

Clinical Significance of Uncommon, Non-Clinical, and Novel Autoantibodies

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Abstract: Autoantibodies are a common mark of autoimmune reaction and their identification in the patients' serum, cerebrospinal fluid, or tissues is generally believed to represent diagnostic or prognostic biomarkers of autoimmune diseases or autoinflammatory conditions. Traditionally, autoantibody testing is an important part of the clinical examination of suspected patients, and in the absence of reliable T cell tests, characterization of autoantibody responses might be suitable in finding causes of specific autoimmune responses, their strength, and sometimes commencement of autoimmune disease. Autoantibodies are also useful for prognostic stratification in clinically diverse groups of patients if checked repeatedly. Antibody discoveries are continuing, with important consequences for verifying autoimmune mechanisms, diagnostic feasibility, and clinical management. Adding newly identified autoantibody-autoantigen pairs to common clinical laboratory panels should help upgrade and harmonize the identification of systemic autoimmune rheumatic disorders and other autoimmune conditions. Herein, we aim to summarize our current knowledge of uncommon and novel autoantibodies in the context of discussing their validation, diagnostic practicability, and clinical relevance. The regular updates within the field are important and well justified.

Keywords: autoimmune diseases, autoantibodies, autoantigen, immunological disorders

Introduction

Growing epidemiological data provide confirmed evidence of a continuous dramatic rise in autoimmune disorders in industrial countries during the last decades.¹ More than 5% of people worldwide may suffer from autoimmune diseases, which collectively include almost 100 conditions, such as mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), scleroderma (SSc), atopic dermatitis, Crohn's disease, ulcerative colitis, lupus nephritis, dermatomyositis, myasthenia gravis, celiac disease, autoimmune thyroid, liver and gastric diseases, autoimmune neuropathies, and many other autoimmune conditions.² The prevalence of autoimmune disorders in the USA now impacts more than 10% of the population and almost 50 million people could experience different appearance of an autoimmune reaction in the USA.³ In addition, autoimmune pathology might be connected with a variety of other diseases, including immunodeficiency, cancer, infections, like SARS-CoV-2, cardiovascular and certain neurodegenerative illnesses.^{4–6} For instance, new data demonstrate that rheumatoid arthritis may be linked with an increased risk of Parkinson's disease, and RA seropositivity presents a higher risk of Parkinson's disease.⁷

Nevertheless, of the tens of millions of people diagnosed with modest and rigorous forms of chronic autoimmune disorders, only about 15% obtain advanced modern therapies, and only 5% achieve sufficient clinical responses. A key obstacle to the development and utilization of efficient novel therapies and treatment concepts has been the complexity and divergence of autoimmune diseases, which markedly limits our understanding of disease immunopathology in different tissues and organs.⁸ The deviations of the disease pathways may be further illustrated by numerous clinical phenotypes⁹ and polyautoimmunity¹⁰ in spite of the generally accepted concept of autoimmune tautology.¹¹ Another important barrier to the development of new medicines designed for differentiated patient populations is the identification of multidimensional biomarkers for mechanistic stratification and for early and specific diagnosis of autoimmune and autoinflammatory conditions.^{12–14}

Specific autoantibodies are conventional indicators of autoimmune diseases, and new data suggest that an increase in autoantibodies' revealed in the US population may reflect a growth of autoimmune diseases seen in the last decades. Even though clinical data have revealed autoantibodies as a prognosticator of certain autoimmune disorders, including inflammatory bowel disease (IBD), SLE, RA, scleroderma, Type 1 diabetes (T1D), and other conditions,^{15–20} new results advocate for more personalized clinical data, like genetics and patterned biomarkers, as well as improved data examination techniques to augment the overall predictive and diagnostic power of antinuclear antibodies (ANA) and other “classical” clusters of autoantibodies.^{21,22}

Traditionally, autoantibody testing is recommended as a part of the patient's workout when it is clinically reasonable and appropriate.²³ Since the discovery of specific autoantibodies in lupus erythematosus in 1948 and 1957,^{24,25} autoantibody tests are generally conducted to assist in diagnosing many autoimmune diseases and conditions and are used in both screening and confirmational settings (Table 1). Sometimes, these immunoassays are utilized to help estimate the severity of the illness, observe the progress of the disease, and measure the effectiveness of treatments. Because of the lack of

Table 1 Examples of Autoantibodies and Associated Autoimmune Diseases

Known Autoantibodies by Groups	Autoimmune diseases
<i>ANA, ENA and other Antibodies</i>	Connective Tissue Diseases: Systemic Lupus Erythematosus (SLE) Systemic Sclerosis (SSc) (Scleroderma) Sjögren's Syndrome (SS) Mixed Connective Tissue Disease (MCTD)
Anti-dsDNA, nucleosomes, histones AB Anti-Ro/SS-A (60kD) AB Anti-La (SS-B) AB Anti-ribonucleoprotein (U1RNP) AB Anti-Sm (Smith) and anti-Sm/RNP AB Anti-RNP (A and 60kD) Anti-DNA topoisomerase I (SCL70) AB Anti-chromatin AB Anti-centromere (centriole) AB (ACA) (pericentrin, ninein, Cep250, Cep110, PCM-1, enolase) Anti-proliferating cell nuclear antigen (DNA) polymerase δ (cyclin) AB Anti-nuclear membrane (lamins A/B/C, lamin associated proteins 1/2) AB Anti-nuclear dots (Sp-100, PML) AB Anti-salivary protein I (SPI) AB Anti-carbonic anhydrase II and IV AB Anti-parotid secretory protein (PSP) AB Anti-angiotensin II type I receptor (AT1R) AB Anti-endothelin-I type A receptor (ETAR) AB	
<i>Anti-Cytoplasmic Antibodies</i>	Systemic Lupus Erythematosus Autoimmune hepatitis (AIH type I) Lupus nephritis (LN) Sjögren syndrome Mixed motor and sensory neuropathy Idiopathic inflammatory myopathies (polymyositis, dermatomyositis, immune-mediated necrotizing myopathy) Raynaud phenomenon Primary biliary cholangitis (PBC) Hashimoto's thyroiditis Systemic sclerosis Rheumatoid Arthritis (RA)
Anti-F-actin AB (anti-smooth muscle (SMA)) Anti-vimentin AB Anti-cytokeratin 8, 18, 19 AB Anti-tubulin AB Anti-cytoplasmic RNP (GW bodies (GWB): GW182, Su/Argonaute-2, RAP-55, Ge-1/hedls, diacyl-phosphatidyl ethanolamine) AB Anti-ribosomal AB: anti-P (P0 (38 kDa)), P1 (19 kDa) and P2 (17 kDa) Anti-mitochondrial AB (AMA): inner (M1, M2, M7) and outer mitochondrial membranes (M3, M4, M5, M6, M8, M9) antigens Anti-Golgi (giantin, golgins) AB (AGA) Anti-rods/rings AB (anti-RR) (inosine monophosphate dehydrogenase (IMPDH), cytidine triphosphate synthase I (CTPSI)) Anti-Jo-1 (tRNA synthetases) AB	

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Table 1 (Continued).

Known Autoantibodies by Groups	Autoimmune diseases
<i>Anti-mitotic Antibodies</i>	Sjögren's syndrome
Anti-mitotic spindle apparatus (MSA) AB (NuMA-1, NuMA-2, centrophilin, HsEg5)	Systemic sclerosis
Anti-midbody (Intracellular bridge) AB (Aurora Kinase B, CENP-E, MSA-2, KIF-14, MKLP-1)	Primary biliary cholangitis
Anti-mitotic chromosome (mitotic chromosome coat, H3, MCA-1) AB	Systemic Lupus Erythematosus
	Raynaud's syndrome
	Polymyalgia rheumatica
<i>Anti-Neutrophil Cytoplasmic Antibodies (ANCA)</i>	Granulomatosis with polyangiitis (GPA) (Wegener's granulomatosis)
Anti-myeloperoxidase (MPO) AB (pANCA)	Microscopic polyangiitis (MPA)
Anti-proteinase 3 (PR3) AB (cANCA)	Eosinophilic granulomatosis with polyangiitis (EGPA) (Churg-Strauss syndrome)
Anti-lysozyme AB	
Anti-elastase AB	
Anti-cathepsin G, B and D AB	
<i>RA Antibodies</i>	Rheumatoid Arthritis (RA)
IgM rheumatoid factor (IgM-RF), IgA-RF, IgG-RF	
Anti-cyclic citrullinated peptide (CCP) AB	
Anti-mutated citrullinated vimentin (MCV, Sa antigen) AB	
Anti-carbamylated protein (CarP) AB	
<i>Myositis Antibodies</i>	Polymyositis
Anti-Jo-1 (tRNA synthetases) AB	Dermatomyositis
Anti-Zo (phenylalanyl-tRNA synthetase) AB	Scleromyositis
Anti-YRS (tyrosyl-tRNA synthetase) AB	Inclusion body myositis
Anti-PL-7 (threonyl-tRNA synthetase) AB	
Anti-PL-12 (alanyl-tRNA synthetase) AB	
Anti-OJ (isoleucyl-tRNA synthetase) AB	
Anti-EJ (glycyl-tRNA synthetase) AB	
Anti-KS (asparaginyl-tRNA synthetase) AB	
Anti-Mi-2 (nucleosome remodeling deacetylase complex Mi-2a (240 kDa) and Mi-2b (218 kDa)) AB	
Anti-signal recognition particle (SRP) (protein-RNA cytoplasmic complex of 7SL RNA and polypeptides (72, 68, 54, 19, 14, 9 kDa) AB	
Anti-transcription intermediary factor-1 γ (TIF-1 γ) AB	
Anti-small ubiquitin-like modifier activating enzyme (SAE1/2) AB	
Anti-melanoma differentiation antigen 5 (MDA-5, CADM-140) AB	
Anti-MJ (nuclear protein 2 (NXP-2)) AB	
Anti-PMS1 (DNA repair enzyme) AB	
Anti-ribonucleoprotein (U1/U2/U3 (fibrillarin) RNP) AB	
Anti-Ku (DNA-dependent protein kinase regulatory subunit (70/80 kDa) AB	
Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) AB	
Anti-cytosolic 5'-nucleotidase 1A (cN1A) AB	

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Table 1 (Continued).

Known Autoantibodies by Groups	Autoimmune diseases
<i>Anti-Phospholipids Antibodies</i>	Anti-phospholipid syndrome (APS)
Anti-cardiolipin (CL) AB Anti-beta-2 glycoprotein I (β 2GPI) AB Anti-phosphatidic acid (P-acid) AB Anti-phosphatidylcholine (PC) AB Anti-phosphatidylethanolamine (PE) AB Anti-phosphatidylglycerol (PG) AB Anti-phosphatidylinositol (PI) AB Anti-phosphatidylserine (PS) AB Anti-annexin 5 (AN5) AB Anti-prothrombin (PT) AB	
<i>Blood cell Autoantibodies</i>	Immune thrombocytopenia (ITP) (immune thrombocytopenic purpura) Autoimmune hemolytic anemia (AIHA) Acquired Thrombotic thrombocytopenic purpura (TTP)
Anti-platelet glycoproteins IIb/IIIa (CD41/61; fibrinogen receptor), GPIb/IX (CD42c/CD42a), GPV (CD42d) AB Anti-RBC AB Anti-ADAMTS13 AB	
<i>Endocrine Autoantibodies</i>	Autoimmune thyroiditis (Graves' diseases, Hashimoto's thyroiditis) Type 1 Diabetes Mellitus Addison's disease (Autoimmune adrenalitis) Schmidt syndrome Autoimmune pancreatitis
Anti-thyroid peroxidase (TPO) AB Anti-thyroglobulin (TG) AB Anti-thyrotropin (thyroid-stimulating hormone) receptor (TR, TSHR) AB Anti-glutamic acid decarboxylase 65 (GAD65) AB Anti-insulinoma-associated protein 2 (IA-2) AB Anti-insulin AB Anti-zinc transporter (ZnT8) AB Anti-pancreatic (glycoprotein 2 (GP2) and CUB zona pellucida-like domain 1) AB Anti-21-hydroxylase AB Anti-17-hydroxylase AB Anti-lactoferrin AB Anti-carbonic anhydrase AB	
<i>Skin Autoantibodies</i>	Scleroderma Dermatitis herpetiformis (Duhring disease) Psoriasis Autoimmune bullous skin diseases (pemphigus vulgaris, pemphigus foliaceus, bullous lupus erythematosus, bullous pemphigoid)
Anti-cathelicidin LL-37 AB Anti-melanocytic ADAMTSL5 AB Anti-lipid antigen PLA2G4D AB Anti-keratin 17 AB Anti-vinculin (vinculin α -actinin) AB Anti-epidermal transglutaminase (eTG) AB Anti-laminin-332 AB Anti-p200/laminin- γ 1 AB Anti-BP180 and anti-BP230 AB Anti-collagen type VII (NC1 and NC2 domains) AB Anti-desmoglein (Dsg) 1 and 3 AB Anti-envoplakin AB Anti-epithelial basement membrane zone (BMZ) AB Anti-Herpes gestationis factor (HGF) AB	

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Table 1 (Continued).

Known Autoantibodies by Groups	Autoimmune diseases
<i>GI Autoantibodies</i>	Autoimmune gastritis
Anti-adenosine triphosphatase (H+/K+-ATPase) of gastric parietal cells AB	Inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis (UC))
Anti-intrinsic factor of parietal cells AB	Celiac disease (CD)
Anti-glycoprotein 2 (GP2) AB	Pernicious anemia
Anti-tissue transglutaminase (tTG) AB	
<i>Kidney Autoantibodies</i>	Goodpastures syndrome (Glomerulonephritis Type I)
Anti-GBM (glomerular basement membrane collagen Type IV protein) AB	ANCA Glomerulonephritis
ANCA	
<i>Liver Autoantibodies</i>	Primary biliary cholangitis (PBC)
Anti-mitochondrial AB (AMA-M2, PDH-E2, BCOADC-E2, OGDC-E2)	Primary sclerosing cholangitis (PSC)
Anti-smooth muscle AB (SMA) (anti-F-actin AB)	AIH type 1
Anti-liver-kidney microsome (LKM) 3 (UGT1A) AB	AIH type 2
Anti-LKM-1 (cytochrome P450 2D6) AB	
Anti-soluble liver antigen/liver-pancreas antigen (SLA/LP) AB	
Anti-liver cytosol type 1 (LC1, formiminotransferase cyclodeaminase) AB	
Anti-Kelch-like 12 AB	
Anti-hexokinase 1 AB	
Anti-glycoprotein 2 (GP2) IgA	
Anti-nuclear membrane (lamins A/B/C, lamin associated proteins 1/2) AB	
Anti-nuclear dots (Sp-100, promyelocytic leukemia (PML) factor) AB	
<i>Nervous System Autoantibodies</i>	Multiple sclerosis (MS)
Anti-myelin AB	Neuromyelitis optica (NMO)
Anti-aquaporin 4 (AQP4) AB	Neuromyelitis optica spectrum disorder (NMOSD)
Anti-acetylcholine receptor (AChR) AB	Myasthenia gravis
Anti-NR2 glutamate receptor AB	Autoimmune encephalitides
Anti-N-methyl-D-aspartate receptor (NMDAR) AB	Autoimmune encephalomyelitis
Anti-γ-aminobutyric acid (GABA) receptors AB	Paraneoplastic neurological syndromes (eg, paraneoplastic encephalomyelitis)
Anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors AB	Lambert Eaton myasthenic syndrome
Anti-metabotropic glutamate receptor type 5 (mGluR5) AB	Anti-MAG neuropathy
Anti-immunoglobulin-like cell adhesion molecule 5 (IgLON5) AB	
Anti-voltage-gated calcium channels (VGCC) AB	
Anti-myelin oligodendrocyte glycoprotein (MOG) AB	
Anti-myelin associated glycoprotein (MAG) AB	
Anti-leucine-rich glioma-inactivated protein 1 (LGII) AB	
Anti-contactin-associated protein 2 (CASPR2) AB	
Anti-glial fibrillary acidic protein (GFAP) AB	
Anti-neuronal nuclear (ANNA-1, anti-Hu/HuD) AB	
Anti-Ri (ANNA-2) AB	
Anti-Yo (Purkinje cell cytoplasmic antibody type 1, PCA-1) AB	
Anti-PCA2 AB	
Anti-Tr (Delta/Notch-like epidermal growth factor-related receptor, DNER) AB	
Anti-Rho-associated coiled-coil containing protein kinase 2 (ROCK2) AB	
Anti-ganglioside (GM1-4, GD1a/b, GD2/3, GT1a/b, GQ1b) AB	
Anti-collapsin response mediator protein 5 (CRMP5/ CV2) AB	
Anti-amphiphysin AB	

Note: AB, autoantibody.

available and approved clinical T cell functional assays, characterization of autoantibody levels might be helpful in recognizing stimuli of specific autoresponses. This can be done by inspecting seroconversion and strength of the autoantibody reaction, which might reflect the onset of an autoimmune disease, especially in individuals with specific genetic predisposition. Specific patterns of autoantibodies might additionally help with extrapolative stratification in clinically diverse groups of patients if checked dynamically.

Although detection of autoantibodies is a common diagnostic tool, the list of “standardly” tested autoantibodies is limited in most clinical laboratories in spite of a broad spectrum of known human self-antigens/autoantibodies pairs. For instance, uncommon or rare types of autoantibodies, detected by fluorescent microscopy on a cellular substrate during an initial screening, include cytoplasmic and mitotic cellular patterns.²⁶ These and other non-clinical autoantibodies are not regularly reported by many clinical laboratories because the clinical relevance of these biomarkers has not been entirely elucidated.²⁷ Another example is novel autoantibodies that have been recently identified in specific cohorts of patients with known autoimmune diseases or animal models of human disease. Unrecognized or unverified clinical potential of new autoantibodies significantly limits their incorporation into standardized approved panels of diagnostic autoantibodies. Regular updates of current data focusing on uncommon, rare, non-clinically granted and novel autoantibodies discussing their validation, prevalence, diagnostic utility, and relevant clinical association are important and well justified.

Autoantibodies: Pathophysiology

A wide repertoire of serum and tissue-localized autoantibodies recognizing key elements of the cell, such as cytoplasmic organelles, nuclear molecules, membrane-bound receptors, or other structural apparatuses, have been identified in autoimmune diseases (Table 1). Although their occurrence may have a significant part in the diagnosis, verification and sorting of a disorder, their specificity is not always proven. For instance, 180 autoantibodies were thus far detected in SLE patients with 122 of them correlating with disease severity.²⁸ Analysis of autoimmune regulator (AIRE)-deficient patients revealed that they collectively expressed antibodies targeting more than 3700 human proteins, which, at least partly, may represent some protective autoantibodies.²⁹ Additionally, many longstanding clinical reports revealed that individuals might have detectable autoantibodies for years prior to manifesting clinical disease,^{30,31} which suggests that perceiving these serum biomarkers may have marked predictive value.

Since the description of natural autoantibodies (against sperm, crystalline lens, and red blood cells) more than 100 years ago by Elie Metchnikoff and Alexander Besredka,^{32–35} the phenomenon of autoimmunological reactivity began to be accepted in spite of the fact that other leading immunologists of the time, including Paul Ehrlich and Sir Frank Macfarlane Burnet expressed dislike of autoimmunity as a physiological reaction.^{36,37} Interestingly, Besredka proposed that antibodies targeting self-antigens (autoantigens) may be targeted by “anti-antibodies”. In addition to Metchnikoff’s and Besredka’s reports demonstrating the existence of autoantibodies, Karl Landsteiner and Julius Donath in 1904 provided a clear indication that autoantibodies are able to induce disease: they demonstrated how “hemolysins” serving as autoantibodies could induce paroxysmal cold hemoglobinuria, an autoimmune hemolytic anemia.³⁸ However, despite the growing clinical data depicting a number of autoimmune diseases, experimental and clinical studies of the autoimmune disease pathogenesis were diminished for several decades.³⁹ However, later the presence of antibodies in injured organs and tissues, and in associated local or systemic inflammation confirmed that autoantibodies are directly and indirectly involved in the development of different autoimmune conditions.

Clinical and experimental results revealed several autoantibody-mediated mechanisms leading to tissue and cell damage. Many autoantibodies are directly injurious following binding to surface membranes of targeted cells or deposition in tissues. Subsequent cytotoxic effects may involve either complement-mediated or antibody-dependent cell-mediated or phagocytosis-mediated, or antibody internalization-dependent mechanisms. The formation of immune complexes in the tissue or the formation of tissue deposits by circulating immune complexes is another common pathway of autoantibody-induced cytotoxic effects. Recognition of different cell surface molecules by autoantibodies might block (an antagonistic effect), stimulate (an agonistic action), or alter the affinity of cell membrane receptors changing the functional activity of cells in the organ or tissue. Furthermore, autoantibodies recognizing certain intracellular proteins can target cell surface molecules because of existing cross-reactivity between the extracellular and intracellular antigens or due to translocation of the intracellular molecules to the outer membrane after cell activation or cell damage. For

instance, amplification of inflammation and extension of assembly of autoantibodies as a result of self-nucleoprotein transport to the cell surface to bind to toll-like receptors has been suggested.⁴⁰ Finally, experimental findings and clinical observations propose that certain autoantibodies are able to enter into living cells.^{41–43}

While autoantibodies directed against extracellular proteins are often directly pathogenic, autoantibodies targeting intracellular antigens are often known as suitable indicative markers of irregular autoimmune reactions. However, the clear antigenicity versus pathogenicity relationship is not always direct.^{44,45}

Interestingly, although autoantibodies are frequently linked with different pathogenic effects, they can additionally demonstrate disease-amending purposes that may be favorable for affected individuals.⁴⁶ For instance, cytokine-neutralizing antibodies with the immunosuppressive activity might cause a less critical symptom in some autoimmune disorders.^{47,48} Also, anti-tumor antibodies might be linked with a better prognosis in patients with different malignancies.^{49–51} Autoantibodies may delay proteolysis of regulatory peptides and hormones and provide transportation to specific cells and tissues.⁵² Many natural polyreactive/autoreactive antibodies created in the absence of exogenous antigens may be crucially involved in the principal protection from certain viral, bacterial and parasitic infections, as well as cancer,^{40,53–55} although their contribution to autoimmune disease has also been demonstrated.⁵⁶ As natural antibodies are recognized for their wide reactivity against self-antigens, whether their targeting of foreign constituents is the common consequence of cross-reactivity against self-antigens is still a matter of debate.^{57,58}

Autoantibodies: Detection

Today, many “old”, “classic”, and modern techniques are available for the detection, identification, and recognition of autoantibodies in biological fluids and tissue samples. Some methodologies are only suitable for biomedical research, and some are utilized on a regular basis for laboratory clinical diagnostics. A broad array of immunoassay platforms includes immunoprecipitation (followed by the analysis of the purified immune complexes), immunodiffusion (Ouchterlony and Mancini assays), immunoblotting or dot immunoblotting, immunohistochemistry, ELISA-based tests including multiplexed versions and bead-based assays, chemiluminescence, immunofluorescence on unmodified or transfected cells (HEp-2, HEK 293, neutrophils) or rodent and primate tissues, and modern high-throughput screening assays utilizing recombinantly produced autoantigens or a yeast surface display-based high-throughput technique.^{45,59} For instance, protein array methodology facilitated the creation of new or improved groups of autoantibody-based biomarkers by the searching for the immune reactivity recognizing hundreds of identified antigens, which can be dotted on microchips for practical screening of tested serum specimens.⁵⁹

Although detecting autoantibody immunoreactivity grants foundational knowledge for the analysis of different autoimmune conditions, autoantibodies may also clarify a substantial portion of the clinical and morphological differences existing in affected patients.⁶⁰ Thus, identification of critical autoantibody panels in tested individuals presents the capacity to disclose important etiologic elements and therapeutic approaches. However, despite the expanding knowledge of immunological regulatory pathways in the last decades, many challenges associated with autoantibodies, especially newly identified, remain unresolved, including the mechanism involved in the breaking of immunological unresponsiveness and recognizing the nature of the autoimmunity-induced damage mediated by these mechanisms.

Extension of Autoantibody-Based Diagnostics with Uncommon Autoantibodies

The continuum of many well-characterized autoantibodies is frequently useful for a specific autoimmune reaction. In the 80 most frequently detected autoimmune disorders, about 110–130 from the approximate 20,000 proteins coded by the human genome cover the prevalent antigenic molecules.⁶¹ Nevertheless, a growing body of novel autoantibodies are constantly identified in many common and especially rare diseases, suggesting additional pathogenetic pathways may exist in the autoimmune conditions linked to the autoantibody formation. At the same time, various autoimmune disorders are considerably underrecognized in clinical practice, primarily because of diverse symptomatic presences making the first diagnosis difficult or often not possible: pathogenic autoimmune reactions remain undiagnosed in more than half of affected individuals.⁴⁴ Appropriate and quick diagnosis of suspected autoimmune conditions is critically

important for a disease prognosis, selection of a proper treatment plan, prior to irreparable injury to the involved tissues and organs occurs.

The identification of autoantibodies is an established hallmark of many autoimmune and autoinflammatory conditions, and the presence of serum autoantibodies, especially in high titer, is important for the diagnosis and sorting of different autoimmune disorders.⁶² For instance, ANA test is a common tool for the initial clinical screening approach for various autoantibodies - the immunofluorescent ANA test is the accepted “gold standard” for autoantibody verification as it can visualize antibody binding to 130–150 possible autoantigens on HEp-2 cells serving as a substrate and source of human antigens. Furthermore, the association of the fluorescent pattern (homogeneous, speckled, nucleolar, etc.) with the class of nuclear autoantibodies (dsDNA, RNP, centromeres, Smith antigen, etc.) and specific autoimmune conditions is well established. However, little is proven regarding the diagnostic utilization of uncommon ANA patterns, probably because of their minimal prevalence,⁶³ although such staining patterns may help in earlier detection or confirmation of developing autoimmune reactions. For instance, certain ANA patterns, such as the nuclear centromere, nuclear large/coarse speckled and mitotic spindle fibers may help in diagnosis of SLE and RA.²⁷ The nuclear envelope pattern may be associated with primary biliary cholangitis (PBC) and may also have a negative prognostic significance.^{64,65} Unfortunately, many clinical labs do not yet consistently state such ANA patterns, and many practitioners may not have the necessary expertise in their interpretation.

In addition, the cytoplasmic and mitotic cellular patterns seen in HEp-2 cells are still underestimated and require further assessment and incorporation in routine clinical practice.²⁶ While reporting non-nuclear HEp-2 cell patterns is considered clinically applicable, there is no strong agreement in considering them as a negative or positive result.⁶⁶ Analysis of ANA reports from 68 countries encompassing 118 laboratories revealed that 55% of laboratories reported cytoplasmic patterns as “ANA positive results”, 33% described isolated cytoplasmic patterns as “ANA negative” with other teams claiming cytoplasmic staining as “ANA negative results with notice” indicating the presence of cytoplasmic staining. Some laboratories reveal cytoplasmic cellular staining on HEp-2 cells with medium or strong positivity only per request from ordering clinicians.⁶⁷ Surprisingly, in answering the questionnaire, several laboratories stated the difficulties in recognizing different cytoplasmic cellular patterns. Alternative survey of 438 medical laboratory professionals and 248 practitioners from 67 countries revealed that cytoplasmic and mitotic cellular patterns are recognized by >70% of participants.⁶⁸

Cytoplasmic cellular patterns may, for example, provide clinically relevant insights into autoimmune liver diseases, including the suspected disease entity. Analyzing subgroups of patients with autoimmune liver diseases with reticular/mitochondrial or cytoplasmic speckled cellular staining, Cha et al reported significant differences. Patients with a reticular cytoplasmic staining showed a higher positive frequency for mitochondrial autoantibodies and a lower positive frequency for smooth muscle autoantibodies and nuclear ANA staining than people who exhibited the speckled cytoplasmic pattern.⁶⁹ Therefore, they concluded that positive cytoplasmic reticular staining patterns should be employed to manage autoimmune liver disease description in presumed clinical cases.

Furthermore, the cytoplasmic speckled IFA patterns in HEp-2 cells are a relatively frequent result and may be related to different autoantibodies. Cytoplasmic fine speckled pattern may be associated with anti-Jo1 antibodies, which are found in patients with anti-synthetase syndrome, while cytoplasmic dense fine speckled pattern could be related to anti-ribosomal P antibodies associated with SLE or anti-PL7/PL12 antibodies associated with anti-synthetase syndrome.^{70,71} The anti-synthetase syndrome is progressively diagnosed and accepted as a pleomorphic entity, which can be clinically seen as isolated arthritis or interstitial lung disease. However, common reflex autoantibody-specific testing usually recognizes only anti-Jo-1. Therefore, evaluation of a new commercially available synthetase profiling dot-blot assay, which includes antibodies targeting Jo1, PL7, PL12, EJ, OJ, KS, ZO, HA, SRP and Ribosome P0 antigens, in serum specimens with a cytoplasmic speckled IFA pattern on HEp-2 cells allowed the detection of a substantial number of individuals with infrequent anti-synthetase antibodies and a partial or atypical clinical picture.⁷⁰ This suggests that appropriate multiplexed testing approaches for identified positive cytoplasmic speckled staining may generate important data and whenever possible must be employed as standard IFA reflex assay.

Mitotic cellular patterns seen in HEp-2 cells generally reflect cell domains involved in mitosis and are judged as the most uncommon of the accepted IFA groups. The importance and interpretation of mitotic patterns and their reasonable clinical connection are still a clinical diagnostic challenge because of the extremely low occurrence of these IFA staining

patterns. However, new evidence suggests the necessity of considering mitotic patterns in routine reports for improved patient stratification.^{72,73} For example, examination of nationwide IFA HEp-2-positive data focusing on anti-mitotic spindle apparatus (MSA) cellular patterns (NuMA/MSA-1, midbody/MSA-2, CENP-F/MSA-3, and centrosome) revealed that the NuMA pattern accompanying chronic idiopathic urticaria was the most common pattern.⁷⁴ MSA-2 as the second rate pattern was associated mainly with SS, RA, and SLE. Similarly, Xi et al reported that MSA-recognizing autoantibodies were predominantly coupled with CTD, mostly SS, RA, and SLE.⁷⁵ The RNP-associated patterns could be indicative of undifferentiated CTD.⁷⁴ Results of another study showed that the NuMA pattern was positive in patients with neurofibromatosis and small vessel vasculitis.²⁷

Furthermore, intercellular bridge or midbody patterns may be also related to SSc, SLE, vasculitis, and cancers.^{63,76} One preliminary study stated an elevated occurrence of liver disease in MSA-positive individuals.⁷⁷ Interestingly, SLE was linked to rare cytoplasmic and mitotic cellular patterns mostly at autoantibody titer $\geq 1:160$.⁷⁶

The International Consensus on ANA Patterns states that the cytoplasmic and to a lesser extent the mitotic HEp-2 IFA staining patterns, are clinically important and should “demand dedicated follow-up testing in daily clinical practice”.⁶⁶

Finally, it is important to note that the International Consensus on Standardized Nomenclature of HEp-2 cell staining patterns in IFA demands that autoantibodies detected in HEp-2 cells must be verified by supplementary monospecific assays. Interestingly, recent data showed that even if recording cytoplasmic staining as ANA-positive was judged meaningful, missing of pattern consideration and recommendation comments may result in improper reflex analyses.⁷⁸ At present, validation of significant ANA nuclear patterns is available in the comprehensive multiplex format and encompasses dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B, RNP/Sm, Sm, Mi-2 α , Mi-2 β , Ku, CENP A, CENP B, Sp100, PML, Scl-70, PM-Scl100, PM-Scl75, RP11, RP155, gp210, PCNA, and DFS70 differentiation. A confirmative cytoplasmic pattern panel comprises AMA-M2, M2-3E, ribosomal P-proteins, Jo-1, SRP, PL-7, PL-12, EJ, and OJ antigens. As cytoplasmic antibodies may be still difficult to recognize in certain laboratories, their monospecific recognition is of specific significance. The introduction and applicable employment of reflex testing based on HEp-2 patterns in clinical practice has been shown to advance the competence of clinical diagnostics of autoimmune diseases while cutting the turn-around time and preserving resources and efforts.^{79,80}

Novel Autoantibodies: New Discoveries and New Tools in Systemic Autoimmune Diseases

Antibody discoveries are continuing, with ultimate consequences for a better understanding of pathophysiology of autoimmune reactions and for diagnostic and therapeutic decision-making. The addition of newly identified autoantibody-autoantigen pairs and other analytical tests to common clinical laboratory panels should help advance and complement diagnostics of systemic rheumatic diseases and other autoimmune conditions.

For instance, the multi-analyte panel, which includes quantitative assessment of autoantibodies and complement activation, may improve the diagnostic specificity of earlier detection and stratification of SLE. The levels of the cell-bound complement activation products (CB-CAPs) – C4d deposition on erythrocytes (EC4d) and B lymphocytes (BC4d) measured by quantitative flow cytometry, are known sensitive and specific in diagnosis and monitoring of adult and pediatric SLE.^{81,82} Furthermore, a cluster analysis methodology revealed a relationship between different subsets of SLE patients and patterns of detectable autoantibodies. The multi-analyte and multi-variate analysis of serum and other biological fluids from patients using modern antigen arrays markedly expands this diagnostic approach.⁴⁰ Therefore, utilization of novel and/or “non-classic” biomarkers for systemic lupus may provide additional opportunities for precise diagnosis, estimation of disease progress, and strategy for efficient treatments.

Furthermore, the development of a new subtle and high-quantity approach for the detection of exoproteome-engaged autoantibodies, which allows the discovery of autoantibodies, which are hard to perceive by other technologies, revealed that the amount of reactive autoantibody in patients with most severe SLE was markedly higher than in individuals with milder forms of the disease.⁸³

Similarly, updated panels are helpful for the diagnosis of SS. In addition to the “classic” antigen for connective tissue disease sorting (ANA, Ro/SSA, La/SSB, RF) (Table 1), additional antigens recognized in animal models (SP1, PSP, CA6,

and α -fodrin) and antigen recognized from other autoimmune reactions (ACA, AMA, and CCP) should be included.⁸⁴ Autoantibodies targeting salivary gland protein 1 (SP1), carbonic anhydrase 6 (CA6), and parotid secretory protein (PSP) were found in IL-14 α transgenic mice, which resemble many clinical aspects of human SS. These novel autoantibodies were later identified in SS patients who express or do not express Ro/SSA and La/SSB autoantibodies and in individuals suffering from the idiopathic dry mouth and dry eye conditions.^{85,86} Importantly, antibodies recognizing SP1, PSP, and CA6 were seen in early SS patients who do not express anti-Ro/SSA and anti-La/SSB antibodies: 45% of those patients expressed anti-SP1 and 5% expressed anti-CA6 antibodies.⁸⁵ In another subpopulation of patients, 76% expressed SP1 and CA6 autoantibodies in comparison with only 31% of people who expressed Ro/SSA and La/SSB autoantibodies.⁸⁷ The prevalence of α -fodrin-targeting antibodies in the serum of SS patients was 38–42%.⁸⁸ Thus, it was suggested that the ideal diagnostic approach for the early assessment of SS may comprise primary HEp-2 cell IFA screening for detection of ANA, AMA, and ACA antibodies, and following reflex tests for specific identification of SSA/Ro60, SSA/Ro52, SSB/La, RF, SP1, CA6, PSP, α -fodrin, and CCP targeting antibodies.⁸⁴

The panel of anti-phospholipid (PL) antibodies may also include a number of “non-criteria” autoantibodies that are not frequently assessed, although their pathogenic role and significance in defining antiphospholipid syndrome (APS) phenotypes are not yet completely determined. Generally, the common diagnostic criteria for APS include the detection of lupus anticoagulant or increased levels of IgG or IgM anti-cardiolipin (CL) or β 2-glycoprotein I (β 2GPI) autoantibodies: triple positivity with positive lupus anticoagulant, anti-CL and anti- β 2GPI autoantibodies are associated with an increased risk for APS, while irregular seropositivity or low levels of these antibodies pose a low risk.⁸⁹ However, about 30 unique PL autoantibodies have been identified, which can recognize anionic phospholipids, such as phosphatidylinositol and phosphatidylserine, or phospholipid-binding proteins, such as distinct domains of β 2GPI, prothrombin and annexin-V.

Non-conventional anti-PL antibodies, for instance, anti-phosphatidylethanolamine and anti-phosphatidylserine antibodies, could be linked to recurrent pregnancy losses, while anti-phosphatidylserine/prothrombin antibodies could be linked to thrombosis.^{90,91} Another recent study reported a connection between specific PL autoantibodies and/or PL autoantibody patterns, and clinically seen subtypes of APS such as arterial thrombosis, CNS manifestations, recurrent thrombosis, and obstetric APS.⁹² Thus, detection of a broad spectrum of anti-PL antibodies permits the identification of APS subtypes and is important for the correct selection of therapeutic approach for this multilayered autoimmune disease. Furthermore, a new multiplexed methodology for PL autoantibody testing was established based on a line immunoassay, which allows a concomitant assessment of a large pattern of anti-PL antibodies. This unique procedure may discriminate PL autoantibodies related to APS from PL autoantibodies seen in infectious diseases and in asymptomatic carriers, as well as assess specific recognition of domain 1 of the β 2GPI by the anti- β 2GPI antibody.⁹²

Novel Autoantibodies: New Discoveries and New Patterns in Other Autoimmune Diseases

Many liver-related autoantibodies have been identified and while many of them are not disease-specific, their identification is required for appropriate diagnosis of autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) because of their inclusion in the scoring system for diagnostic purposes. However, autoantibodies are not generally involved in detecting primary sclerosing cholangitis (PSC), the third major autoimmune liver pathology. Furthermore, in spite of the fact that autoantibodies can frequently be detected in AIH, they display a relatively low disease specificity. However, in PBC, specific antibodies, notably antimitochondrial antibodies (AMA), are considered a characteristic marker.⁹³ Interestingly, among various subtypes of AMA (M1-M9), only anti-M2, -M4, -M8 and -M9 autoantibodies have shown association with PBC. Thus, the clinical availability of tests detecting all AMA subtypes and their specific patterns may improve the diagnostic and prognostic power of AMA tests for PBC. Similarly, the detection of AMA immunoglobulin subtypes, including IgG, IgA, and IgM, may be incorporated as the IgG3 and IgA anti-pyruvate-dehydrogenase E2 (PDC-E2) AMA can serve as a sign of disease progression.⁹⁴

Although 10% of PBC patients may be AMA-negative, almost half of them display relatively PBC-specific ANA patterns (nuclear dots and nuclear envelope) (Table 1). Therefore, the addition of related antibodies recognizing sp100,

gp210, and Lamin-B-receptor in the “ideal PBC antibody” multiplexed panel may be a reasonable request. Two new autoantibodies recently described in PBC, recognizing Kelch-like 12 and hexokinase 1,⁹⁵ should be added to the “ideal panel”, though their connection with the severity or progression of the disease was not proven. Similarly, new antibodies targeting biliary epithelial cells in patients suffering from PSC have been recently described,⁹⁶ although their utility for clinical practice has not yet been determined.

Almost 25 new autoantibody categories were recently identified in individuals with certain neurological abnormalities, with some of them being proven to play a pathogenic role and acting as novel clinical manifestations. Identification of additional autoantibodies recognizing specific antigenic molecules in neural or neuroglial cells opens vital diagnostic and therapeutic prospects. For instance, besides antipsychotic medicine, tranquilizers, and psychotherapy, some patient groups may obtain instant immune-based therapy, such as depletion of B or plasma cells and antibody elimination.⁹⁷ In fact, antibodies binding to cell membrane molecules relating to voltage-gated potassium channels (VGKC) were discovered in 2001 in patients exhibiting seizures and memory loss.⁹⁸ In 2005, antibodies targeting aquaporin 4 (AQP4) in individuals with neuromyelitis optica (NMO) were identified by using the cell-based assay (CBA) with transfected human embryonic kidney (HEK) cells.⁹⁹ Another team in 2007 reported antibodies recognizing glutamate receptors in the brain in patients characterized by noticeable neuropsychiatric symptoms and ovarian teratoma.¹⁰⁰ Many new neurological antigens or intracellular epitopes have been also discovered in the last years and include flotillin 1 and 2, neurochondrin, sodium/potassium-transporting ATPase subunit α -3 (ATP1A3), drebrin (DBN1), Rho-associated kinase 2 (ROCK2), carnitine palmitoyltransferase 1C (CPT1C), solute carrier family 4, sodium bicarbonate cotransporter member 4 (SLC4A4), Kelch-like protein 11 (KLHL11), regulator of G-protein signaling 8 (RGS8), septin 5, syntaxin 1B (STX1B), glutamate receptor ionotropic δ -2 (GRID2), phosphodiesterase 10A (PDE10A), and AP-3 complex subunit beta-2 (AP3B2).^{97,101}

Dermatomyositis as an idiopathic inflammatory myopathy is distinguished by the detection of myositis-specific and myositis-associated antibodies in almost 80% of investigated patients (Table 1).¹⁰² Recently, a few new myositis-specific antibodies and target antigens have been identified, which is helpful for recognizing the pathogenesis of this disease.^{103,104} Anti-MJ (or p140) autoantibodies, originally described in children with dermatomyositis,¹⁰⁵ target the nuclear protein 2 (NXP-2)/MORC3 matrix protein complex, which regulates p53-induced cell senescence. Anti-NXP2 antibodies were also seen in individuals with idiopathic inflammatory myopathies with 20–30% prevalence in dermatomyositis, 8–9% in polymyositis, and 15% in connective tissue myositis.^{106,107}

Anti-melanoma differentiation-associated gene 5 (MDA-5, CADM-140) antibodies were originally identified in East-Asian patients with dermatomyositis with a prevalence of 19–35%.¹⁰⁸ MDA5 belongs to the retinoic-acid-inducible gene (RIG)-I-like receptor family and acts as a cytoplasmic pattern-recognition receptor sensing virus double-stranded RNA (dsRNA) and bacterial nucleic acids.¹⁰⁹ The predictable clinical manifestations of idiopathic inflammatory myopathy patients expressing anti-MDA5 antibodies are amyopathic myositis and fast-progressing interstitial lung disease, associated with a high level of mortality. Detection of MDA5 autoantibodies also frequently correlates with skin and oral ulceration. Interestingly, a higher proportion of patients expressing MDA5 autoantibodies were seen in remission after two years than patients without detectable autoantibodies.¹¹⁰

Autoantibodies against the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), detected in individuals with immune-mediated necrotizing myositis treated with statin,¹¹¹ target enzyme that converts HMG-CoA to mevalonic acid during the biosynthesis of cholesterol. The concentration of HMGCR autoantibodies is associated with the necrotizing myositis severity. Individuals with HMGCR autoantibodies showed an insistent autoimmune reaction even after the termination of therapy. This is in contrast to non-immune statin myopathy, which is usually resolved after discontinuing statin treatment.¹¹²

The detection of abovementioned new dermatomyositis antibodies can be performed by only a few laboratories because no generally approved commercial tests are currently offered. An establishment of clinically available and approved immunoassays for the detection of these antibodies should make these tests broadly accessible to accelerate diagnostic potential. The addition of newer autoantigens to the available panels may both accelerate the diagnostic processes and add significant data on the stratification of affected individuals according to tissue location, risk of malignization, and general patient prognosis.

Improvements in diagnostic testing for autoimmune dermatologic diseases were initiated by the identification of serum autoantibodies recognizing intercellular markers in epithelial cells in patients with pemphigus in 1964.¹¹³ Autoimmune skin blistering diseases belong to the group of various dermatoses with the detectable level of antibodies targeting autoantigens in the skin and mucous membranes: autoantibody binds to structural elements of the dermal-epidermal junction in pemphigoid diseases and autoantibody binds to desmosomal proteins connecting neighboring keratinocytes in pemphigus.¹¹⁴ For disease diagnosis, both serum and tissue assays are most helpful, although biopsy testing is commonly preferential. Even if serum assays are not as sensitive as immunofluorescence on tissue slices, they are needed to differentiate disease subtypes. For instance, three types of Bullous Lupus Erythematosus are distinguished by the presence of autoantibodies: type I with autoantibodies recognizing the NC1 and NC2 domains of type VII collagen; type II with autoantibodies bind to other proteins of the basement membrane zone, including BP180, BP230, and laminin-332 or laminin-331; and type III with undefined epidermal and dermal autoreactivity.¹¹⁵

Detection of serum autoantibodies in dermatological diseases is also helpful in monitoring disease development as the presence of autoantibodies correlates with disease activity.¹¹⁶ However, in several dermatologic diseases, including linear IgA disease and pemphigoid subgroups, the antigenic profile has not yet been outlined. At the same time, like in mucous membrane pemphigoid, diagnostic challenges are attributed to multiple heterogeneous autoantigens.

New Assays and New Algorithms

Proficient description of autoantibody profiles with respect to clinical signs, therapeutic sensitivity, and disease prognosis is an important ongoing effort. The utilization of novel methodologies and techniques plays a key role in this progress since the repertoire of identified antigens may represent only a small pool of total autoreactivity detected by multivariate technology. For instance, the application of proteomics methods, which are in use for infectious disease serodiagnosis, to autoimmune diseases has recently revealed 15 antigens in patients with pemphigus showing negative desmoglein results.¹¹⁶ Thus, proteomic technology may provide the basis for multiplex biomarker assays for autoantibody profile assessments and can be applied to delineating the immunologic mechanisms involved in autoantibody-mediated disease pathogenesis.

Although autoantibodies recognizing platelet antigens are accepted as the primary source of immune thrombocytopenia (ITP), their screening is not included in the suggested for the diagnostic panels, and no “gold standard test” for ITP rather than a count of thrombocytes has been recommended.¹¹⁷ The main reason is the relatively low specificity and sensitivity of current platelet autoantibody assays resulting in the fact that ITP is commonly confirmed by rejection of alternative reasons for thrombocytopenia.¹¹⁸ This lack of assurance causes misdiagnosis and empirical treatment strategies.¹¹⁹ Therefore, the dependable recognition of anti-platelet antibodies is crucial for the clinical verification of ITP and the prevention of misdiagnosis. The meta-analysis demonstrated that antibody-based assays for ITP had an acceptable specificity with a relatively small sensitivity. However, in spite of the revealed low sensitivity of both direct (autoantibodies recognizing the platelet surface markers) and indirect (plasma or serum autoantibodies) assays, direct assays had superior sensitivity and were useful for ruling in ITP.¹¹⁹ Recent optimization of the diagnostic procedure and modified algorithm for potential ITP cases, which was based on the immobilization of platelet antigens using direct monoclonal antibody binding demonstrated its practicability for the identification of platelet autoantibodies.¹¹⁷

Anti-Cytokine and Anti-Complement Autoantibodies

Alterations in circulating concentrations of anti-cytokine antibodies, which change cytokine accessibility and activity, were detected in specific autoimmune abnormalities. Therefore, certain intimidating clinical conditions associated with anti-cytokine antibodies attracted extensive attention recently. Cytokine-bound antibodies were detected in individuals with SLE, SS, and RA.¹²⁰ Increased concentrations of circulating anti-cytokine antibodies may cause immunodeficiency: for instance, anti-IL-17 antibodies may be associated with autoimmune polyendocrine syndrome type I, and anti-IFN- γ antibody may be linked with mycobacterial infections.^{121–123} Autoantibodies to cytokines may serve as worsening prognostic markers, as anti-IL-8 and anti-IL-1 α antibodies in RA, or better prognostic markers, like IL-6 autoantibody in SSc or osteopontin autoantibody in RA.⁴⁸ Alike, IL-1 α autoantibody may be connected with the non-destructive phenotype of chronic polyarthritis. Ching et al reported high levels of type 1 IFN autoantibodies in 42% of individuals with SLE.¹²⁴ Furthermore, although autoantibodies to IFN- α and IFN- ω were initially utilized clinically as signs of risk

for myasthenia gravis and thymoma, they have recently been demonstrated to be a highly sensitive screening test for the autoimmune polyglandular syndromes. These are uncommon groups of organ-specific autoimmune diseases characterized by the clinical signs of several autoimmune reactions in an affected individual resulting from the autoimmune regulator (AIRE) gene mutations and abnormal self-tolerance.^{125,126}

While cytokine-neutralizing antibodies may be associated with autoimmunity, immunological dysfunction, and immunodeficiency, most diagnostic laboratories do not routinely offer these assays. A rare exemption may be the assessment of anti-GM-CSF antibodies in the cases of autoimmune pulmonary alveolar proteinosis.¹²⁶

Autoantibodies targeting different complement proteins, convertases, and complement regulators were also reported, such as autoantibodies recognizing C1q or C1 inhibitor, factor H/B, and C3 convertases (C3bBb, C4bC2b, formerly C4b2a) and C5 convertases (C4b2a3b, C3bBbC3b). Anti-complement antibodies can modulate regulation of classic and alternative complement cascades provoking autoimmune and renal diseases, including C3 glomerulopathy, atypical hemolytic uremic syndrome, membranoproliferative glomerulonephritis, dense deposit disease, and SLE. For instance, C3 nephritic factors (C3NF), a group of autoantibodies (both IgM and IgG) to the alternate pathway C3 convertase, which stabilizes C3bBb and upregulates its activity. The result is a prominently reduced C3 serum level.¹²⁷ C3NF can be seen in immune complex-associated membranoproliferative glomerulonephritis, dense deposit disease or partial lipodystrophy.

C1q autoantibodies serve as the accepted markers checked for the assessment of lupus nephropathy. Their increased levels in serum are seen in about 20% to 50% of patients with SLE.^{128,129} The estimated positive predictive value of C1q autoantibodies for lupus nephritis is approximately 58%; the negative predictive value is about 90–100%.¹³⁰ Autoantibodies to complement factor H of the complement alternative pathway, are one of two main pathogenic mechanisms that cause atypical hemolytic uremic syndrome.¹³¹ These complement-regulating autoantibodies can also be seen in SLE and RA.¹³² Interestingly, serum antibodies targeting factor H have been demonstrated in the individuals with neuromyelitis optica spectrum disorder (NOSD).¹³³ The formation of antibodies to lectin pathway components, such as anti-mannose binding lectin and anti-Ficolin-3 autoantibodies, can be developed in individuals with SLE.¹³⁴ Autoantibodies targeting complement receptors have also been found and characterized in several autoimmune pathologies.¹³⁴

Thus, antibodies against complement components may be seen in different clinical conditions, including SLE, SS, rheumatoid vasculitis, dense deposit disease, polyarteritis nodosa, IgA nephropathy, HIV, atypical hemolytic uremic syndrome, and other diseases. The pathogenic function of antibodies targeting the complement components is not always obvious and, in many cases, should be further refined. These data may lead to the development and validation of specific antibody panels that can guide the practitioners regarding the autoantibody contribution is likely, pending, actual, or declining during therapy, particularly in the developing period of complement-focusing biological therapy.¹²⁹

Autoantibodies: Immunoglobulin Subtypes

Detection of specific immunoglobulin subtypes in autoantibody screening is another important but undeveloped direction in the diagnosis of autoimmune disorders. The pathogenic role of autoantibodies is governed by the immunoglobulin constant domain that reflects the antibody isotype and subclass, and autoimmune responses may have their own unique isotype and subclass profile.¹³⁵

Even if IgG1 is responsible for the majority of autoantibody reactivity, in some cases IgE and IgA may play an important role. For instance, glycoprotein 2 recognition by IgA autoantibodies was associated with severe forms of Crohn's disease.¹³⁶ Antigenic molecules such as glycoprotein 2 and CUB zona pellucida-like domain 1 are members of the protein groups that regulate innate immunity reactions in the gut and were reported as unique targets of pancreatic autoantibodies in Crohn's disease. New data from the analysis of patients with different demyelinating diseases of the CNS, like neuromyelitis optica spectrum disorder and multiple sclerosis, demonstrate that anti-myelin oligodendrocyte glycoprotein (MOG) IgA can be used as a diagnostic indicator for a separate form of AQP4/MOG-IgG double-seronegative individuals with CNS demyelination syndrome.¹³⁷ Furthermore, IgA antibodies targeting dsDNA predict disease severity and glomerulonephritis development in SLE, equally to dsDNA IgG antibody, and appeared in SLE patients without detectable dsDNA-recognizing IgG.^{138,139} Therefore, the detection of both IgG and IgA subtypes of immunoglobulins targeting dsDNA should improve diagnostic sensitivity and prognostic value of the test.

IgE autoantibodies targeting various antigenic epitopes in the skin participate in disease progress and persistence. This group of autoantibodies was detected in people with chronic atopic dermatitis, signifying the development of autoimmune reactions in the skin from an allergic inflammatory process, and new data showed a connection between clinical severity and IgE reactivity.¹⁴⁰ Other clinical data suggested that the detection of anti-BP180 IgE antibodies in serum or IgE deposit in the basement membrane zone suggests the specific clinical features of infiltrated lesions of bullous pemphigoid, such as urticarial plaques or nodules.¹⁴¹ Additionally, BP180 IgE autoantibody concentration in the serum correlates with disease progression and skin reactivity.¹⁴²

Isotypes of antibodies are rarely assessed in most autoimmune conditions, which creates a gap in our understanding of the pathophysiology and in creating practical testing panels of autoimmune diseases.¹⁴³ Particular isotypes and subclasses may perform a specific pathophysiological role and govern a disease phenotype that may involve a different therapy strategy. Future investigations have to direct profiling both isotype and subclass of autoantibodies in wider detail and integrate antibody assessment in addition to total IgG reactivity or deposition in experimental model systems and preclinical and clinical settings.

Future Directions

The term “autoantigenomics” has been recently introduced as a novel omics subcategory combining omics and autoantibody screening comprehensive tactics such as combinatorial serology or immune system-characterizing transcriptomics and proteomics.¹⁴⁴ Combinations of methods, such as key component investigation, hierarchical cluster analysis, pathway analysis combined with utilization of databases like Gene Ontology and Reactome Pathway, and machine learning approaches should help identify groups of antigens and individuals expressing allocated outlines. Groups of self-antigens with comparable reactive or longitudinal characteristics allow the selection of autoantibody ranges in an inclusive pattern for improved disease understanding and better therapeutic targets. Clusters of patients allow the identification of subgroups in a certain disease that should support stratification or treatment selection. Furthermore, autoantigenomic approaches may help identify novel single or panel autoantibody candidates.¹⁴⁴ For instance, serum autoantibodyome approach reveals 77 common autoantibodies detected in healthy individuals.¹⁴⁵

This “autoantigenomics” concept is perfectly supported by the “clinlabomics” concept – the establishment of a new diagnostic strategy by combining medical laboratory results and artificial intelligence (AI).² Utilization of robotic technologies to obtain substantial characteristic information (including autoantibody results) from available biological samples (blood, secretions, tissue) and diagnostic laboratory assays together with comprehensive analytical statistics, available databases, and machine learning has an incredible opportunity to streamline diagnostic intricacy, improve verification competence, optimize workflow proficiency and laboratory utilization, and increase the power of clinical prediction. Clinical immunology laboratories are particularly well suited to leverage machine learning because they produce large, complex, and highly structured data sets. Autoantigenomics thus should also help in predicting laboratory test values, encouraging accuracy of laboratory assay clarification, and developing result interpretation models able to provide additional references regarding the laboratory analysis a provider ought to request. In fact, an introduction of a novel attention-based enhancement framework to facilitate neural networks in expediting the machine learning process, which was designed for the recognition of rare ANA patterns, demonstrated its potential as an effective and dependable tool to improve identification of uncommon autoantibodies in clinical practice.¹⁴⁶

Conclusions

Autoantibodies play major etiological and pathobiological roles in different groups of diseases including autoinflammatory reactions, cancer, cardiovascular diseases, metabolic abnormalities, trauma, immunodeficiencies, infectious diseases, and certain neuropathic and neurodegenerative disorders. However, although specific self-antigen-recognizing antibodies frequently reflect the development or presence of pathological processes, detectable autoantibodies may additionally demonstrate protecting activities, which might be favorable in certain pathophysiological states. This raises the concerns about correlation between specific antibody presence and disease progression and about the utilization of autoantibodies as a personalized tool to calculate the anticipated autoreactivity and prognosis.⁹ In fact, individualized autoantibody titer, isotype, subclass, allotype, and N-glycosylation may explain most of the objective variations revealed between patients.

Examination of whether newly identified autoantibodies may be associated with, or contribute to, the etiology or pathogenesis of specific autoimmune diseases is an important ongoing effort. To ascertain these autoantigens and autoantibodies and further expose the potential molecular mechanisms of a disease, it is of great importance to develop and adopt a comprehensive screening approach to identify novel antigens in autoimmune disorders. One approach is the design, fabrication, and validation of novel antigen arrays that harbor presumed autoantigens, previously purported in the literature to be relevant, to categorize potentially pathogenic autoantibodies in the serum of affected patients. Some of the disease-defining target epitopes might be connected with distinguishing clinical and phenotypic variants and disease course, and the discovery of different autoantibodies might provide new insights into the previously unclear mechanisms of the pathological condition. Encountering effector autoreactivity in affected individuals thus holds the promise to reveal important etiological and risk elements and treatment approaches comparable to the analysis of genetic abnormalities and polymorphisms.⁸³ For instance, new technological advances in protein microarray methodology, such as human protein (HuProt) arrays, provide unbiased screening of autoantibodies at the proteome level. In the past, this approach to identify and validate autoantibodies as biomarkers for several autoimmune diseases has demonstrated its feasibility.^{147–149}

Revealing the pathophysiological pathways directing the initiation and maintenance of the personalized immunoreactivity and the specific involvement of various self-antigens in disease will also advance our understanding of the development of the disease and offer major opportunities for understanding more general mechanisms of autoimmunity. This will not only provide a new tool to diagnose autoimmune disease manifestations and predict the appearance of organ manifestation but also accelerate the immunotherapy revolution in autoimmune disorder management by advancing efficacy of novel therapies, like CAR-T cells and “inverse vaccines”.

Abbreviations

AB, antibody; ACA, anti-centromere antibody; AChR, acetylcholine receptor; ADAMTS13, A Disintegrin and Metalloprotease with ThromboSpondin type 1 repeats, number 13; AIH, autoimmune hepatitis; AIHA, autoimmune hemolytic anemia; AIRE, autoimmune regulator; AMA, anti-mitochondrial antibody; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibody; ANNA, anti-neuronal nuclear antibody; AP3B2, AP (adaptor protein)-3 complex subunit β -2; APS, antiphospholipid syndrome; ATP1A3, sodium/potassium-transporting ATPase subunit α -3; AQP4, aquaporin 4; BMZ, basement membrane zone; CA6, carbonic anhydrase 6; CBA, cell-based assays; CCP, citrullinated cyclic peptide; CD, celiac disease; CPT1C, carnitine palmitoyltransferase 1C; CRMP5, collapsin response mediator protein 5; CTD, connective tissue disease; DFS70, dense fine speckled; dsDNA, double stranded DNA; ENA, extractable nuclear antigens; GABA, γ -aminobutyric acid; GFAP, glial fibrillary acidic protein; GP2, glycoprotein 2; GRID2, glutamate receptor ionotropic δ -2; HEK 293, human embryonic kidney 293 cells; HEp-2, human larynx epithelioma cell line; HGF, Herpes gestationis factor; IBD, inflammatory bowel disease; IIF, indirect immunofluorescence; ITP, immune thrombocytopenia; LKM, liver-kidney microsome; MAG, myelin-associated glycoprotein; MCTD, mixed connective tissue disease; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MSA, mitotic spindle apparatus; NMDAR, N-methyl-D-aspartate receptor; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorder; NuMA, nuclear mitotic apparatus; PBC, primary biliary cholangitis; PCA, Purkinje cell cytoplasmic antibody; PDE10A, phosphodiesterase 10A; PSC, primary sclerosing cholangitis; PP, parotid secretory protein; RA, rheumatoid arthritis; RF, rheumatoid factor; RGS8, regulator of G-protein signaling 8; RNP, ribonucleoprotein; ROCK, Rho-associated coiled-coil containing protein kinase; ROCK2, Rho-associated kinase 2; SARD, systemic autoimmune rheumatic disease; SLA/LP, soluble liver antigen/liver-pancreas antigen; SLC4A4, solute carrier family 4, sodium bicarbonate cotransporter, member 4; SLE, systemic lupus erythematosus; Sm, Smith antigen; SMA, smooth muscle antibody; SP1, salivary gland protein 1; SS, Sjögren's syndrome; SSc, scleroderma, systemic sclerosis; T1D, Type 1 diabetes; Tg, thyroglobulin; TPO, thyroid peroxidase; TR, thyrotropin receptor; tTG, tissue transglutaminase; TTP, thrombotic thrombocytopenic purpura; UC, ulcerative colitis; VGKC, voltage-gated potassium channels.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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