



Invited critical review

Autoantibodies in pre-clinical autoimmune disease



Zhi-De Hu ^a, An-Mei Deng ^{b,*}

^a Department of Laboratory Medicine, General Hospital of Ji'nan Military Command Region, Ji'nan, PR China

^b Department of Laboratory Diagnosis, Changhai Hospital, Second Military Medical University, Shanghai, PR China

ARTICLE INFO

Article history:

Received 10 March 2014

Received in revised form 17 June 2014

Accepted 18 June 2014

Available online 24 June 2014

Keywords:

Autoantibodies

Autoimmune disease

Pre-clinical phase

ABSTRACT

The presence of autoantibodies is characteristic of autoimmune diseases. It is widely accepted that autoantibodies provide crucial diagnostic and prognostic information for autoimmune diseases. Indeed, numerous studies have demonstrated that the appearance of autoantibodies precedes the clinical onset of autoimmune diseases. We performed a literature review regarding the appearance of autoantibodies that preceded the clinical onset of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, primary biliary cirrhosis, inflammatory bowel disease, and multiple sclerosis. Herein we review and comment on the major findings of these studies.

© 2014 Elsevier B.V. All rights reserved.

Contents

1. Introduction	14
2. Evidence that autoantibodies precede AID	15
2.1. Rheumatoid arthritis (RA)	15
2.2. Systemic lupus erythematosus (SLE)	15
2.3. Sjögren's syndrome (SS)	15
2.4. Primary biliary cirrhosis (PBC)	15
2.5. Inflammatory bowel disease (IBD)	16
2.6. Multiple sclerosis (MS)	16
3. Comments on available studies	16
3.1. Test methods	16
3.2. Biological variations	16
3.3. Thresholds	16
3.4. Confounding factors	17
3.5. Study designs	17
4. Conclusions	17
Conflicts of interest	17
Acknowledgments	17
References	17

1. Introduction

The onset and development of autoimmune disease (AID) are the consequence of interactions between genetic and environmental factors, which results in dysregulation of the immune system, and is

characterized by the presence of autoantibodies and autoreactive T cells. Under these circumstances, immune system antimicrobial defenses react against normal components of the body and result in organ-specific or systemic immunopathology. Thus, one of the most frequent characteristics of AID is the presence of circulating autoantibodies. These autoantibodies provide crucial diagnostic and prognostic information for the management of AID.

Autoantibodies are not necessarily specific for AID, as the autoantibodies can also appear in the blood of healthy individuals or under

* Corresponding author at: Department of Laboratory Diagnosis, Changhai Hospital, Second Military Medical University, Shanghai 200433, China. Tel./fax: +86 21 31162061. E-mail address: amdeng70@163.com (A.-M. Deng).

some special situations, such as infections and the pre-clinical phase of AID. Recently, the association between autoantibodies and risk of AID has attracted considerable attention. Numerous studies have shown that detectable autoantibodies precede the clinical onset of AID [1–3], and therefore could be a possible tool for AID screening or early diagnosis. We performed a literature review regarding evidence of autoantibodies during the pre-clinical phase of AIDs and the implications of these findings.

2. Evidence that autoantibodies precede AID

2.1. Rheumatoid arthritis (RA)

Rheumatoid factor (RF) is the most widely used autoantibody to diagnose RA [4]. A positive RF was considered an important criterion by the American Rheumatism Association (AHR) in 1958 [5], the American College of Rheumatology (ACR) in 1987 [6], and the ACR/European League Against Rheumatism (EULAR) in 2010 [7]; anti-cyclic citrullinated peptide (anti-CCP) has now been included in the diagnostic criteria of the EULAR [7]. The testing methods used for RF have greatly improved during recent years [4], from the classic Waaler–Rose test (agglutination of sheep red blood cells sensitized with rabbit IgG) and latex particle assays to automated techniques, such as nephelometry and ELISA. This review focuses on published reports that were based on automated RF assays.

Numerous studies have demonstrated that autoantibodies can be detected in the blood of individuals who are free of RA symptoms, but who developed RA years later, including RF [8–17], anti-CCP [9–16,18–21], anti-mutated citrullinated vimentin (anti-MCV) [15], and anti-peptidylarginine deiminase 4 (anti-PAD-4) [14]. Some studies have also shown that RF [22–24], anti-CCP [22–25], and anti-MCV [23] are detectable in the blood of undifferentiated arthritis (UA) patients, with UA defined as an inflammatory arthritis for which no definitive diagnosis can be made [26]. For patients with arthralgias, RF, anti-CCP [27], and anti-carbamylated protein (anti-CarP) antibodies [22] are associated with a higher risk of RA. These studies [8–21], with sample sizes for RA ranging from 49 to 183, and a median follow-up time ranging from 1 to 5 years, all reported that various isotypes of autoantibodies were increased in individuals who were free of RA at the time of blood sampling, but developed RA later, as compared with those who did not develop RA during the follow-up period. The positive rates for RF or anti-CCP antibodies in pre-clinical RA patients range from 10% to 60%, which is higher than healthy controls [9–20].

The prevalence of these antibodies increase at times close to the onset of RA [9–11,13,14,17,19,20,28,29], and these incremental increases remain after the diagnosis of RA was established [14,28]. In addition, the higher the anti-CCP antibody titer and the younger the individual, the shorter the time between a positive autoantibody test and the onset of RA [9,12,18]. The median time between a seropositive result and the onset of RA symptoms was longer for anti-CCP-positive individuals than IgM-RF-positive individuals [12,13]. Thus, the presence of IgM-RF is the consequence of inflammation, whereas the earlier appearance of anti-CCP antibodies suggests a pathophysiologic process [12]. These autoantibodies are also associated with the future clinical characteristics of RA. Anti-CCP antibodies, but not RF, for example, are highly associated with the development of erosive RA [9].

Unlike previous studies that were based on a nested case–control design, Nielsen et al. [30] conducted a general population-based cohort study with a sample size of 9712 and a maximum follow-up period of approximately 28 years to determine the association between detectable RF and future risk of RA; RA occurred in 183 cases. Nielsen et al. [30] found that the cumulative incidence of RA increased as the titer of RF increased. For women 50–69 years of age who smoked and had RF values >100 IU/mL, 1 in 3 developed RA within 10 years from the time of blood sampling.

Taken together, it is clear that the presence of RF and anti-CCP antibodies usually precedes the onset of RA. These results indicate that in

apparently healthy individuals, as well as individuals with UA and arthralgias and positive RF or anti-CCP titers, careful follow-up should be provided to aid in an early diagnosis.

2.2. Systemic lupus erythematosus (SLE)

One early study in which 23 asymptomatic pregnant women with positive anti-Ro or anti-La titers were followed for many years reported that four developed SLE, which suggested that anti-Ro or anti-La antibodies preceded the development of SLE [31]. In another study, Aho et al. [32] reported that 10 of 16 SLE patients were positive for antinuclear antibodies (ANA) before the onset of SLE, which was a much higher rate than controls.

The results from the U.S. Department of Defense Serum Repository (n = 130; longest follow-up time, 9.4 years) showed that the presence of ANA, anti-Ro, anti-La, anti-dsDNA, anti-Sm, anti-phospholipid (APL), and anti-rNP antibodies and RF preceded the onset of SLE [33–37]. Among SLE patients, 88% were positive for at least 1 of these autoantibodies, which was a much higher percentage than healthy controls, and the prevalence of these autoantibodies increased after diagnosis [33,34]. Anti-Ro, anti-La, and APL antibodies were the earliest detectable autoantibodies during the pre-clinical phase of RA [33,37]. In addition, the presence of these autoantibodies was associated with incipient severe SLE. For example, patients who were positive for anti-dsDNA antibodies often developed renal disease [34,35], patients who were positive for IgG RF were more likely to develop arthritis [35], and positive APL was associated with malar rash and photosensitivity [36]. In addition, regular patterns exist among these autoantibodies. For example, the majority of La-positive pre-clinical SLE patients were also Ro-positive, and a significant overlap was observed between anti-dsDNA antibody-positive patients and anti-chromatin antibody-positive patients [37].

Additional evidence was reported by Eriksson et al. [38]. Similar to the study performed with the U.S. Department of Defense Serum Repository, Eriksson et al. [38] also found that anti-Ro, anti-rNP, anti-histone, anti-La, and anti-dsDNA antibodies and ANA were detectable in 63% (n = 38) of patients' serum samples 4.2 years before the onset of SLE symptoms and a confirmed diagnosis. ANA had the highest sensitivity (45.7%), followed by anti-dsDNA (20%) and anti-Ro antibodies (20%). Anti-Ro antibody was detected first in the serum of these patients, 6.6 years before the onset of SLE symptoms and 8.1 years before a diagnosis was made [38].

2.3. Sjögren's syndrome (SS)

In a study that followed 23 asymptomatic pregnant women who were positive for anti-Ro or anti-La antibodies for 5–9 years reported that 2 patients developed SS, which suggested that seropositivity for anti-Ro or anti-La preceded the development of SS [31]. In another study, which was also based on pregnant women, it was shown that almost all mothers developed SS symptoms during a 9-year follow-up (mean follow-up, 4.5 years) [39].

A recently published nested case–control study (n = 44) showed that 66% of SS patients were positive for ANA, RF, and anti-La or anti-Ra antibodies approximately 5 years before the onset of SS [40]. The time that patients were positive for these antibodies persisted for up to 18 years before the onset of SS [40]. The seropositive rate for these antibodies increased nearer the onset of SS [40].

In conclusion, the evidence that autoantibodies are predictors of SS is limited. Additional well-designed studies, particularly general population-based cohort studies, are needed to establish a relationship between other autoantibodies and SS.

2.4. Primary biliary cirrhosis (PBC)

PBC in the early stage is often asymptomatic. One-half of PBC patients will become symptomatic during a 5-year period, and almost all

PBC patients will develop symptoms during a 20-year period [41]. Therefore, an early diagnosis or prediction is particularly important.

In 1986, Mitchison et al. [42] reported on 29 patients who were positive for anti-mitochondrial antibodies (AMA), had normal liver function, and were free of PBC symptoms. The biopsy results suggested that these patients exhibited PBC-like pathologic findings [42]. After follow-up for 4–13 years, PBC-related symptoms were observed in 5 patients and alkaline phosphatase (AKP) levels were elevated in 11 patients [42]. After follow-up for 18 years, with the exception of 5 patients who died due to a non-liver disease, 22 of 24 patients developed PBC-related symptoms and all 24 of these patients had abnormal liver function test results [43]. Repeat biopsies suggested that 40% of these patients had progressed to PBC [43].

In another study, Kisand et al. [44] followed 8 patients who were free of PBC symptoms and were positive for anti-pyruvate dehydrogenase complex (anti-PDC) or PDC-E2. After a 9-year follow-up, 3 of 8 patients had elevated AKP levels [44].

Therefore, it seems that the appearance of AMA can precede clinical symptoms, abnormal liver function, and elevated AKP levels associated with PBC. The current diagnostic criteria for PBC are two of the following: (1) positive AMA; (2) elevated AKP; and/or (3) histologic evidence of non-suppurative destructive cholangitis and destruction of interlobular bile ducts [45]. These diagnostic criteria may facilitate the early diagnosis of PBC for patients who have no characteristic symptoms. It would be very interesting to determine whether or not AMA-positive patients with normal AKP levels and the absence of histologic features of PBC in apparently healthy individuals can predict the onset of PBC.

2.5. Inflammatory bowel disease (IBD)

In 2005, Israeli et al. [46] reported that 10 of 32 Crohn's disease (CD) patients were positive for anti-*Saccharomyces cerevisiae* antibodies (ASCA) 38 months prior to diagnosis, while no uninfected controls were positive for ASCA. The seropositive rates for ASCA increased nearer the onset of CD [46]. There is no evident association between ASCA and ulcerative colitis (UC); no UC patients ($n = 8$) were positive for ASCA before disease onset [46]. In contrast to ASCA, perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) are less frequently associated with CD; only 2 of 8 UC cases and 2 of 32 CD cases were positive for pANCA, although no controls were positive [46].

It was recently shown that ASCA IgA, ASCA IgG, pANCA, anti-OmpC, and anti-CBir1 titers were increased before the onset of IBD that had occurred a mean of 4.5 years previously [47]. Using a logistic regression model to combine these autoantibodies, an algorithm was established to predict the onset of UC ($n = 167$) and CD ($n = 77$); these results showed that this algorithm had a higher predictive accuracy for UC and CD [47]. More importantly, the predictive accuracy of this algorithm increased as the clinical onset of CD or UC approached [47].

2.6. Multiple sclerosis (MS)

The majority of MS patients initially present with a clinically isolated syndrome (CIS), which is characterized by inflammatory demyelinating lesions in the optic nerve, spinal cord, or brain stem. Therefore, CIS patients have a high risk for developing MS and should be regarded as a target population for MS screening or early diagnosis.

In 2003, Berger et al. [48] reported that positive anti-myelin oligodendrocyte glycoprotein (anti-MOG) and anti-myelin basic protein (anti-BMP) antibodies based on immunoblot analysis in CIS patients were associated with MS. Berger et al. [48] reported that only 23% (9/39) of CIS patients who were negative for anti-MOG and anti-BMP antibodies developed MS during a 4-year follow-up. In contrast, for patients who were positive for anti-MOG and anti-BMP antibodies, 95% develop MS within 7 months [48]. That study demonstrated that anti-MOG and anti-MBP antibodies are particularly useful for predicting MS among CIS patients. To date, many studies have reported on the

value of anti-MOG and anti-BMP antibodies for MS prediction; however, the results of these studies varied [49–58], possibly due to the differences in study subjects and test methods.

Four studies partially supported the findings of Berger et al. [55–58]. Kuhle et al. [56] found that anti-MOG antibodies only predicted MS for female patients with at least one MRI lesion. Rauer et al. [57] reported that although the incidence of MS was not affected by the presence of anti-MOG and anti-MBP antibodies, antibody-positive patients developed significantly earlier relapses than patients who were antibody-negative [57]. In another study, it was found that 8 of 11 CIS patients who were seropositive for anti-MOG antibodies, but negative for anti-MBP antibodies, and 14 of 18 CIS patients who were seropositive for anti-MOG and anti-MBP antibodies developed MS, whereas none of the 9 patients who were seronegative for anti-MOG and anti-MBP antibodies developed MS within 20 months [58]. Tomassini et al. [55] concluded that the diagnostic criteria for MS could affect the predictive accuracy of anti-MOG and anti-MBP antibodies. There was no significant association between anti-MOG and anti-MBP antibody titers and future MS when using McDonald's criteria as the reference [55]; however, if Poser's criteria were used as the reference, anti-MOG and anti-MBP antibody titers could predict the onset of MS [55].

Four studies that used the same method as Berger et al. [48] did not detect an association between anti-MOG and anti-BMP antibody titers and future MS among CIS patients [49–52], which may have been due to the sampling time from CIS onset, cerebrospinal fluid oligoclonal band status, and genetic background [54]. In addition to immunoblot tests, studies based on flow cytometry [51] and ELISA [53,55] results did not demonstrate a positive association between autoantibodies and MS risk. In addition, it should be noted that anti-MOG-antibody status is not stable in individuals, as serial measurements have demonstrated that this antibody is associated with variable results [53].

3. Comments on available studies

3.1. Test methods

It should be noted that when interpreting clinical significance, the test method used must be taken into consideration. Currently, most autoantibody tests use immunoassays, which results may have interference due to the presence of cross-reacting substances, anti-analyte antibodies, or anti-reagent antibodies [59]. Indeed, different test methods can yield varying test results. For example, the positive rate for first generation anti-CCP test methods (ES 300 analyzer; Roche Diagnostics; Mannheim, Germany) was 41% in the same cohort [13], while the positive rate for a second-generation anti-CCP ELISA (Axis-Shield, Dundee, UK) was 59% [20]. Therefore, improving test performance and standardizing assays for autoantibodies are important. It would be very interesting to determine whether or not those patients who have false-positive autoantibody results (after testing error is completely excluded) have a higher risk for an AID.

3.2. Biological variations

Some studies have shown that autoantibodies are not always stable in individuals [8,13,37,53]. For example, one study showed that only 69.2% of pre-RA patients who were positive for anti-CCP antibodies remained positive during a follow-up test [13]. Some autoantibodies can also be affected by infections [60]. Therefore, when interpreting autoantibody test results, possible biological variations should be taken into consideration and repeated testing, if indicated, may provide more reliable results.

3.3. Thresholds

Some studies have shown that higher autoantibody levels are associated with a higher risk for an AID [18,30] and that there is a shorter

interval between a positive autoantibody result and the onset of an AID [11,18,48]. To determine an optimal threshold (clinical decision level) for a positive autoantibody result to predict AID is particularly important. It should be noted that the majority of studies have used the upper limit of a reference interval (quantitative test) as the threshold; however, a reference interval only reflects the distribution of autoantibodies in an apparently healthy cohort, but does not take into consideration future disease risks [61]. Therefore, additional studies are needed to explore the optimal thresholds to be used for autoantibodies for disease prediction.

3.4. Confounding factors

Some studies have shown that autoantibodies are associated with inflammatory markers [16], chemokines [10], and genetic [62,63] and environmental factors [4]. It is very important to verify whether or not autoantibodies are independently associated with disease risk. In some of these studies, when considering these factors, some of these autoantibodies were no longer independently associated with an AID [10,11,13,19]. Therefore, positive autoantibody results should be interpreted within the clinical context. A rule or algorithm that takes genetic factors, laboratory findings, and clinical symptoms or signs into consideration may be a good tool for predicting the onset of an AID. For example, two algorithms developed by van de Stadt et al. [64] and van der Helm-van Mil et al. [65] had a high accuracy for predicting RA among patients with UA and arthralgias.

3.5. Study designs

The majority of the studies cited used a nested case–control design, which cannot be used to determine to what extent detectable autoantibodies predict the onset of AIDs. The question is as follows: How many AID-free patients with positive autoantibody results will develop an AID. To answer this question, prospective, large-sample, general population-based cohort studies are needed to rigorously explore the predictive value of autoantibodies for AIDs. Given the low prevalence of AIDs, autoantibody testing in the general population is of no benefit [25,66]; however, for individuals who are at high risk for an AID, autoantibody testing may be beneficial. High-risk patients include those with UA who have a high risk for developing RA in the short term [23,25,62], patients with arthralgia [27], and women 50–69 years of age who smoke, and patients with RF results >100 IU/mL, 1 in 3 of whom will develop RA within 10 years from the time of blood sampling [30].

4. Conclusions

To date, many studies have identified that detectable autoantibodies are associated with a risk for future AID. In general, the existing studies demonstrate that the following: (1) the presence of autoantibodies can precede the clinical onset of AID; (2) the positive rates (qualitative tests) and the levels (quantitative tests) of autoantibodies increase nearer the onset of AID; (3) detectable autoantibodies during the pre-clinical phase are associated with future clinical characteristics of AID; and (4) the duration of positivity for autoantibodies may be underestimated because the time when autoantibodies first appeared in blood were not recorded for some subjects. In addition, when interpreting positive autoantibody results for patients who are free of AIDs, the test methods, thresholds, biological variations, and confounding factors should be taken into consideration. To better understand the associations between detectable autoantibodies and risk for future AIDs, well-designed, prospective cohort studies are needed.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (Nos. 81273282 and 81302541).

References

- [1] Deane KD. Preclinical rheumatoid arthritis (autoantibodies): an updated review. *Curr Rheumatol Rep* 2014;16:419.
- [2] Bizzaro N. Autoantibodies as predictors of disease: the clinical and experimental evidence. *Autoimmun Rev* 2007;6:325–33.
- [3] Bizzaro N. The predictive significance of autoantibodies in organ-specific autoimmune diseases. *Clin Rev Allergy Immunol* 2008;34:326–31.
- [4] Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. *Dis Markers* 2013;35:727–34.
- [5] Diagnostic criteria for rheumatoid arthritis: 1958 revision by a Committee of the American Rheumatism Association. *Ann Rheum Dis* 1959;18:49–51 [French transl 51–42; Spanish transl 52–43].
- [6] Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
- [7] 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.
- [8] Halldorsdottir HD, Jonsson T, Thorsteinsson J, Valdimarsson H. A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. *Ann Rheum Dis* 2000;59:149–51.
- [9] Majka DS, Deane KD, Parrish LA, et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. *Ann Rheum Dis* 2008;67:801–7.
- [10] Kokkonen H, Mullazehi M, Berglin E, et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. *Arthritis Res Ther* 2011;13:R13.
- [11] Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–9.
- [12] Bos WH, Nielen MM, Dijkmans BA, van Schaardenburg D. Duration of pre-rheumatoid arthritis anti-cyclic citrullinated peptide positivity is positively associated with age at seroconversion. *Ann Rheum Dis* 2008;67:1642.
- [13] Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
- [14] Kolfenbach JR, Deane KD, Derber LA, et al. Autoimmunity to peptidyl arginine deiminase type 4 precedes clinical onset of rheumatoid arthritis. *Arthritis Rheum* 2010;62:2633–9.
- [15] Turesson C, Bergstrom U, Jacobsson LT, Truedsson L, Berglund G, Saxne T. Increased cartilage turnover and circulating autoantibodies in different subsets before the clinical onset of rheumatoid arthritis. *Ann Rheum Dis* 2011;70:520–2.
- [16] Nielen MM, van Schaardenburg D, Reesink HW, et al. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. *Ann Rheum Dis* 2006;65:535–7.
- [17] Jonsson T, Thorsteinsson J, Valdimarsson H. Elevation of only one rheumatoid factor isotype is not associated with increased prevalence of rheumatoid arthritis—a population based study. *Scand J Rheumatol* 2000;29:190–1.
- [18] Chibnik LB, Mandl LA, Costenbader KH, Schur PH, Karlson EW. Comparison of threshold cutpoints and continuous measures of anti-cyclic citrullinated peptide antibodies in predicting future rheumatoid arthritis. *J Rheumatol* 2009;36:706–11.
- [19] Berglin E, Padyukov L, Sundin U, et al. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. *Arthritis Res Ther* 2004;6:R303–8.
- [20] van de Stadt LA, de Koning MH, van de Stadt RJ, et al. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum* 2011;63:3226–33.
- [21] Sokolove J, Bromberg R, Deane KD, et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* 2012;7:e35296.
- [22] Shi J, van de Stadt LA, Levarht EW, et al. Anti-carbamylated protein antibodies are present in arthralgia patients and predict the development of rheumatoid arthritis. *Arthritis Rheum* 2013;65:911–5.
- [23] van der Linden MP, van der Woude D, Ioan-Facsinay A, et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. *Arthritis Rheum* 2009;60:2232–41.
- [24] Kudo-Tanaka E, Ohshima S, Ishii M, et al. Autoantibodies to cyclic citrullinated peptide 2 (CCP2) are superior to other potential diagnostic biomarkers for predicting rheumatoid arthritis in early undifferentiated arthritis. *Clin Rheumatol* 2007;26:1627–33.
- [25] van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50:709–15.
- [26] Wevers-de Boer KV, Heimans L, Huizinga TW, Allaart CF. Drug therapy in undifferentiated arthritis: a systematic literature review. *Ann Rheum Dis* 2013;72:1436–44.
- [27] Bos WH, Wolbink GJ, Boers M, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. *Ann Rheum Dis* 2010;69:490–4.

- [28] Jorgensen KT, Wiik A, Pedersen M, et al. Cytokines, autoantibodies and viral antibodies in premonitory and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. *Ann Rheum Dis* 2008;67:860–6.
- [29] del Puente A, Knowler WC, Pettitt DJ, Bennett PH. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. *Arthritis Rheum* 1988;31:1239–44.
- [30] Nielsen SF, Bojesen SE, Schnohr P, Nordestgaard BG. Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. *BMJ* 2012;345:e5244.
- [31] Waltuck J, Buyon JP. Autoantibody-associated congenital heart block: outcome in mothers and children. *Ann Intern Med* 1994;120:544–51.
- [32] Aho K, Koskela P, Makitalo R, Heliovaara M, Palosuo T. Antinuclear antibodies heralding the onset of systemic lupus erythematosus. *J Rheumatol* 1992;19:1377–9.
- [33] Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349:1526–33.
- [34] Arbuckle MR, James JA, Kohlase KF, Rubertone MV, Dennis GJ, Harley JB. Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. *Scand J Immunol* 2001;54:211–9.
- [35] Heinlen LD, McClain MT, Merrill J, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. *Arthritis Rheum* 2007;56:2344–51.
- [36] McClain MT, Arbuckle MR, Heinlen LD, et al. The prevalence, onset, and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus. *Arthritis Rheum* 2004;50:1226–32.
- [37] Heinlen LD, McClain MT, Ritterhouse LL, et al. 60 kD Ro and nRNP A frequently initiate human lupus autoimmunity. *PLoS One* 2010;5:e9599.
- [38] Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapää-Dahlqvist S. Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden. *Arthritis Res Ther* 2011;13:R30.
- [39] McCune AB, Weston WL, Lee LA. Maternal and fetal outcome in neonatal lupus erythematosus. *Ann Intern Med* 1987;106:518–23.
- [40] Jonsson R, Theander E, Sjöström B, Brokstad K, Henriksson G. Autoantibodies present before symptom onset in primary Sjögren syndrome. *JAMA* 2013;310:1854–5.
- [41] Prince MI, Chetwynd A, Craig WL, Metcalf JV, James OF. Asymptomatic primary biliary cirrhosis: clinical features, prognosis, and symptom progression in a large population based cohort. *Gut* 2004;53:865–70.
- [42] Mitchison HC, Bassendine MF, Hendrick A, et al. Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? *Hepatology* 1986;6:1279–84.
- [43] Metcalf JV, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. *Lancet* 1996;348:1399–402.
- [44] Kisand KE, Metskula K, Kisand KV, Kivik T, Gershwin ME, Uibo R. The follow-up of asymptomatic persons with antibodies to pyruvate dehydrogenase in adult population samples. *J Gastroenterol* 2001;36:248–54.
- [45] Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. *Hepatology* 2009;50:291–308.
- [46] Israeli E, Grotto I, Gilburd B, et al. Anti-*Saccharomyces cerevisiae* and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005;54:1232–6.
- [47] van Schaik FD, Oldenburg B, Hart AR, et al. Serological markers predict inflammatory bowel disease years before the diagnosis. *Gut* 2013;62:683–8.
- [48] 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.
- [49] Kuhle J, Pohl C, Mehling M, et al. Lack of association between antimyelin antibodies and progression to multiple sclerosis. *N Engl J Med* 2007;356:371–8.
- [50] Pelayo R, Tintore M, Montalban X, et al. Antimyelin antibodies with no progression to multiple sclerosis. *N Engl J Med* 2007;356:426–8.
- [51] Chan A, Decard BF, Franke C, et al. Serum antibodies to conformational and linear epitopes of myelin oligodendrocyte glycoprotein are not elevated in the preclinical phase of multiple sclerosis. *Mult Scler* 2010;16:1189–92.
- [52] Lim ET, Berger T, Reindl M, et al. Anti-myelin antibodies do not allow earlier diagnosis of multiple sclerosis. *Mult Scler* 2005;11:492–4.
- [53] Pittock SJ, Reindl M, Achenbach S, et al. Myelin oligodendrocyte glycoprotein antibodies in pathologically proven multiple sclerosis: frequency, stability and clinicopathologic correlations. *Mult Scler* 2007;13:7–16.
- [54] Berger T, Reindl M. Lack of association between antimyelin antibodies and progression to multiple sclerosis. *N Engl J Med* 2007;356:1888–9 [author reply 1888–1889].
- [55] Tomassini V, De Giglio L, Reindl M, et al. Anti-myelin antibodies predict the clinical outcome after a first episode suggestive of MS. *Mult Scler* 2007;13:1086–94.
- [56] Kuhle J, Lindberg RL, Regeniter A, et al. Antimyelin antibodies in clinically isolated syndromes correlate with inflammation in MRI and CSF. *J Neurol* 2007;254:160–8.
- [57] Rauer S, Euler B, Reindl M, Berger T. Antimyelin antibodies and the risk of relapse in patients with a primary demyelinating event. *J Neurol Neurosurg Psychiatry* 2006;77:739–42.
- [58] Greeve I, Sellner J, Lauterburg T, et al. Anti-myelin antibodies in clinically isolated syndrome indicate the risk of multiple sclerosis in a Swiss cohort. *Acta Neurol Scand* 2007;116:207–10.
- [59] Sturgeon CM, Viljoen A. Analytical error and interference in immunoassay: minimizing risk. *Ann Clin Biochem* 2011;48:418–32.
- [60] Palazzi C, Buskila D, D'Angelo S, D'Amico E, Olivieri I. Autoantibodies in patients with chronic hepatitis C virus infection: pitfalls for the diagnosis of rheumatic diseases. *Autoimmun Rev* 2012;11:659–63.
- [61] Horn PS, Pesce AJ. Reference intervals: an update. *Clin Chim Acta* 2003;334:5–23.
- [62] Feitsma AL, Toes RE, Begovich AB, et al. Risk of progression from undifferentiated arthritis to rheumatoid arthritis: the effect of the PTPN22 1858 T-allele in anti-citrullinated peptide antibody positive patients. *Rheumatology (Oxford)* 2007;46:1092–5.
- [63] Perricone C, Agmon-Levin N, Ceccarelli F, Valesini G, Anaya JM, Shoenfeld Y. Genetics and autoantibodies. *Immunol Res* 2013;56:206–19.
- [64] van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. *Ann Rheum Dis* 2013;72:1920–6.
- [65] van der Helm-van Mil AH, le Cessie S, van Dongen H, Breedveld FC, Toes RE, Huizinga TW. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007;56:433–40.
- [66] Shmerling RH. Autoantibodies in systemic lupus erythematosus—there before you know it. *N Engl J Med* 2003;349:1499–500.