Biomarkers in connective tissue diseases

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1. To understand the utility of autoantibodies in managing connective tissue disease.
2. To identify statistical measures of commonly ordered autoantibody tests.
3. To recognize important associations between particular biomarkers and clinical manifestations of disease.

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Autoimmune connective tissue diseases are clinically variable, making biomarkers desirable for assessing future disease risk, supporting early and accurate diagnosis, monitoring disease activity and progression, selecting therapeutics, and assessing treatment response. Because of their correlations with specific clinical characteristics and often with disease progression, autoantibodies and other soluble mediators are considered potential biomarkers. Additional biomarkers might reflect downstream pathologic processes or appear because of ongoing inflammation and damage. Because of overlap between diseases, some biomarkers have limited specificity for a single autoimmune connective tissue disease. This review describes select current biomarkers that aid in the diagnosis and treatment of several major systemic autoimmune connective tissue disorders: systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and anti-neutrophil cytoplasmic antibody–associated vasculitides. Newly proposed biomarkers that target various stages in disease onset or progression are also discussed. Newer approaches to overcome the diversity observed in patients with these diseases and to facilitate personalized disease monitoring and treatment are also addressed. (J Allergy Clin Immunol 2017;140:1473-83.)

Key words: *Connective tissue diseases, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, vasculitis, biomarkers*

Autoimmune connective tissue disorders are a heterogeneous group of diseases that affect connective tissue in various organs resulting from poorly controlled autoimmune responses, complement activation, interferon dysregulation, and associated inflammation. Although their clinical presentations vary, these diseases share significant genetic risk factors, as demonstrated by cross-analysis of genome-wide association studies[1] and common regulatory mechanisms of autoimmune diseases.[2] Environmental and female-associated factors also play critical roles in development of autoimmune diseases.[3-7] In nearly all systemic

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Abbreviations used

AAV: ANCA-associated vasculitides
ACA: Anti-centromere antibody
ACPA: Anti-citrullinated protein antibody
ACR: American College of Rheumatology
ANA: Anti-nuclear autoantibody
ANCA: Anti-neutrophil cytoplasmic antibody
Anti-CarP: Antibodies against carbamylated proteins
BLyS: B-lymphocyte stimulator
CCP: Cyclic citrullinated peptide
CRP: C-reactive protein
DAS28: Disease Activity Score-28 joints
dcSSc: Diffuse cutaneous systemic sclerosis
DLCO: Diffusing capacity of the lungs for carbon monoxide
dsDNA: Double-stranded DNA
EGPA: Eosinophilic granulomatosis with polyangiitis
ESR: Erythrocyte sedimentation rate
GDF-15: Growth differentiation factor 15
GPA: Granulomatosis with polyangiitis
HES: Hypereosinophilic syndrome
ILD: Interstitial lung disease
lcSSc: Limited cutaneous systemic sclerosis
LN: Lupus nephritis
MPA: Microscopic polyangiitis
MPO: Myeloperoxidase
NT-proBNP: N-terminal prohormone of brain natriuretic peptide
PAH: Pulmonary arterial hypertension
PR3: Proteinase-3
RA: Rheumatoid arthritis
RF: Rheumatoid factor
RNAP III: RNA polymerase III
SLE: Systemic lupus erythematosus
SSA: Sjögren syndrome type A antigen
SSc: Systemic sclerosis

autoimmune rheumatic diseases evaluated to date, autoantibody production and immune dysregulation precede clinical onset,[8-15] although a significant amount of this information is not yet integrated to standard clinical care. Ongoing research is focused on improving biomarkers for diagnosis, prognosis, treatment selection, and optimized therapy. This review describes current and new emerging biomarkers for major connective tissue diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), and anti-neutrophil cytoplasmic antibody (ANCA)–associated vasculitides.

SLE

SLE is a systemic autoimmune disease characterized by production of anti-nuclear autoantibodies (ANAs). Early and accurate diagnosis and disease monitoring are hindered by its heterogeneous presentation and clinical course. Serologic and urinary biomarkers are either in use or are emerging as potential biomarkers for SLE. These autoantibodies (Table I), complement products, and cytokines/chemokines/soluble mediators have the potential to facilitate diagnosis, identify subjects at greater risk for SLE, and monitor disease activity or specific organ involvement (Fig 1).

Autoantibodies

ANAs are a hallmark of SLE. Nearly all patients with SLE exhibit ANAs at diagnosis, with a 1:80 immunofluorescent titer showing up to 98% sensitivity but 75% specificity for SLE classification.[16] ANAs are also found in patients with many other autoimmune diseases, malignancies, or hepatic diseases; unaffected family members of patients with lupus; and even up to 14% of healthy subjects,[17] especially with increasing age. Therefore a positive ANA value serves as a necessary but insufficient criterion for SLE classification or diagnosis but not as a definitive test.[18] Patients with a negative ANA test result are extremely unlikely to have any lupus-specific autoantibodies. Therefore through the Choosing Wisely campaign, the American College of Rheumatology (ACR) recommends testing for specific autoantibodies only when a positive ANA level and clinical suspicion are present.[19] Repeat testing is not indicated in subjects with positive ANA results because changes in ANA titers alone show no clinical correlation with increased disease activity or worsening prognosis. Testing of ANAs and other autoantibodies in preclinical disease states or to identify subjects for potential preventive interventions will require additional studies and guidelines.[20]

Anti–double-stranded DNA (anti-dsDNA) antibody responses have high specificity (92% to 96%) and moderate sensitivity (57% to 67%) for SLE.[21] They constitute a criterion for SLE classification by ACR criteria (requiring 4/11 criteria for classification) and by the Systemic Lupus International Collaborating Clinics criteria (requiring 4/17 criteria or dsDNA plus biopsy-proven lupus nephritis [LN]).[22-24] Anti-dsDNA forms immune complexes with nucleosomes observed in patients with SLE, leading to immune complex deposition in the kidney.[25] Furthermore, anti-dsDNA antibodies show cross-reactivity to α-actinin and can bind to mesangial cells in the kidney.[26] Immune complexes formed by anti-dsDNA antibodies in the kidney can activate the complement cascade, leading to damage in patients with glomerulonephritis.[27] Patients with proliferative LN have increased anti-dsDNA as early as 4 years before diagnosis, and an increase of greater than 1 IU/mL/y was specific for LN.[28] Anti-dsDNA with low complement levels also associates with mucocutaneous, renal, and hematologic flare within 1 year.[29] In patients with clinically stable SLE and increasing levels of anti-dsDNA (≥25%) and C3a (≥50%), the free released product of complement activation, treatment with moderate prednisone can avert severe clinical flares.[30]

Although less common (sensitivity, 26% to 31%) antibodies against the Sm antigen are highly specific (95% to 99%) for SLE and can associate with early mortality.[31] About 30% to 70% of patients with SLE have anti-Ro/Sjögren syndrome type A antigen (SSA), and Ro/SSA is associated with subacute lupus erythematosus, sicca symptoms, and secondary Sjögren syndrome. Anti-Ro/SSA antibodies can bind to either of 2 antigenic proteins: 52-kDa and 60-kDa Ro. Antibodies to 60-kDa Ro/SSA are more frequently observed in patients with SLE and correlate with photosensitivity, cutaneous vasculitis, and hematologic disorders.[32] Antibodies to a related antigen, La/SSB, are present in approximately 10% of patients with SLE and associated with lower prevalence of renal disease.[32] Anti-ribosomal P antibodies, similar to anti-Sm antibodies, are very specific for SLE but occur in only approximately 20% of white patients with SLE. Anti-ribosomal P is enriched in neuropsychiatric[33] and pediatric-onset disease.[34] A number of other autoreactivities have been reported in patients with SLE.[21] Of interest are anti-nucleosome responses, which correlate with disease activity in clinically quiescent patients,[35] and anti-cardiolipin responses, which are implicated in

TABLE I. Autoantibody specificities in patients with connective tissue diseases and their association with disease phenotype

Antibody	Associated disease phenotype	Other associated diseases	Reference
SLE			
Anti-dsDNA	LN Neuropsychiatric lupus erythematosus		21
Anti-Sm	LN		31
Anti-Ro/SSA (Ro60)	LN Neonatal lupus erythematosus Subacute cutaneous lupus erythematosus	Sjögren syndrome	32
Anti-La/SSB	LN Neonatal lupus erythematosus Subacute cutaneous lupus erythematosus	Sjögren syndrome	32
Anti–ribosomal P	LN Neuropsychiatric lupus erythematosus Pediatric-onset SLE		33, 34
Anti-RNP	SLE	Systemic sclerosis	21, 31
Anti-cardiolipin	SLE, antiphospholipid syndrome		10
RA			
RF	RA	SLE, Sjögren syndrome	60, 61
ACPA	RA		61, 65
Anti-CarP	RA		73, 74
RA33	RA		60
Systemic sclerosis			
Anti-centromere	Limited cutaneous systemic sclerosis PAH		83, 84, 85
Scl70	Diffuse cutaneous systemic sclerosis Pulmonary fibrosis Renal crisis		83, 84, 85
Anti–RNAP III	Diffuse cutaneous systemic sclerosis Renal crisis		83, 86
Anti-Th/To	Limited cutaneous systemic sclerosis PAH Pulmonary fibrosis		87
ANCA-associated vasculitis			
Anti-PR3 ANCA	GPA	MPA, EGPA	109, 110
Anti-MPO ANCA	Microscopic polyangiitis EGPA	GPA	109, 110

Anti-CarP, Anti-carbamylated protein antibodies; *PR3*, proteinase 3; *RNP*, ribonuclear protein.

thrombosis and recurrent fetal loss and are associated with a complex clinical outcome, with patients meeting a higher number of ACR criteria.[10]

Because no single autospecificity is sufficient for SLE diagnosis, recent efforts have focused on detecting autoantibody signatures encompassing combinations of autoreactivities. One autoantigen array covers antigens related to 8 distinct autoimmune diseases.[36] Another microarray-based test has a reported sensitivity of 94% and specificity of 75%. The test is validated as a clinical test to exclude a diagnosis of SLE if no compelling clinical evidence exists or to support a low likelihood of SLE.[37]

Autoantibodies are typically detectable before diagnosis; 63% to 88% of subjects have autoantibodies before disease classification (0.1-9 years).[8,9] Anti-Ro/SSA is among the earliest detectable specificities, whereas anti-dsDNA antibodies appear closer to classification (approximately 3 years before).[8] Therefore autoantibodies can serve as a biomarker of disease risk before SLE onset. In subjects meeting less than 4 ACR classification criteria for SLE, those who later reached SLE classification had higher baseline ANA levels and increased IgG autoreactivity in IgG profiling of more than 80 autoantigens.[38] In a follow-up study of previously unaffected relatives of patients with SLE, 89% of patients who later reached SLE classification had positive ANA levels at baseline compared with 48% of those who remained unaffected.[13] Therefore a positive ANA level alone is not a definitive marker for increased risk of future disease classification, and additional predictive markers are needed.

Complement

Levels of complement C3 and C4 are used to monitor SLE disease activity. Reduced C3 and C4 levels are associated with more severe disease, and reduced C1q, C3, and C4 levels can precede disease flare.[39] Complement is activated in patients with SLE through immune complex deposition. Therefore levels of cell-bound complement C4 activation products on erythrocytes are increased in patients with SLE and have 22% higher sensitivity for SLE than reduced C3/C4. ANA positivity, anti-MCV negativity, anti-dsDNA positivity, increased erythrocyte C4d levels, and increased B-cell C4d levels demonstrated 80% sensitivity for SLE and 87% specificity against other rheumatic diseases.[40] These findings have been confirmed and refined to a commercially available weighted SLE risk score with a 2-tier design.[41]

Emerging biomarkers

Soluble mediators. Recent studies have suggested specific soluble mediators as potential biomarkers for SLE onset and SLE

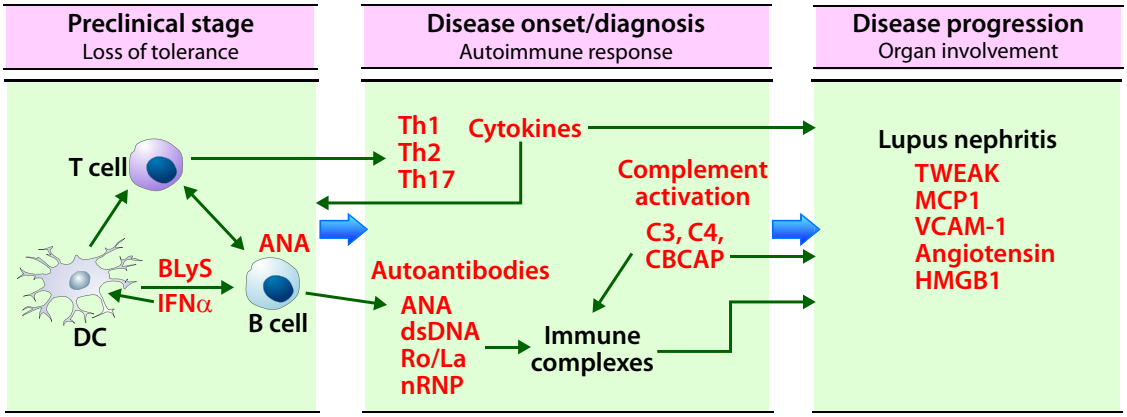

FIG 1. Biomarkers in patients with SLE. The initial stage in SLE pathogenesis is loss of tolerance to self-antigens. Genetic predisposition and environmental factors, such as viral infections, can lead to antinuclear antibody generation by means of epitope spreading. Disease onset or diagnosis occurs after amplification of autoimmune response through interactions between innate and adaptive immune cells (dendritic cells, T cells, and B cells); differentiation of T cells into T_H1, T_H2, and T_H17 subsets; and maturation and class-switching of B cells to secrete autoantibodies. These autoantibodies form immune complexes, fix complement, and activate both classical and nonclassical complement. This results in decreased complement C4 and C3 levels and an increase in levels of cell-bound C4d *(CBCAP)*. Increased levels of cytokines, autoantibodies, and CBCAP and decreased levels of C3 and C4 in circulation are the most useful biomarkers at this stage. Passive deposition of immune complexes or *in situ* immune complex formation in end organs, such as kidneys, together with complement activation and high levels of secreted proinflammatory cytokines causes further organ damage. Urinary biomarkers are a convenient and effective way to monitor renal disease progression in SLE. *ANA*, Antinuclear antibodies; *DC*, dendritic cells; *dsDNA*, anti-dsDNA antibodies; *MCP1*, monocyte chemoattractant protein 1; *nRNP*, nuclear ribonuclear protein; *TWEAK*, TNF-like weak inducer of apoptosis; *VCAM-1*, vascular adhesion molecule 1.

disease flares. In a survey of nearly 30 immune or inflammation-based soluble mediators, several soluble mediators were increased more than 3.5 years before SLE classification, including IL-5, IL-6, and IFN-γ. Levels of some cytokines (B-lymphocyte stimulator [BLyS] and a proliferation-inducing ligand) were increased closer to diagnosis. In this preclinical period a combination of IFN-γ, IL-4, IL-6, ANAs, and anti-Ro distinguished patients from control subjects with 84% accuracy compared with 58% accuracy with ANA positivity alone. Thus evaluating immune pathway dysregulation in conjunction with ANA positivity can help identify subjects at higher risk for SLE.[11]

Flares are a significant risk factor for end-organ damage in patients with SLE, and soluble mediators are promising biomarkers of imminent SLE flare. Levels of BLyS in patients with SLE are associated with anti-dsDNA levels and disease activity.[42] A baseline BLyS concentration of 2 ng/mL or greater predicted SLE flare at week 52 in a combined analysis of data from phase II, worldwide clinical trials.[43]

In a longitudinal study of patients with SLE, reduced levels of regulatory cytokines, such as IL-1 receptor antagonist, TGF-β, and IL-10, preceded disease flares. A combined soluble mediator score incorporating 52 analytes was increased in patients with impending flare compared with either matched stable patients or the same patients during a clinically stable period.[44,45] This score accurately predicted flares in both European American and African American study groups. Accurate prediction of SLE flares might allow early treatment or prevention of flares.

Urinary biomarkers. Glomerulonephritis, a major cause of morbidity and mortality in patients with SLE, currently requires renal biopsy for definitive diagnosis. Urinary biomarkers would be useful for identifying patients with LN at the highest risk of end-stage renal disease or distinguishing between LN and other forms of renal disease in patients with lupus. Urinary levels of TNF-like weak inducer of apoptosis correlated with disease activity in a large multicenter longitudinal study.[46] Other possible urinary biomarkers include monocyte chemoattractant protein 1, high-mobility group box 1 protein, vascular cell adhesion molecule 1, and angiostatin.[47-50] Levels of these markers correlate with histologic changes in renal tissue and can distinguish between active and inactive LN.

Interferons. Interferons are consistently associated with SLE. Serum IFN-α activity associates with autoantibodies, as well as BLyS.[51,52] IFN-α levels correlated with the number of autoantibody specificities in preclinical samples obtained from patients later classified as having SLE, suggesting a role for IFN-α in autoantibody accrual.[12] Interestingly, IFN-γ activity was increased before autoantibody positivity, and autoantibody positivity preceded increases in IFN-α levels. IFN-γ regulates both IFN-α and B-cell differentiation and possibly drives B-cell maturation and class-switching in early stages of SLE pathogenesis. However, only a subset of adults with SLE show interferon activity in serum.[51]

Interferon response is most often measured indirectly as an "interferon signature" defined by upregulation of sets of interferon-regulated genes. In a large longitudinal monitoring study, 84.8% of pediatric patients with lupus demonstrated an interferon signature.[53] A similar interferon signature has been reported in about half of adults with SLE, and interferon activity levels have been associated with autoantibody production and disease activity.[54-56] Longitudinal monitoring of whole-blood gene expression profiles and parallel disease activity in a large pediatric cohort demonstrate 7 different clusters of reactivity and association with various gene expression modules.[53] Although these data associate interferons and interferon-associated gene regulation with SLE, some aspects of the

interferon signature might be relatively stable over the course of the disease based on paired analyses of longitudinal data.[57,58] Indeed, high serum IFN-α activity appears to be a complex heritable trait.[51] Therefore interferons can influence SLE predisposition, whereas, together with dysregulation of other immune pathways, such as BLyS, T_H, and inflammatory mediators, they can influence disease pathogenesis.

Because of the heterogeneity among patients with SLE, personalized treatment/monitoring based on molecular mechanisms involved might be beneficial. Using an unbiased approach with modular gene expression panels that include interferon signature genes, Banchereau et al[53] stratified patients with pediatric SLE into separate groups supported by patient genotypes. Such patient stratification might enable studying the effectiveness of biomarkers for disease activity or treatment response in a particular subset of patients with a relevant molecular mechanism of disease pathogenesis.

RA

RA, a systemic autoimmune rheumatic disease afflicting up to 0.8% of the population, is characterized by synovitis leading to irreversible joint destruction. Effective management of RA requires initiation of therapy with disease-modifying antirheumatic drugs within months after disease onset to maximize outcomes.[59] Even a brief delay can have a significant effect on disease progression. New autoantibody specificities can develop over time, and therefore periodic monitoring might be warranted, especially in arthralgia-positive patients who do not yet have an RA diagnosis. However, once autoantibodies are present in patients with clinical RA, serial monitoring is not necessary because fluctuations in titers over time are not associated with disease activity or further prognosis.

Autoantibodies

Two of the most common autoantibodies in patients with RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) (Table I).

RF. RF is directed against the Fc portion of the IgG class of antibodies. Although RF occurs in 70% to 80% of patients with RA, it also occurs in patients with SLE, Sjögren syndrome, or systemic infections, as well as approximately 10% of healthy subjects.[60] For RA disease classification, RF has a sensitivity of 69% and specificity of 85%.[61] Higher levels of RF are associated with more severe disease marked by disease progression, rheumatoid nodules, and various extra-articular manifestations.[62] Based on the higher sensitivity and clinical utility of ACPAs, RF has become more of a historical test and is usually now only tested in combination with anti–citrullinated peptide (CCP).

Antibodies against citrullinated proteins. ACPAs target proteins or peptides in which arginine residues have been converted to citrulline. HLA-DRB1 alleles are the strongest genetic association for seropositive RA. HLA-DRB1 interacts with cigarette smoking to increase the risk of ACPA-positive RA but not seronegative RA,[63] and IgA ACPA responses have been found in the sputum of patients with preclinical or early clinical RA.[64] ACPAs also appear before the onset of clinical disease, making them valuable markers for diagnosis and prognosis. See England et al[65] for a more extensive review of ACPA pathogenesis in patients with RA.

Clinically, antibodies against CCPs are used in RA diagnosis. In a meta-analysis anti-CCP had a pooled sensitivity of 67% and 95% specificity for RA, and anti-CCP positivity was associated with increased risk of radiographic progression.[61]

Anti-CCP positivity predicted progression to RA in a 3-year follow-up study of patients with undifferentiated arthritis.[66] Anti-CCP and RF were strongly associated with extra-articular manifestations.[62] Anti-CCP positivity and initial DAS28 (Disease Activity Score-28 joints) scores were associated with EULAR response to abatacept in analyses to predict factors of efficacy using data from the Orencia and Rheumatoid Arthritis registry.[67] A high anti-CCP titer was an independent predictor of decrease in DAS28 scores and EULAR good response to rituximab.[68] In patients with recent-onset RA, IgA anti-CCP was observed in 29% of patients, along with IgG. Patients with positive results for IgA anti-CCP had a more severe disease course over 3 years compared with those with negative IgA anti-CCP results.[69] A higher number of anti-CCP isotypes was associated with significantly more radiographic damage during the disease course over 10 years of follow-up.[70] Even though anti-CCP isotypes might not provide significant improvement in diagnosis compared with anti-CCP IgG, the isotypes can have possible prognostic implications.

Anti-perinuclear factor antibodies and anti-keratin antibodies recognize citrullinated epitopes on the same autoantigen, filaggrin or profilaggrin, and can serve as early diagnostic markers. Anti-Sa recognizes citrullinated vimentin[71] and shows a high specificity of 92% to 100% and a moderate sensitivity of 32% to 43%.[72] Several other citrullinated antigens have been identified in patients with RA, including fibronectin, filaggrin, fibrinogen, α-enolase, and collagen.

Autoantibodies with other specificities. Antibodies against carbamylated proteins (anti-CarP) are detected in 45% of patients with RA, including anti-CCP–negative patients. Targets of anti-CarP in patients with RA include vimentin, fibrinogen, and albumin.[73] Anti-CarP responses have been associated with mortality in patients with seropositive RA and specifically with respiratory causes of death in a Spanish cohort.[74] Anti-A2/anti-RA33 antibodies occur in more than 60% of patients with RA and are also seen in patients with SLE. If diagnoses of SLE or mixed connective tissue disease can be excluded, the specificity of anti-A2/anti-RA33 antibodies for RA can be as high as 96%. The specificity of anti-BiP antibodies for RA has been reported to be 96%, making these antibodies promising additional candidates for the classification or diagnosis of RA.

Acute-phase reactants

The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are increased in patients with RA compared with control subjects. ESR and swollen joint count were associated with radiographic disease progression in a systemic literature review including 57 studies with disease activity measurements in 13 to 1433 patients.[75] ESR and CRP levels are also measured as components of RA disease activity indices, such as DAS28, which are used in trials for clinical disease monitoring.

Cytokines

Many cytokines and chemokines are active in the joints of patients with RA, and these cytokines are critical in inflammation, joint damage, and RA-associated comorbidities (Fig 2).[76] Indeed, a number of different cytokines, such as TNF-α, IL-6, and IL-1RA, are successfully targeted in RA treatment, as are small-molecule

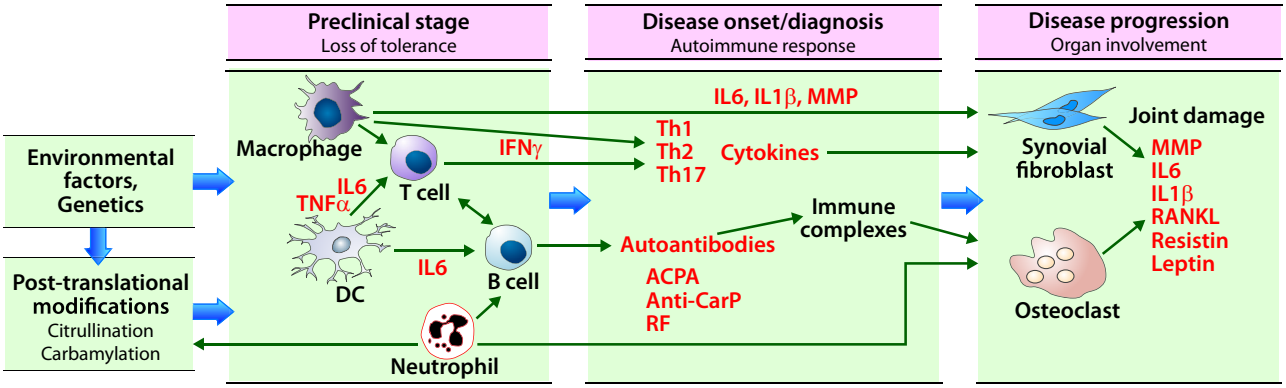

FIG 2. Biomarkers in patients with RA. The initial stage in RA pathogenesis is loss of tolerance to self-antigens. Genetic predisposition and environmental factors, such as viral infections, can lead to loss of tolerance and autoantibody generation through posttranslational modification of self-proteins. Disease onset or diagnosis occurs after amplification of the autoimmune response through interactions between innate and adaptive immune cells (dendritic cells, T cells, and B cells), leading to secretion of proinflammatory cytokines (IL-6 and TNF-α) that activate macrophages and neutrophils. Both macrophages and neutrophils contribute to the adaptive immune response. Antigen-exposed T cells differentiate into T_H1, T_H2, and T_H17 subsets, and maturation and class-switching of B cells leads to autoantibody secretion. Increased levels of cytokines and autoantibodies are the most useful markers at this stage. The inflammatory cytokines secreted by macrophages, T cells, and reactive oxygen species secreted by neutrophils activate synovial fibroblasts and induce osteoclast maturation from pro-osteoclasts. The ensuing inflammation causes joint damage. Soluble mediators secreted by macrophages, T cells, synovial fibroblasts, and osteoclasts are major biomarkers at this stage. *Anti-CarP*, Anti-carbamylated protein antibodies; *DC*, dendritic cells; *MMP*, matrix metalloproteinase; *RANKL*, receptor activator of nuclear factor κB ligand.

inhibitors, such as Janus kinase inhibitors that regulate cytokine secretion pathways. In addition, in serial samples preceding RA classification in a military cohort, the number of increased cytokines and chemokines predicted time to RA diagnosis/classification.[77] A commercially available blood test monitors RA disease activity with a score calculated from concentrations of 12 serum biomarkers: vascular cell adhesion molecule 1, epidermal growth factor, vascular endothelial growth factor A, IL-6, TNF receptor type 1, matrix metalloproteinases 1 and 3, cartilage glycoprotein 39 (YKL-40), leptin, resistin, serum amyloid A, and CRP. Changes in scores correlate with changes in other indicators of RA disease activity, including the DAS28 index, ESR, and CRP. In addition, this score decreased significantly in patients who responded to TNF inhibitors based on EULAR criteria, whereas patients with higher scores showed greater radiographic progression over 52 weeks of TNF inhibitor treatment.[78]

Emerging biomarkers

Biologics, such as TNF inhibitors, anti–IL-6 receptor antibodies, anti-CD20 antibodies, and cytotoxic T lymphocyte–associated antigen 4–immunoglobulin have shown efficacy in patients with RA. Several new biomarkers have been proposed to identify patients who might respond to these therapies. The response to rituximab is associated with RF positivity and normal levels of $CD19^+$ B cells together with increased $CD19^+CD27^-IgD^-$ B-cell counts.[79] Treatment with infliximab leads to decreases in the chemokines CXCL10/interferon-inducible protein 10, CCL2/monocyte chemoattractant protein 1, and CCL4/macrophage inflammatory protein 1β.[80] Serum concentrations of the myeloid-related protein 8/14 protein complex at baseline were proposed predictors of response to biological therapy (adalimumab, infliximab, or rituximab)[81] and methotrexate[82] in patients with active RA and might be useful for monitoring response to treatment across different mechanisms of action. Additional expanded biomarker studies are needed to help select the ideal therapy at the ideal time and dose for an individual patient.

SSc (SCLERODERMA)

SSc is a systemic autoimmune disease characterized by extensive fibrosis in the skin and internal organs. Patients with limited cutaneous systemic sclerosis (lcSSc) show restricted distal skin sclerosis, a long history of Raynaud phenomenon, and better prognosis. Diffuse cutaneous systemic sclerosis (dcSSc) has much more extensive skin involvement and earlier and more severe organ manifestations.[83] Major complications of SSc include skin and musculoskeletal complications, pulmonary arterial hypertension (PAH), interstitial lung disease (ILD), digital vasculopathy, renal crisis, and cardiac and gastrointestinal manifestations. Autoantibodies and other serologic markers, when present, can be very useful in ascertaining potential organ involvement, monitoring needs, or overall prognosis. However, as with other systemic autoimmune rheumatic diseases, monitoring levels once detected is usually unwarranted.

Autoantibodies

SSc is diagnosed based on clinical features, which are supplemented by the ANA profile (Table I). Anti-centromere antibodies (ACAs) occur in approximately 20% to 42% of patients in North America, mostly in patients with lcSSc.[84,85] Anti-centromere is 97% specific for lcSSc against other connective tissue diseases, with a positive predictive value of 89.5%.[85] The most likely severe complication in patients with positive ACA results is PAH, whereas digital ulcers and myocardial and kidney involvement are rare.

Anti–topoisomerase I (Scl70) antibodies are also highly specific (99.5%) and predictive (98%) for SSc.[85] They occur in 14% to 42% of patients in North America,[85] with the vast majority

having dcSSc. These autoantibodies associate with progressive pulmonary fibrosis, digital ulcers, and hand disability.

Anti–RNA polymerase III (RNAP III) has 98% to 100% specificity for SSc and occurs in 16% to 20% of patients, mostly in the dcSSc subset.[84,85] Anti–RNAP III is associated with hand disability and renal involvement and rarely with pulmonary fibrosis.[84] Similar to Scl70, anti–RNAP III is associated with higher rates of SSc-related mortality. Patients with anti-RNAP antibodies are about twice as likely as ACA-positive and 4-fold as likely at anti-Topo1–positive subjects to have cancer within 3 years of SSc onset.[86]

Anti-Th/To and U3-RNP antibodies target nucleolar antigens. Th/To autoantibodies are directed against subunits of RNase P and RNase myeloid-related protein. They occur in 2% to 5% of patients with SSc, 8.4% with lcSSc, and 0.6% with dcSSC. Anti-Th/To antibodies might be a marker for PAH.[87] Anti-U3 RNP antibodies target fibrillarin and are found in 18.5% of African American patients. These patients had a younger age of onset, higher frequency of digital ulcers and pericarditis, but lower lung severity scores and no difference in survival.[88] Anti-U3 RNP is most frequent in male and African American subjects with SSc and is associated with muscle involvement and increased risk of PAH.[84] Anti-U11/U12 RNP antibodies have been reported in 3.2% of patients with SSc who had no other SSc-associated autoantibodies. The presence of anti-U11/U12 RNP antibodies was associated with pulmonary fibrosis (79% of antibody positive vs 37% of antibody negative) and a 2.25-fold greater risk of death.[89] In a recent study anti-U11/U12 RNP antibodies were associated with myopathy, as well as severe gastrointestinal disease and severe Raynaud phenomenon, in patients with SSc and cancer.[90]

Emerging biomarkers

Because the major complications of SSc include pulmonary dysfunction and ILD, lung proteins have been studied as potential biomarkers for SSc. Serum levels of both Krebs von den Lungen protein (KL-6) and surfactant protein D are increased in patients with SSc and associate with decreased forced vital capacity and diffusing capacity of the lungs for carbon monoxide (DLCO). Increased levels are more frequent in Scl70-positive patients than in ACA-positive patients.[91] One of the more extensively studied biomarkers in patients with SSc is the N-terminal prohormone of brain natriuretic peptide (NT-proBNP). Higher circulating levels of NT-proBNP are associated with more severe PAH and greater risk of mortality.[92] However, NT-proBNP is not specific to PAH and can result from cardiac dysfunction.

Fibrosis is a major player in SSc pathogenesis. TGF-β is a key regulator of fibrosis, but its utility as a biomarker is limited by technical difficulties in measuring its free circulating form. Mediators regulated by or related to the TGF-β family have been studied in patients with SSc. TGF-β regulates cartilage oligomeric matrix protein (also known as thrombospondin-5), and sera from patients with SSc have increased cartilage oligomeric matrix protein levels, which correlate with the extent of skin involvement.[93] Levels of growth differentiation factor 15 (GDF-15), a distant member of the TGF-β family, are increased in sera from patients with SSc. GDF-15 levels strongly correlate with a modified Rodnan severity score for skin involvement and negatively correlate with forced vital capacity and DLCO.[94,95] Levels of GDF-15 are increased in patients with SSc with ILD compared with other patients with SSc, and higher levels of GDF-15 at baseline were predictive of worse lung disease severity scores at 30 months of follow-up. Therefore GDF-15 might be a prognostic marker for lung function. Interestingly, GDF-15 levels correlate with NT-proBNP and identified PAH with higher specificity and sensitivity compared with NT-proBNP.[96]

Along with TGF family proteins, levels of certain proinflammatory proteins are increased in patients with SSc and associated with fibrosis. Levels of the proinflammatory cytokine IL-6 are increased in sera of patients with SSc with anti-Scl70 or anti–RNAP III but not in patients with anti-centromere.[97] Increased IL-6 levels are associated with skin fibrosis, lung fibrosis, and increased mortality[98,99] but have not been correlated conclusively to disease activity. Levels of CXCL4, a proinflammatory chemokine that regulates several immune and nonimmune cells, were greater in patients with SSc compared with those in control subjects. Increased CXCL4 levels associated with faster progression of skin fibrosis and PAH and faster decrease in DLCO.[100] S100A8 and a dimer of S100A8/A9 are calcium-binding proteins involved in inflammatory processes. Their levels are higher in sera and bronchoalveolar lavage fluid from patients with SSc.[101,102] An independent study found increased levels in patients with lcSSc with lung fibrosis and Scl70 positivity but observed no correlation to pulmonary function tests.[103] Further studies are required to identify and validate specific biomarkers and their roles in pathogenesis and to enable early prediction of patients who will need a lung transplantation.

ANCA-ASSOCIATED VASCULITIDES

ANCA-associated vasculitides (AAV) are a group of autoimmune diseases characterized by the presence of ANCAs (Table I). AAV include eosinophilic granulomatosis with polyangiitis (EGPA; Churg-Strauss syndrome), microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA; Wegener granulomatosis).

EGPA (Churg-Strauss syndrome)

EGPA is an AAV distinguished by a history of allergic disease in the majority of patients, as well as the presence of eosinophilic infiltration in extravascular granulomas. EGPA starts often as chronic rhinitis followed by eosinophilia, which progresses to small-vessel vasculitis with associated symptoms. EGPA shares several features with asthma and hypereosinophilic syndrome (HES), making early diagnosis challenging. Currently, EGPA is often a diagnosis of exclusion and based on associated organ damage. Therefore identifying biomarkers for early diagnosis is important. ANCAs are detected in a third of patients (mostly p-ANCA directed against myeloperoxidase [MPO]). ANCA-positive patients are less likely to have heart and nonhemorrhagic lung involvement compared with ANCA-negative patients.

EGPA is characterized by increased numbers of circulating eosinophils (>1500 cells/μL). Glucocorticoid treatment drastically decreases eosinophil numbers, and eosinophilia is usually not an adequate biomarker for EGPA once a patient has started treatment.[104] The chemokine eotaxin-3 (CCL26) is one of the most widely studied biomarkers of pathologic significance in EGPA. Eotaxin-3 is secreted by epithelial cells and acts as a chemoattractant for eosinophils. Higher serum levels of eotaxin-3 were associated with active EGPA, although levels

decreased significantly on treatment.[105] Interestingly, eotaxin-3 levels were lower in patients with HES, thereby making it potentially useful in diagnosis.[105] At a cutoff of 80 pg/mL, eotaxin-3 has 87.5% sensitivity and 98.6% specificity for EGPA,[105] suggesting that eotaxin-3 might be a highly sensitive and specific marker for distinguishing active EGPA from eosinophilic (asthma and HES), rheumatic (SLE and SSc), and other AAV diseases. Serum IgG_4 levels are also increased in patients with active EGPA and correlate with the number of organ manifestations and Birmingham vasculitis activity score.[106]

CCL17/thymus and activation-regulated chemokine is secreted by PBMCs and is a chemoattractant for T_H2-type cells that are important in EGPA pathogenesis. Levels of CCL17 were increased in patients with active EGPA compared with control subjects and patients with inactive disease.[107] CCL17 levels decreased drastically after initiation of glucocorticoid therapy but increased before clinical relapse.[107]

A study of eicosanoid levels in excreted breath condensate found increased levels of the arachidonic acid metabolite 12-hydroxy-eicosatetraenoic acid in patients with active EGPA compared with those with inactive EGPA, HES, and asthma and healthy control subjects.[108] Although progress is being made, EGPA management would benefit from biomarkers to aid with initial and early diagnosis and longitudinal information about associations of emerging biomarkers with disease outcomes.

MPA

MPA is another AAV affecting the arterioles, capillaries, and venules, thereby involving the skin, nerves, gastrointestinal tract, lungs, kidneys, and joints. ANCAs in MPA are often directed against MPO.[109] However, MPO-ANCAs are not specific for MPA because they occur in patients with EGPA, necrotizing crescentic glomerulonephritis, sarcoidosis, IgA nephropathy, and infections.[110] MPA flares are often accompanied by increases in anti-MPO titers and increased levels of ESR, CRP, or both. Levels of a C-terminal fragment of apolipoprotein A1, AC-13, were increased in patients with MPA compared with those in patients with EGPA, GPA, and RA and healthy subjects, with reduced levels after treatment. Therefore AC-13 might serve as a specific marker for MPA disease activity.[111] Necrotizing glomerulonephritis is common in patients with MPA, and anti-MPO–associated glomerulonephritis is less responsive to standard-of-care treatments and has worse renal survival.[112] A serum creatinine level of greater than 4.6 mg/dL at initial MPA diagnosis was shown to be a good predictive factor for development of end-stage renal failure with 92.3% sensitivity and 84.6% specificity.[113] A recent study identified differential expression of genes associated with Toll-like receptor signaling in peripheral neutrophils in patients with MPA; however, evaluating the diagnostic potential of these patterns requires further research.[114]

GPA (Wegener granulomatosis)

The hallmarks of GPA include necrotizing granulomatous inflammation in the respiratory tract and pauci-immune vasculitis, primarily in the lung and kidneys. ANCAs in patients with GPA are directed mostly against proteinase-3 (PR3) and much less frequently against MPO.[115] PR3-ANCA has a high sensitivity and specificity for the diagnosis of active GPA (>90%), although ANCA-negative cases do occur.

Even with improved therapies, up to 50% of patients with GPA might face relapse within 5 years. Although an increase in ANCA levels is generally believed to precede relapse, many ANCA level increases are not followed by a clinical relapse, and relapses can occur without a preceding ANCA level increase.[116] However, ANCA level increases correlated with clinical relapses in patients with renal involvement, and the avidity of anti-PR3 antibodies increased during the period preceding clinical relapse but not during the preceding ANCA level increase.[117] Patients with severe GPA exhibit lower levels of Fc glycosylation in PR3-ANCA,[118] and the glycosylation profile of total IgG at the time of an ANCA level increase predicted a clinical relapse in patients with severe disease.[119]

Several cytokines have been proposed to contribute to systemic inflammation in patients with GPA and could potentially serve as biomarkers of disease activity or upcoming flare. Serum levels of S100A8/A9 are increased in patients with active AAV compared with those in patients in remission or healthy control subjects, and serum S100A8/A9 levels can identify PR3-positive patients at risk of relapse.[120] Serum levels of high-mobility group box 1 protein are significantly greater in patients with GPA than in control subjects.[121] Recently, a reduction in the number of regulatory T cells was reported during disease flare in patients with GPA, whereas expansion of both the regulatory T and T_H2 cell compartments was observed during remission.[122] The utility of immunophenotyping in assisting disease monitoring requires further evaluation.

SUMMARY/CONCLUSIONS

Autoimmune connective tissue diseases are complex disorders driven by environmental, genetic, and immunologic mechanisms. Antibodies are used clinically for diagnosis but fall short because of low specificity and limited understanding of their role in pathogenicity. Moreover, autoantibodies alone are often not sufficient to identify subjects at risk of disease. Implementing preventive measures will require biomarkers that can predict disease progression within a specific time frame or in patients with milder disease. Newer studies have proposed several new biomarkers that could serve this purpose, but several areas still need extensive research. Despite overlapping mechanisms of pathogenesis between autoimmune connective tissue diseases, these diseases are diverse, with varied clinical presentations and therapeutic efficacies. Therefore the underlying immunologic mechanisms can vary, and it is unlikely that a single biomarker will be suitable in all diseases. Indeed, even within a single disease, multiple biomarkers might be required to account for mechanistic and clinical heterogeneity between patients. A multivariate approach accounting for several aspects of the disease, as has been developed for SLE and RA, might prove useful to support diagnosis, monitor disease progression/prognosis, and select appropriate therapy for individual patients. Confirming the clinical validity of these approaches will require longitudinal analyses with sufficient power on well-characterized clinical cohorts.

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What do we know?

- **The spectrum of autoimmune connective tissue disorders shows a large degree of overlap in disease pathogenesis.**
- **Autoantibodies are one of the most useful biomarkers to support diagnosis and in some disease states can be used to monitor progression or response to treatment.**
- **Antibodies to dsDNA are more specific for SLE and, along with several soluble mediators, can assist in identifying subjects at increased risk of SLE and predicting disease flares.**
- **ACPAs are more specific for RA and precede disease onset. A circulating soluble mediator score has been proved to be useful in monitoring disease progression.**
- **ACA, Scl70, RNAP III, and Th/To autoantibodies are observed in patients with SSc, and although their roles in pathogenic mechanisms are unknown, they might allow patients to be divided into specific subsets based on clinical features.**
- **Patients with EGPA have eosinophilia, but this cannot distinguish between EGPA and other rheumatic or eosinophilic pathologies once treatment is initiated. A third of patients with EGPA have MPO-ANCA.**
- **GPA is characterized by the presence of PR3-ANCA. ANCA levels can be used to monitor disease progression in patients with renal involvement.**
- **A multivariate approach that encompasses different measures of pathologic processes, such as autoantibodies and soluble mediators, might prove most informative for early accurate diagnosis and monitoring and predicting disease progress.**

What is still unknown?

- **A precise understanding of disease pathogenesis and which mechanisms are disease specific or shared among autoimmune rheumatic diseases**
- **Which cytokines, chemokines, and soluble mediators will be the most informative in predicting flares in patients with SLE, RA, and AAV**
- **Which biomarkers can monitor response to treatment across diseases or within a specific autoimmune connective tissue disease**
- **Which biomarkers can be used to monitor disease progression in patients with SLE, SSc, GPA, and EGPA**
- **Biomarkers to aid early EGPA diagnosis**
- **Whether biomarkers can be used for early diagnosis of EGPA or to identify high-risk patients with AAV for prevention trials**

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