the neuron synaptic cleft [271]; thus, it is considered a synaptic antigen. Neurological syndromes associated to anti-GAD antibodies include the stiff-person syndrome (SPS), epilepsy, limbic encephalitis, downbeat nystagmus, cerebellar ataxia, and progressive encephalomyelitis with rigidity and myoclonus (PERM) [271, 272]. Anti-GAD is considered specific for an autoimmune neurological syndrome only when detected at high titer (more than 500 UI/mL) using ELISA assays,

especially in the cerebrospinal fluid (CSF), as low titer anti-GAD is regularly present in the peripheral blood of patients with type 1 diabetes mellitus [272, 273]. Approximately 80% of the patients with GAD-associated neurologic disease have concomitant organ-specific autoimmune disorders, such as type 1 diabetes mellitus, thyroiditis, pernicious anemia, or vitiligo [271]. Paraneoplastic association is very rare, but when a neoplasia is present, there is partial improvement after tumor treatment [271, 273].

AIE constitutes a group of inflammatory brain diseases characterized by prominent neuropsychiatric symptoms associated with autoantibodies against neuronal cell-surface proteins, ion channel molecules, and neurotransmitter receptors [274, 275]. Autoantibodies target molecules of the neuronal cell surface involved mostly in gabaergic and glutamatergic neurotransmission leading to a heterogeneous presentation of neuronal dysfunction. Autoantibodies may have agonistic or antagonistic receptor effects, block ion channel pores, disrupt the interaction with neighboring molecules, alter receptor localization at the membrane, or cause receptor internalization [276]. The epitope specificity of the autoantibodies influences the functional consequences upon the affected cells. Diverse conditions may trigger AIE, including tumors (paraneoplastic) and infections (parainfectious), but many cases are cryptogenic [275, 277–279]. AIE is the main consideration in the differential diagnosis of infectious encephalitis and its clinical manifestations include acute and subacute psychosis, various behavior changes, movement disorders, reduced level of consciousness, and seizures [279]. It is estimated that 20% of all encephalitis cases in northern Europe are immune-mediated [280] and 47% of encephalitis in patients under 30 years of age in the California Encephalitis Project were found to have AIE [281]. The estimated AIE annual incidence is 8–13 cases/100,000, occurring in all ages, although some types affect predominantly children and young adults [282, 283].

The most common type of AIE is anti-*N*-methyl-*D*-aspartate receptor (anti-NMDAR) encephalitis, characterized by acute/subacute psychosis, seizures, memory and speech impairment, as well as abnormal movements such as orofacial dyskinesias, dystonia, and choreoathetosis. Patients are usually children or young adults and may be severely ill, requiring mechanical ventilation and admission to intensive care unit due to decreased level of consciousness, refractory status epilepticus, autonomic dysfunction, or central hypoventilation [282]. Complementary investigation shows normal brain MRI in half of the patients and EEG findings include seizures or slow waves, although 30% of the cases show a specific pattern known as “extreme delta brush” [284]. Clinical criteria for anti-NMDAR encephalitis have been established and the presence of antibodies to the GluN1 subunit of the NMDA receptor is required for definite diagnosis [275].

Early autoantibody detection is of paramount importance as recent studies showed that the interval between initial symptoms and treatment is associated with prognosis in anti-NMDAR encephalitis [285]. Despite severe symptoms or prolonged coma, 81% of the patients show substantial recovery at 24 months upon early treatment, with an estimated mortality of 7% at 24 months [282]. Patients with AIE are treated with steroids combined with intravenous gamma globulin (IVIG) or plasma exchange. Approximately 50% fail to respond to the first-line therapy, requiring second-line treatment with rituximab and/or cyclophosphamide [275, 279, 282].

Other autoantibodies associated with AIE are anti-leucine-rich glioma-inactivated-1 (LGI1), anti-contactin-associated protein-like 2 (CASPR2), anti- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), anti-gamma-aminobutyric acid B receptor (GABA_BR), anti-gamma-aminobutyric acid A receptor (GABA_AR), anti-glycine, anti-dipeptidyl peptidase-like protein 6 (DPPX), anti-IgLON family member 5 (IgLN5), anti-metabotropic glutamate receptor 1 (mGluR1), and anti-neurexin. Certain autoantibodies are associated with specific syndromes recognizable on clinical grounds [274], such as ataxia, hyperexcitability syndrome, Morvan syndrome, brainstem impairment, or fasciobrachial dystonic seizures [269, 286–295]. Tables 12 and 13 summarize the most common clinical syndromes, phenotypes, and associated autoantibodies in autoimmune neurological syndromes.

Response to immunotherapy, recurrence rates, and frequency of paraneoplastic etiology vary among AIE subtypes [279]. For example, anti-AMPA encephalitis has a paraneoplastic basis in 64% of the cases, while anti-LGI1 encephalitis is rarely associated with malignancy [289, 295]. Moreover, tumors also vary according to the AIE subtypes. Thus, anti-NMDAR encephalitis frequently associates with ovary teratoma, whereas 40% of anti-CASPR2 encephalitis patients harbor a thymoma [287, 296]. Early testing and identification of onconeurologic autoantibodies can contribute to personalize paraneoplastic screening.

Autoantibodies targeting intraneuronal antigens are classically named onconeural antibodies (e.g., Anti-Hu, anti-amphiphysin, anti-Ma) because they are most frequently associated with paraneoplastic neurological syndromes. Paraneoplastic encephalomyelitis involves two or more regions of the nervous system and consequently the neurological involvement may present as different syndromes (Table 14). The identification of onconeural antibodies allows a personalized approach and the possibility of early detection and treatment of an incipient underlying neoplastic disease. In fact, each onconeural autoantibody has particular associations with specific neurologic manifestations and underlying neoplasia (Table 13).

Table 13 Autoantibodies, clinical manifestations, and tumor associations in autoimmune encephalitis and paraneoplastic syndromes

Autoantibody	Most frequent clinical manifestations	Tumor association
<i>Autoantibodies against intracellular antigens</i>		
Hu (ANNA-1)	Limbic encephalitis, encephalomyelitis, brainstem encephalitis, peripheral neuropathy	Lung, neuroendocrine, genitourinary
Ri (ANNA-2)	Cerebellar ataxia, brainstem encephalitis, opsoclonus-myoclonus, dystonia	Lung, breast, gynecological
Yo (PCA-1)	Cerebellar ataxia	Breast, ovarian cancer
PCA-2	Cerebellar ataxia, limbic encephalitis, brainstem encephalitis, peripheral neuropathy	SCLC
CV2/CRMP-5	Encephalomyelitis, myelitis, chorea, cerebellar ataxia	Breast, lung, thymoma
Tr (DNER)	Cerebellar ataxia, opsoclonus-myoclonus	Hodgkin lymphoma
Ma	Limbic encephalitis, brainstem encephalitis, parkinsonism	Testicular germ cell
GAD	SPS, PERM, cerebellar ataxia	Thymoma
Amphiphysin	SPS, PERM, cerebellar ataxia, encephalomyelitis, isolated myelopathy, peripheral neuropathy	Breast, lung
<i>Autoantibodies against membrane-bound antigens</i>		
LGI-1	Limbic encephalitis, refractory status epilepticus	Mostly non-paraneoplastic
CASPR2	Neuromyotonia (Morvan's syndrome), limbic encephalitis	Thymoma, lung
AMPA	Limbic encephalitis, refractory status epilepticus	Breast, lung, thymoma
GlycR	Hyperexcitability syndrome, stiff-person syndrome	Hodgkin, thymoma
NMDAR	Encephalitis with orofacial dyskinesia	Ovarian teratoma
GABA _A R	Encephalitis, refractory status epilepticus	Thymoma
GABA _B R	Limbic encephalitis, seizures, cerebellar ataxia, opsoclonus-myoclonus	SCLC
mGluR1	Cerebellar ataxia	Hodgkin lymphoma
mGluR5	Limbic encephalitis, Ophelia syndrome	Hodgkin lymphoma
AQP4	NMOSD	Lung, breast, thyroid, urogenital, thymoma

Adapted from Tirthani E, Said MS, Smith RG et al. Paraneoplastic encephalomyelitis. [Updated 2021 May 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK564519/>

ANNA anti-neuronal nuclear antibody, PCA Purkinje cytoplasm antibody, SCLC small cell lung carcinoma, CRMP5 collapsin response-mediator protein 5, GAD glutamic acid decarboxylase, SPS stiff-person syndrome, PERM progressive encephalomyelitis with rigidity and myoclonus, LGI1 leucine-rich glioma-inactivated-1, CASPR2 contactin-associated protein-like 2, AMPA α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, GlycR glycine receptor, NMDAR N-methyl-D-aspartate receptor, GABA_AR gamma-aminobutyric acid A receptor, GABA_BR gamma-aminobutyric acid B receptor, mGluR1 metabotropic glutamate receptor 1, mGluR5 metabotropic glutamate receptor 5, AQP4 aquaporin 4, NMOSD neuromyelitis optica spectrum disorders

The Manifold Conundrums of Autoantibody Determination

The determination of autoantibodies is not as straightforward as the determination of simple analytes, such as electrolytes

and common biochemical analytes (glucose, albumin, creatine phosphokinase, hemoglobin, etc.). A simple analyte is homogeneous in terms of chemical and physical properties, and this allows the development of laboratory assays that will perform equally in samples from any individual. In addition,

Table 14 Anatomical and clinical heterogeneity in paraneoplastic neurologic disease

Nervous system area	Main clinical presentation	Observations
Temporal lobes	Limbic encephalitis	Anti-Hu, anti-PCA-2, anti-Ma
Limbic areas	Paraneoplastic limbic encephalitis	~20% PEM cases; anti-Yo/Tr
Brainstem	Paraneoplastic rhombencephalitis, SPS, opsoclonus-myoclonus	~30% PEM cases; anti-Hu/Ma, anti-amphiphysin
Cerebellum	Paraneoplastic cerebellar degeneration	~15% PEM cases; anti-Yo/Tr
Spinal cord and dorsal root ganglia	Myelitis, subacute sensory neuropathy, SPS	May coexist with PEM, most anti-Hu
Autonomic nervous system	Autonomic dysfunction	~15% PEM

PEM paraneoplastic encephalomyelitis, SPS stiff-person syndrome

it is relatively easy to establish reference material for standardization and quality assessment, which contributes to the excellence of laboratory methods for determination of simple analytes. In contrast, autoantibodies represent a polyclonal immune response to a given autoantigen formed by a collection of immunoglobulins highly heterogeneous in terms of serum concentration, isotype, Fc glycosylation, epitope targeting, and avidity. Consequently, the collection of autoantibodies to antigen X in patient A is necessarily distinct from the collection of autoantibodies to antigen X in patient B. On the other hand, the various commercially available immunoassays have peculiarities in terms of the preferentially expressed epitopes, avidity requirement, sensitivity to serum concentration, isotype specificity, etc. As a consequence, the various available immunoassays for anti-X antibody frequently yield different results for the same anti-X-positive sample [92, 297–299]. This heterogeneity poses an overwhelming challenge in the establishment of reference materials for standardization and quality assessment, as any chosen reference material tends not to be representative to all individuals to be assayed.

One other perplexing aspect of autoantibody testing is the possibility of interference of natural autoantibodies, which occur frequently in the normal population and belong to the pool of natural antibodies. These are immunoglobulins produced by B1 lymphocytes with no previous immunization and being derived from germ-line genes not modified by mutations induced by antigenic stimulation [300, 301]. Isoagglutinins that react with blood group proteins A and B represent a prototype natural antibody. In general, natural autoantibodies occur at low titer and present low avidity and broad cross-reactivity. Natural autoantibodies can cause positive reactivity in very sensitive immunoassays, yielding clinically unexpected results. In particular, solid phase immunoassays (ELISA, western blot, other solid phase immunoassays) are particularly prone to detect antibodies with low concentration and low avidity that frequently can cause reactivity at the low positive range [302]. Therefore, the interpretation of results from these sensitive immunoassays should take into consideration the intensity of reactivity of the sample of interest with respect to the cut-off value for that assay.

These limitations imply that a positive result from an autoantibody immunoassay should be strictly interpreted taking into consideration the clinical context. A request for autoantibody immunoassays in a low pre-test probability context frequently generates unexpected positive results that may ensue misdiagnosis, inappropriate treatment, and increase in health costs. On the other hand, the detection of a specific autoantibody in individuals with ill-defined clinical disease may contribute to early diagnosis and treatment, promoting optimal outcomes for the patient as well as savings in health costs [303]. The thin line separating the above scenarios requires clinical expertise, optimal laboratory practice, and communication between the clinician and the laboratory clinical pathologist.

Fine-Tuning the Results of Autoantibody Assays Ahead of the “Positive/Negative Paradigm” as a Step Forward to PM

Possibly because of early methodological approaches (e.g., gel immunodiffusion/immunoprecipitation), the “qualitative paradigm” is prevalent in the interpretation and reporting of autoantibodies. Usually, a patient is frequently referred to as being “positive” or “negative” for a given autoantibody. However, current immunoassays provide semi-quantitative results that allow exploring the quantitative dimension of the humoral autoimmune response. In fact, various autoantibodies have specific clinical relevance at higher autoantibody serum concentrations. For example, anti-cardiolipin antibodies are considered a criterion for the classification of antiphospholipid syndrome only at titer equal to or greater than 40GPL or 40MPL [53] and increasing titers of anti-dsDNA antibodies are frequently associated with active lupus nephritis [27]. The “semi quantitative paradigm” is also relevant to the HEp-2 IFA, as the titer plays an important role in the clinical relevance of a positive HEp-2 IFA test in a given individual. By using receiver-operator characteristic (ROC) curve analysis, it has been shown that the performance of the HEp-2 IFA test for the diagnosis of systemic autoimmune diseases is significantly higher at titer equal to or greater than 1/1280 and that low titers are frequently observed in healthy individuals and patients with non-autoimmune diseases [8, 15, 233]. The result-specific likelihood ratio has been shown to increase with increasing HEp-2 IFA titer [304]. Therefore, aiming at the advancement of PM, the mindset of clinicians and clinical pathologists regarding autoantibody test results should be changed toward the “semi quantitative paradigm.”

Another opportunity for fine-tuning the interpretation of autoantibody tests is the recognition of specific patterns. The HEp-2 IFA test is widely used for detection of autoantibodies to a vast array of autoantigens in several systemic autoimmune diseases [11, 12]. However, this test has low specificity, as a positive result is observed also in healthy individuals [7, 8, 133, 232, 305] and in patients with non-autoimmune diseases [15, 233]. Nonetheless, there is opportunity for increasing the specificity of HEp-2 IFA results by the refined definition of the HEp-2 IFA pattern. In analogy to the importance of pattern definition in chest and brain MRI, the definition of the HEp-2 IFA pattern can discriminate various specificities of a positive HEp-2 IFA test, indicating the most probable involved autoantibody specificities and the possible clinical relevance. Thus, the homogeneous nuclear pattern (AC-1) is frequently associated with antibodies to dsDNA, nucleosome, and histones, and is frequently found in patients with SLE, juvenile rheumatoid arthritis, and autoimmune hepatitis [11]. In contrast, the dense fine speckled nuclear pattern (AC-2) is strongly associated with antibodies

to DFS70 and rarely occurs in systemic autoimmune diseases, but is frequently observed in healthy individuals [8, 306] and patients with non-autoimmune diseases [233, 307–309]. Recognizing the importance of HEp-2 IFA pattern in the interpretation of the test, an international group of specialists launched the ICAP, an initiative committed to define and harmonize the nomenclature of HEp-2 IFA patterns [12]. The most relevant and frequent patterns have been consented and organized into a classification algorithm and each HEp-2 IFA pattern was assigned an alpha-numeric code (AC-#, for Anti-Cell). The ICAP website www.anapattern.org displays an interactive content including the HEp-2 IFA classification tree with representative images from each pattern, recommended nomenclature, immunologic associations, clinical relevance, pertinent bibliography, and frequently asked questions.

Concluding Remarks

Precision Medicine has been pursued along the history of medicine and in retrospect it is clear that a tremendous progress has been made, as nosology groups and subgroups have been defined progressively allowing continuous optimization of therapeutic strategies for each of these subgroups. However, it is also clear that much progress is needed to achieve the ultimate fulfillment of the conception of precision medicine. The path for such progress has been increasingly accelerated in the last decades with the steady incremental discovery of various biomarkers, progressive understanding of the phenotypic relevance of thousands of polymorphic genes, determination of the microbiome associated with physiological and pathological scenarios, determination of the epigenetic modifications in various biologic states, availability of multiplex assays, and use of artificial intelligence and machine learning required to meaningfully extract trends, conjunctions, and focal points in very large datasets. Autoantibodies and other immunological parameters constitute important elements in the complex network being integrated for the establishment of a more advanced degree of medicine personalization in the near future.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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