Autoantibodies in Autoimmune Diseases: Clinical and Critical Evaluation

Efstathia K. Kapsogeorgou PhD and Athanasios G. Tzioufas MD

Department of Pathophysiology, School of Medicine, National University of Athens, Athens, Greece

ABSTRACT: Autoimmune diseases constitute a diverse group of disorders characterized by cellular and humoral responses against self. The humoral autoimmune responses are directed against various cellular and extracellular components. These responses are highly specific for each autoimmune disease and result in the production of autoantibodies that characterize certain disease entities, representing a valuable tool for the diagnosis of autoimmune diseases. Furthermore, certain autoantibodies are helpful in the prognosis of disease development, progression and severity, as well as in the classification of patients with distinct disease subtypes. Today, the value of autoantibodies in the follow-up of patients is limited, but preliminary data suggest that they may be useful in predicting response to treatment.

IMAJ 2016; 18: 519–523

KEY WORDS: autoimmune diseases, autoantibodies, antinuclear antibodies (ANA), antiphospholipid antibodies (aPL), autoantibodies against aminoacyl-tRNA synthetases

Autoimmune diseases are a highly heterogeneous group of chronic disabling disorders characterized by immune responses against self organs, tissues and cells, resulting in damage to one or more organs, in case of organ-specific or systemic autoimmunity, respectively. Worldwide, they affect approximately 7–10% of the general population with a preference for women over men, and represent the second cause for hospitalization in internal medicine departments and the third cause of morbidity and mortality [1]. Although the etiopathogenesis of autoimmune diseases remains largely unknown, multiple factors – environmental, genetic, hormonal and neuropsychiatric – seem to participate in their development. The clinical presentation of autoimmune diseases varies enormously, even in the same disease entity, extending from benign to severe and organ-specific to systemic disease. Autoimmune disorders are the result of deregulated cellular and/or humoral (autoantibodies) autoimmune responses that lead to severe tissue/organ damage and disease.

Certain autoantibodies have a significant diagnostic utility

Despite their heterogeneity, almost all autoimmune disorders are characterized by the production of autoantibodies, e.g., antibodies recognizing various elements of self, proteins, lipids, DNA, etc. A variety of autoantibodies against distinct autoantigens have been recognized, with autoantibody responses characteristic for certain disease entities. Autoantibodies undoubtedly have outstanding diagnostic value in autoimmune diseases. In addition, autoantibodies have proven to be valuable in disease prediction and prognosis, and potentially in patient stratification. In this review we discuss the clinical significance of autoantibodies and present a critical evaluation of their value in autoimmune diseases.

AUTOANTIBODIES

Antibodies reacting with self components exist in non-pathological conditions and are called natural antibodies. These are polyreactive antibodies, mainly of IgM subtype, produced by B lymphocytes in the absence of external antigen stimulation, such as previous infection, vaccination, other foreign antigen exposure or passive immunization. Natural antibodies recognize self, altered self and foreign antigens. They display low affinity for autoantigens and, in contrast to autoantibodies, are considered protective. They have been reported to activate the classical complement pathway leading to lysis of enveloped virus particles long before the activation of adaptive immune response, while also recognizing epitopes arising from oxidation that occurs during inflammatory responses, as well as oligosaccharides or other cell surface molecules that are expressed during fetal differentiation and on tumor cells. Thus, natural antibodies have been implicated and/or are thought to play a significant role in the first-line defense against pathogens, tissue homeostasis, tumor necrosis and autoimmune diseases. In fact, lower levels of natural autoantibodies have been associated with the development of various autoimmune disorders [2].

In contrast to natural antibodies, autoantibodies recognize high affinity self antigens, whereas their transfer in experimental models was found to result in autoimmunity. The exact mechanisms and the triggering factors that govern the production of autoantibodies against self elements have not been delineated. Nevertheless, it is well established that the development of autoantibodies is a highly regulated process that involves typical anti-

Table 1. Autoantibodies in systemic autoimmune diseases and targets

Antigen location		Autoantibodies	Autoimmune disease
Nucleus	Nucleosomes	anti-DsDNA	SLE
	Nucleosomes	anti-histones	SLE
	snRNPs	anti-Sm	SLE
	snRNPs	anti-U1RNP	MCTD, SLE, SS, SSc
	RNPs	anti-Ro/SSA	SS, SLE
	RNPs	anti-La/SSB	SS, SLE
	Centromere	ACA	Limited scleroderma
	Topoisomerase-I	anti-Scl-70	SSc
Cytoplasm	tRNA-synthetases	anti-Jo-1	Myositis
	Mitochondria	AMA	PBC
	PR3, MPO	ANCA	Vasculitis
	Cytoskeleton elements	ASMA	Autoimmune hepatitis
Cell membrane	Phospholipids	Lupus anticoagulant	APS
	Phospholipids	anti-cardiolipin	APS
	Phospholipids	anti-β2GP1	APS
	Acetylcholine receptor	anti-acetylcholine receptor	Myasthenia gravis
Modified proteins	Citrullinated proteins	anti-CCP	RA
Extracellular	Antibodies (Fc portion)	RF	RA, SS, SLE, SSc

gen-driven immune responses arising from inefficient clearance of apoptotic material, molecular mimicry and/or modification of self-antigens during inflammatory responses. Furthermore, inefficient removal and/or regulation of autoreactive B cell clones that escape central tolerance have been incriminated [3]. The autoantibodies recognize a variety of molecules that are often intracellular and thus "invisible" in the immune system, including nucleic acids, lipids and proteins, located in almost all cellular compartments, as well as extracellular components, such as antibodies. Thus, autoantibodies directed against the nucleus, the cytoplasm (organelles and/or other cytoplasmic components), the cell membrane, the cytoskeleton and neurological synapses have been described in various autoimmune diseases [Table 1]. Despite the plethora of humoral responses against variable autoantigens in autoimmune diseases, the pattern of autoantibody expression is highly specific and characterizes each autoimmune disorder. Hence, autoimmune responses against various components of the translational machinery characterize distinct autoimmune entities, including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS) and myositides, while responses against cytoskeleton or antibodies are more often seen in rheumatoid arthritis (RA) [Table 1]. The autoimmune responses are mainly polyclonal and target multiple epitopes within the same or interacting autoantigens, whereas immunization of experimental animals with fragments of the autoantigens results in intra- and inter-molecular spreading of the immune response, similar to that observed after immunization with foreign antigens, supporting the idea that autoimmune responses are antigen driven and specific. Despite the significance of humoral responses in autoimmune diseases, the role of autoantibodies in their pathogenesis is not evident. With the exception of neonatal lupus, a direct pathogenetic role of autoantibodies has not been proved, suggesting that they might represent a result and not a cause of autoimmune responses. In this context, despite the extensive study of autoantibodies, the mechanisms underlying the specificity of autoimmune humoral responses in each disease are not clear and need to be elucidated. However, the disease specificity of certain autoantibodies has been proved extremely useful for the diagnosis of autoimmune diseases; moreover, several lines of evidence suggest that autoantibodies may have significant value in the prediction, prognosis and possibly in the follow-up of the disease [4].

Table 2. Autoantibodies with high diagnostic utility

Autoantibodies	Autoimmune disease
anti-dsDNA	SLE
anti-Sm	SLE
anti-La/SSB	SS
anti-β2GPI	APS
anti-CCP	RA
anti-Scl-70	SSc
anti-tRNA synthetases	Myositis
anti-acetylcholine receptor	Myasthenia gravis
anti-PR3	Wegener's granulomatosis

AUTOANTIBODIES IN THE DIAGNOSIS OF AUTOIMMUNE DISEASES

The diagnosis of autoimmune diseases, particularly an early diagnosis, is often complicated by the heterogeneity of clinical symptoms and phenotypes, the non-specific symptoms that overlap among distinct autoimmune entities (such as arthralgias, fatigue and rashes), and the fact that patients do not present with the full spectrum of features that usually develop over time and may not be specifically associated with a particular disease. Thus, the definite diagnosis of autoimmune diseases may be time consuming and may require repeated evaluation and monitoring of patients, imposing a significant burden on both health costs and patients' quality of life. Autoantibodies, in conjunction with other clinical and laboratory findings, have been proved valuable for disease diagnosis and some have been included in the diagnostic criteria of certain autoimmune syndromes. Thus, the diagnosis of SLE, RA, SS, antiphospholipid syndrome (APS) vasculitides, scleroderma, primary biliary cirrhosis (PBC), etc., is partially based on the detection of one or more autoantibodies [Tables 1 and 2]. Most of these autoantibodies have long been

Diagnosis of autoimmune diseases cannot be based solely on autoantibodies and must also take clinical and other laboratory markers into account

identified; however, the development of immunologic detection methods has led to the identification of numerous new autoantibody specificities whose diagnostic and clinical value needs to be validated. Some of the major autoantibodies used in the routine diagnostic evaluation of patients with suspected autoimmune disorder are described here briefly.

Antinuclear autoantibodies (ANA) represent a broad heterogeneous group of antibodies reacting with various cellular components that are detected by immunofluorescence in neoplastic cell lines or tissues. ANA detection is semi-quantitative and their titer is estimated as the last positive dilution of serum. ANA titers ≥ 1/160 dilution are considered a strong indicator of autoimmune diseases (approximately 90% sensitivity/specificity), whereas below that level ANA can be found in various situations, such as in healthy people, in the elderly, in viral infections, etc. Several patterns of ANA staining involving nuclear and/or cytoplasmic patterns have been recognized. Although most of them are not disease specific, certain patterns, such as anti-mitochondrial (AMA), anti-centromere (ACA) or anti-ribosomal, characterize distinct disease entities (PBC, limited scleroderma or myositides, respectively). Despite their specificity, their presence is not in itself sufficient to endorse diagnosis since they can also be found in other disorders where they usually characterize distinct disease subsets. For example, AMA and ACA have been described in 4–27% and 1.7–13% of SS patients, respectively. AMA have been associated with liver involvement in SS, whereas ACA positivity determines a distinct patient subgroup from ACA-negative SS patients with shared features of both SS and sclerosis [5]. Although ANA are not disease specific, they are of major importance in SLE since they are included in the SLE classification criteria [6]. Furthermore, their low cost renders them an ideal test for screening autoimmunity. However, ANA positivity requires further analysis using specific immunological methods to verify autoantibody specificities.

Autoantibodies can be used for the prognosis, but not the follow-up, of disease and can also predict response to treatment

Autoantibodies recognizing distinct components of the nucleosomes or the transcriptional/translational cellular machinery characterize certain autoimmune disorders [Table 1]. Thus, antibodies against DNA or histones are frequently found in SLE, with anti-DNA antibodies to be included in SLE classification criteria. Furthermore, autoantibodies against topoisomerase-I or Scl-70 characterize diffuse systemic sclerosis (SSc). Anti-Sm autoantibodies that recognize the proteins comprising the common core of U1, U2, U4 and U5 small nuclear ribonucleoprotein (snRNP) particles (B/B', D1, D2, D3, E, F, G) are specific for SLE, being found in 5–30% of SLE patients, and constitute one of the SLE classification criteria [6]. Antibodies to the U1 snRNP complex, which is known to play a major role in splicing of pre-messenger RNA, characterize mixed connective tissue disease (MCTD). However, they can also be found in SLE and SSc. Anti-Ro/La antibodies are directed against Ro52/TRIM21, Ro60/TROVE2 and La/SSB proteins that directly interact (the latter two) or associate with hY RNAs (hY1, hY3, hY4 and hY5 RNAs) [5]. Historically, the specificities against Ro52/TRIM21 and Ro60/TROVE2 were not distinguished and were described as anti-Ro/SSA antibodies. Both anti-Ro/SSA and anti-La/SSB autoantibodies are predominantly found in patients with SS or SLE, whereas in SS they represent one of the classification criteria [7]. Although autoantibodies against Ro/SSA and La/SSB autoantigens frequently co-exist, they can also be found alone (with anti-La/SSB monospecificity to be rare) [5]. These autoantibodies have not been found to have a direct pathogenetic role in SS. On the contrary, maternal Ro/SSA autoantibodies, and to a lesser extent La/SSB, have been incriminated in the development of neonatal lupus syndrome (NLS) by passing to the fetal circulation, causing injury to several organs, mainly skin and heart. NLS presents with a photosensitive annular transient skin rash, reversible alteration of hematological and hepatic function, and irreversible cardiac disease where the major finding is heart block with or without cardiomyopathy (complete heart block occurs in nearly 2%) [8]. Interestingly, the development of NLS seems to be regulated by the development of an idiotypic-anti-idiotypic network of antibodies in the mother. According to the idiotypic/anti-idiotypic network theory, the antibodies recognizing a specific epitope on an antigen (idiotypic) induce an immune response against them, which leads to the production of anti-idiotypic antibodies that neutralize the idiotypic antibodies [9]. Indeed, anti-La/SSB-positive mothers who gave birth to a healthy child had higher titers of anti-idiotypic antibodies in their sera, compared with mothers who gave birth to a child with NLS, suggesting that anti-idiotypic antibodies may be protective, most probably by binding to pathogenic anti-La/SSB antibodies, thereby blocking their entrance to the fetal circulation [10].

Anti-neutrophil cytoplasmic antibodies (ANCA) are a heterogenous group of autoantibodies that react with antigens in the cytoplasm of neutrophil granulocytes. The diagnostic test mainly involves indirect immunofluorescence in fixed neutrophils. ANCA are distinguished according to the fluorescence staining patterns to cytoplasmic ANCA (c-ANCA), perinuclear ANCA (p-ANCA) and atypical ANCA. c-ANCA mainly react with proteinase-3 (PR3), and p-ANCA with myeloperoxidase (MPO). Atypical ANCA may represent reactions against several proteins such as bacterial permeability increasing factor (BPI), cathepsin G, elastase, lactoferrin and lysozyme. The detection of ANCA by immunofluorescence can be followed by identification of the respective autoantigen by specific enzyme-linked immunosorbent assay (ELISA). ANCA have been proved valuable for the diagnosis and differential diagnosis of small vessel vasculitides (ANCA-associated vasculitides), such as Wegener's granulomatosis, microscopic polyangiitis and Churg-Straus syndrome [11].

Anti-smooth muscle antibodies (ASMA) recognize various elements of the cytoskeleton, including actin, microfilaments,

microtubules or intermediate filaments [12]. They are also detected by indirect immunofluorescence assay. In addition to ANA positivity they are indicative of type-1 autoimmune hepatitis [13].

Antibodies to cell surface molecules have also been described. Thus, autoantibodies against the phospholipids of the cell membrane, namely antiphospholipid autoantibodies (aPL) – including anticardiolipin antibodies (aCL), lupus anticoagulant and antibodies to β2-glycoprotein I (β2GPI) – characterize and possibly have a pathogenetic role in antiphospholipid syndrome (APS) [14], whereas anti-acetylcholine receptor (AChR) autoantibodies characterize the vast majority of patients with myasthenia gravis [15].

As mentioned earlier, autoantibodies that recognize other antibodies and specifically the Fc portion of immunoglobulin G (IgG), namely rheumatoid factor (RF), have been described. Although RF is often found in patients with RA (around 80%), it is also frequent in other autoimmune diseases such as SS (70%), SLE (30%) and scleroderma (30%), as well as in chronic infections (50%). Thus, RF alone has low diagnostic value. However, in addition to other autoantibodies that react with chemically modified molecules, it has high diagnostic value for RA [16]. The autoantibodies against chemically modified molecules recognize peptides and proteins that are citrullinated (anticyclic citrullinated peptides, anti-CCP), a process that occurs during inflammation where arginine amino acid residues in proteins are enzymatically converted into citrulline residues. Anti-CCP are mainly directed against citrullinated filaggrin, vimentin, keratin and antiperinuclear factor [17]. Due to their high specificity for RA they are a good diagnostic tool for early diagnosis of the disease, prompting their recent inclusion into RA classification criteria [18]. Nevertheless, like all autoantibodies, anti-CCP can also be found in other autoimmune diseases where they are usually associated with certain clinical phenotypes.

Finally, autoantibodies can be extremely helpful in the differential diagnosis of distinct clinical syndromes of one autoimmune entity. Hence, autoantibodies against aminoacyl-tRNA synthetases have been proved useful in the diagnosis of distinct syndromes belonging to idiopathic inflammatory myopathies (IIM) [19]. In this context, Jo-1, which reacts with the histidyl-tRNA synthetase that catalyzes the binding of the histidine to its cognate tRNA during protein synthesis, is strongly associated with anti-synthetase syndrome. This is characterized by myositis, interstitial pulmonary disease, non-erosive arthritis, "mechanic's hands," skin rashes, and constitutional symptoms such as fever. Autoantibodies to PL-7 (threonyl-) and PL-12 (alanyl-) tRNA synthetases have been connected to interstitial pulmonary disease, gastrointestinal manifestations and less frequently myositis, when compared to patients positive for anti-Jo-1 [20]. On the other hand, autoantibodies to Mi-2, which is a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, characterize typical dernatomyositis and are found in approximately 20% of patients. Autoantibodies to the signal recognition particle (SRP), which recognizes the signal peptide on the growing peptide chain and targets the complex in the ER membrane, have been found in up to 5% of patients with IIM and are also connected to a distinct clinical and histopathological subset of IIM [21]. This is characterized by necrotizing myopathy that includes both necrotic and regenerating myofibers, as well as lower numbers of infiltrating lymphocytes compared to polymyositis. In addition, patients with anti-SRP antibodies frequently reveal an unusually severe weakness, acute onset, rapid disease progression, and resistance to treatment [21]. In the same context, autoantibodies against aquaporin-4 can distinguish between Devic's disease (also known as neuromyelitis optica) and multiple sclerosis [22].

As evident from the above, a wide range of autoantibodies characterizing various autoimmune disorders have been described. Although most of them are used for the diagnosis of autoimmune diseases, a limited number have significant diagnostic value [Table 2]. Despite the abundance of data, research is still focused on the detection of novel autoantibodies, especially in neurologic autoimmune entities. Furthermore, efforts are being made in evaluating and identifying patterns of autoantibody expression that could serve as biomarkers of disease development, follow-up and response to treatment.

AUTOANTIBODIES IN THE PROGNOSIS OF DISEASE DEVELOPMENT, SEVERITY, FOLLOW-UP AND TREATMENT

Several types of autoantibodies, such as ANA, anti-Ro/SSA and anti-La/SSB, precede diagnosis for at least 2 to 3 years [reviewed in 4], suggesting that they may contribute to early diagnosis. The clinical significance of this is disputed since these antibodies are not totally specific and their presence is insufficient to predict the development of autoimmune disease. However, they may indicate that autoantibody-positive individuals should be followed on a regular basis in order to facilitate an early diagnosis.

Furthermore, autoantibodies may be useful in the prognosis of disease severity. Thus, the presence of both RF and anti-CCP antibodies is associated with systematic radiographic joint damage, persistence of synovitis, and need for more aggressive treatment [23]. In SLE, autoantibodies to ribosomal P proteins (RibP) have been associated with neuropsychiatric manifestations as well as nephritis [4,24,25], whereas aPL antibodies at diagnosis have been correlated with severe clinical outcome, such as nephritis, CNS involvement, thrombocytopenia, clotting events and increased risk of pregnancy complications correlated to APS [reviewed in 4]. Furthermore, as mentioned earlier, the presence of anti-Ro/SSA and/or anti-La/SSB has been implicated in increased risk for development of neonatal lupus syndrome. On the other hand, anti-myelin antibodies, such as anti-myelin oligodendrocyte glycoprotein and anti-myelin basic protein, were reported to predict the progression of multiple sclerosis from a clinically isolated to a clinically definite syndrome [26].

Although autoantibodies have a role in disease prognosis, this does not apply to follow-up. The levels of expression of most autoantibodies do not significantly change during the disease course, and even when they do change this has not had clinical value. Autoantibodies against double-stranded DNA may have a role in the follow-up of lupus nephritis [27].

Finally, attempts have been made to use certain autoantibodies to predict therapeutic responses. Thus, the presence of anti-synthetase and anti-Mi-2 autoantibodies in rituximab-treated refractory adult and juvenile dermatomyositis and adult polymyositis strongly predicted clinical improvement in patients with refractory myositis [28]. In ANCA-associated vasculitides, PR3-positive patients are reported to respond better to rituximab compared with cyclophosphamide/azathioprine, whereas PR3-positive patients who underwent renal transplantation were twice as likely to experience a post-transplant relapse compared to the MPO-positive patients [29,30].

CONCLUSIONS

Autoantibodies have been proved a valuable tool for the diagnosis of autoimmune diseases. However, the diagnosis of autoimmune diseases cannot be based solely on autoantibody expression, since even those with high specificity for an autoimmune disorder can be found in multiple entities. Furthermore, certain autoantibodies are helpful in disease prognosis and the classification of patients in distinct subsets with a variable clinical presentation of an autoimmune entity. Although their utility in the follow-up of the disease is doubtful, preliminary data suggest that autoantibodies may be useful for the prediction of response to treatment. Extensive studies applying contemporary techniques are underway to identify novel autoantibodies that could serve as biomarkers for disease diagnosis, prognosis, follow-up and response to treatment.

Correspondence
Dr. A.G. Tzioufas
Dept. of Pathophysiology, School of Medicine, National University of Athens, 75 Mikras Asias Street, Athens 11527, Greece
Phone: (30210) 746-2513
Fax: (30210) 746-2664
email: agtzi@med.uoa.gr

References

1. Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J Autoimmun* 2009; 33: 197-207.
2. Schwartz-Albiez R, Monteiro RC, Rodriguez M, Binder CJ, Shoenfeld Y. Natural antibodies, intravenous immunoglobulin and their role in autoimmunity, cancer and inflammation. *Clin Exp Immunol* 2009; 158 (Suppl 1): 43-50.
3. Suurmond J, Diamond B. Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity. *J Clin Invest* 2015; 125: 2194-202.
4. Damoiseaux J, Andrade LE, Fritzler MJ, Shoenfeld Y. Autoantibodies 2015: From diagnostic biomarkers toward prediction, prognosis and prevention. *Autoimmun Rev* 2015; 14: 555-63.
5. Kyriakidis NC, Kapsogeorgou EK, Tzioufas AG. A comprehensive review of autoantibodies in primary Sjogren's syndrome: clinical phenotypes and regulatory mechanisms. *J Autoimmun* 2014; 51: 67-74.
6. Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677-86.
7. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
8. Izmirly PM, Buyon JP, Saxena A. Neonatal lupus: advances in understanding pathogenesis and identifying treatments of cardiac disease. *Curr Opin Rheumatol* 2012; 24: 466-72.
9. Jerne NK. Towards a network theory of the immune system. *Ann Immunol* (Paris) 1974; 125C: 373-89.
10. Stea EA, Routsias JG, Clancy RM, Buyon JP, Moutsopoulos HM, Tzioufas AG. Anti-La/SSB antiidiotypic antibodies in maternal serum: a marker of low risk for neonatal lupus in an offspring. *Arthritis Rheum* 2006; 54: 2228-34.
11. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013; 65: 1-11.
12. Toh BH. Smooth muscle autoantibodies and autoantigens. *Clin Exp Immunol* 1979; 38: 621-8.
13. Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. *World J Gastroenterol* 2008; 14: 3374-87.
14. Vlachoyiannopoulos PG, Samarkos M, Sikara M, Tsiligros P. Antiphospholipid antibodies: laboratory and pathogenetic aspects. *Crit Rev Clin Lab Sci* 2007; 44: 271-338.
15. Mori S, Shigemoto K. Mechanisms associated with the pathogenicity of antibodies against muscle-specific kinase in myasthenia gravis. *Autoimmun Rev* 2013; 12: 912-17.
16. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000; 43: 155-63.
17. Wiik AS, van Venrooij WJ, Pruijn GJ. All you wanted to know about anti-CCP but were afraid to ask. *Autoimmun Rev* 2010; 10: 90-3.
18. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69: 1580-8.
19. Mahler M, Miller FW, Fritzler MJ. Idiopathic inflammatory myopathies and the anti-synthetase syndrome: a comprehensive review. *Autoimmun Rev* 2014; 13: 367-71.
20. Hervier B, Devilliers H, Stanciu R, et al. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. *Autoimmun Rev* 2012; 12: 210-17.
21. Hengstman GJ, ter Laak HJ, Vree Egberts WT, et al. Anti-signal recognition particle autoantibodies: marker of a necrotising myopathy. *Ann Rheum Dis* 2006; 65: 1635-8.
22. Drori T, Chapman J. Diagnosis and classification of neuromyelitis optica (Devic's syndrome). *Autoimmun Rev* 2014; 13: 531-3.
23. Schoels M, Bombardier C, Aletaha D. Diagnostic and prognostic value of antibodies and soluble biomarkers in undifferentiated peripheral inflammatory arthritis: a systematic review. *J Rheumatol Suppl* 2011; 87: 20-5.
24. Karassa FB, Afeltra A, Ambrozic A, et al. Accuracy of anti-ribosomal P protein antibody testing for the diagnosis of neuropsychiatric systemic lupus erythematosus: an international meta-analysis. *Arthritis Rheum* 2006; 54: 312-24.
25. Tzioufas AG, Tzortzakis NG, Panou-Pomonis E, et al. The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: a large cohort of consecutive patients and patients with active central nervous system disease. *Ann Rheum Dis* 2000; 59: 99-104.
26. Berger T, Rubner P, Schautzer F, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N Engl J Med* 2003; 349: 139-45.
27. Manson JJ, Ma A, Rogers P, et al. Relationship between anti-dsDNA, anti-nucleosome and anti-alpha-actinin antibodies and markers of renal disease in patients with lupus nephritis: a prospective longitudinal study. *Arthritis Res Ther* 2009; 11: R154.
28. Aggarwal R, Bandos A, Reed AM, et al. Predictors of clinical improvement in rituximab-treated refractory adult and juvenile dermatomyositis and adult polymyositis. *Arthritis Rheum* 2014; 66: 740-9.
29. Geetha D, Lee SM, Shah S, Rahman HM. Relevance of ANCA positivity at the time of renal transplantation in ANCA associated vasculitis. *J Nephrol* 2015. **Dec 8** doi: 10.1007/s40620-015-0253-6 [Epub ahead of print]
30. Unizony S, Villarreal M, Miloslavsky EM, et al. Clinical outcomes of treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis based on ANCA type. *Ann Rheum Dis* 2016; 75: 1166-9.