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Predictive autoimmunity using autoantibodies: screening for anti-nuclear antibodies

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Abstract

Background: Early detection of antinuclear antibodies (ANA) in asymptomatic subjects is useful to predict autoimmune diseases years before diagnosis. ANA have been determined by indirect immunofluorescence (IIF) using human epithelial type 2 (HEp-2) cells, which is considered the gold standard technique. Multiplex technology (BioPlex ANA Screen) has been introduced for ANA evaluation in recent years. Nevertheless, concordance between BioPlex and IIF is low and there is no harmonization between both methods for detection of autoantibodies. This study has aimed to clarify the clinical significance of autoantibodies detected by BioPlex ANA Screen in subjects with undiagnosed clinical suspicion of autoimmune disease and to determine the predictive value of autoantibodies detected by BioPlex ANA Screen.
Methods: A 3-year follow-up study was performed of 411 subjects without a clear diagnosis of autoimmune diseases in whom autoantibodies were detected by BioPlex ANA Screen that were negative by IIF on HEp-2 cells.
Results: At 3 years of follow-up, 312 (76%) subjects were positive for autoantibodies by IIF and 99 subjects continued to be negative. A diagnosis of autoimmune disease was found in most of the subjects (87%).
Conclusions: BioPlex ANA Screen has greater sensitivity than IIF on HEp-2 cells for autoantibodies detection. Early detection of these antibodies by BioPlex can predict possible development of autoimmune diseases.

Keywords: BioPlex ANA Screen; high sensitivity; indirect immunofluorescence; positive predictive value; predictive autoantibodies; systemic autoimmune rheumatic diseases.

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Introduction

Antinuclear antibodies (ANA) are a heterogeneous group of autoantibodies [1] that can be detected in the sera of subjects with systemic autoimmune rheumatic diseases (SARD), such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SSj), systemic sclerosis (SS), rheumatoid arthritis (RA), idiopathic inflammatory myopathies (IIM) and systemic vasculitides (SV) [2–4]. Autoantibodies are very helpful in the diagnosis, prognosis assessment and monitoring of the clinical evolution of patients with SARD [2, 4–6].

Classically, the indirect immunofluorescence (IIF) assay on human epithelial type 2 (HEp-2) cells is the preferred method for ANA detection, it being the established gold standard for ANA screening [5]. IIF allows the detection of a large number of nuclear and cytoplasm antigens, however, the IIF assay has some downsides. It is laborious and time consuming due to the large number of serial dilutions and visual determination of the staining patterns [7]. The assay is subjective as it relies heavily on human interpretation [8]. Heterogeneity in microscopes, light power, lens magnification and Hep-2, substantially contributes to its variability [9]. Its limitations can be minimized using automated microscopes, although not all ANA patterns can be recognized by such systems. Another limitation of IIF is its lack of

specificity because these antibodies are present in other autoimmune diseases, infections, tumors and in 25% of apparently healthy individuals [5, 10, 11].

Demand for ANA testing has increased significantly in the last few years, highlighting the need for new faster, time efficient techniques that are preferably automated to ensure consistency and reliability. The BioPlex ANA Screen is a Luminex-based assay using magnetic beads. ANA determination by the BioPlex 2200 ANA Screen has high reproducibility and it is a high throughput analysis. However, at this time, not all antigens can be analyzed by this technique as it simultaneously only detects 13 autoantibodies of clinical significance (i.e. Ro52, Ro60, La, Sm, Sm/RNP, RNP-A, RNP-68, Scl70, centromere B, dsDNA, chromatin, Jo1, ribosomal P proteins) [12, 13].

There are several comparative studies that have reported autoantibodies detection by IIF and by the BioPlex ANA Screen. In one of these, the analysis of 510 healthy individuals for autoantibodies concluded that BioPlex is more specific than IIF for ANA screening and that the BioPlex ANA Screen is a useful tool for a high-throughput screening of healthy populations [13]. Another comparison between both techniques in routine samples with the diagnosis of autoimmune diseases showed that there were numerous discrepancies for ANA screening, but that good agreement exists for anti-dsDNA and anti-extractable nuclear antigens detection. However, a study on 236 patients with a systemic rheumatic disease upon diagnosis concluded that the sensitivity of BioPlex is higher than IIF except for SLE and SS [2, 14]. More studies are required to clarify the discrepancy between both techniques in order to harmonize the ANA detection test.

In recent years, several studies have revealed the importance of early detection of autoantibodies as predictors for autoimmune diseases. The analysis of sera samples in 130 patients before diagnosis of SLE showed that the autoantibodies appeared as early as 10 years prior to the diagnosis of SLE. The same study revealed that 88% of patients had at least one SLE autoantibody present prior to the diagnosis [15]. Others studies have confirmed that the presence of autoantibodies can predict the development of autoimmune diseases before their clinical onset [16–19]. Detection of predictive autoantibodies is important for disease prevention and mitigating clinical impact [20, 21]. The BioPlex ANA Screen may be a good candidate for screening and follow-up of high-risk subjects.

This study had two aims. The first was to clarify the clinical significance of the autoantibodies detected by BioPlex ANA Screen. The second aim was to establish the predictive value of BioPlex ANA Screen in subjects with clinical suspicion but without a diagnosis of autoimmune diseases.

Patients and methods

Study design and patients

We performed a 3-year follow-up prospective cohort study of 411 subjects without autoimmune diseases. Sera samples were collected for 1 year (from October 2011 to October 2012) from routine tests in the Autoimmunity Laboratory of Hospital 12 de Octubre (Madrid, Spain). Mean age (±standard deviation) of the patients was 52±16 years. The women to men ratio was 6:1 (85% women, 15% men). More than 95% of the patients were Mediterranean Caucasian. Physicians in the rheumatology department determined the diagnosis of autoimmune diseases and clinical manifestations according to the American College of Rheumatology criteria. This study complies with the Spanish legislation and European Community directives for cross-sectional studies.

Selection criteria were: (1) Autoantibodies detected by the BioPlex ANA Screen were not found by the IIF on HEp-2 cells at the time of patient inclusion in the study, (2) At least one annual review was performed during the study period.

Ethical issues

The study was submitted to the Ethics Committee for Clinical Research of the Hospital on the 12th October and received a favorable report (Reference No. CEIC-16/383). No informed consent was required.

Autoantibodies detection by the IIF assay on HEp-2 cells

The assay for ANA was performed using an IIF on Hep-2 cells (Inova Diagnostics, Inc, San Diego, CA, USA). Dilutions of 1:80 and 1:160 were performed with phosphatase-buffered saline for screening test. In brief: 30 μL of each diluted serum was incubated on one well with fixed HEp-2 cells. After the cells were incubated and rinsed off, they were incubated with FITC-IgG-conjugated antibody (Reference No. 508113). The results were analyzed using a Nikon Eclipse fluorescence microscope with a magnification ×400.

IIF negative sera (IIF–) was defined as having a negative result in a dilution of 1:80. Detection of nucleolar, nuclear (homogeneous, coarse speckled, fine speckled, peripheral and centromere) or cytoplasmic patterns (diffuse and fine speckled) at a dilution of 1:160 was considered as a positive result (IIF+). Conversion from IIF– to IIF+ was defined as three or more consecutive positive ANA-IIF sera samples in a dilution of 1:160 or higher.

Autoantibodies detection by BioPlex ANA Screen

The BioPlex 2200 ANA Screen (Bio-Rad Laboratories, Hercules, CA, USA) is an automated assay that employs fluorescently dyed

Table 1: The frequencies of the 13 autoantibodies detected by BioPlex ANA Screen in a cohort of 411 subjects BP+/IIF– without diagnosis of an autoimmune disease.

Autoantibodies prevalence (BioPlex ANA Screen)	
Ro60	40.6%
Ro52	27.3%
RNP-A	24.3%
La	18.9%
Chromatin	15.6%
Centromere B	13.1%
Sm-RNP	11.7%
dsDNA	11.4%
Topo I/Scl70	7.1%
Sm	6.3%
Ribonucleoprotein P	3.9%
RNP-68	1.5%
Jo 1	1.2%

magnetic beads for simultaneous detection of 13 autoantibody specificity levels within a single serum sample. The BioPlex 2200 ANA Screen is run on the BioPlex 2200 System, a fully-automated, random access multiplex testing platform. The BioPlex ANA assay can detect autoantibodies against: dsDNA, chromatin, ribosomal P, Ro52 (SS-A), Ro60 (SS-B), La (SS-B), Sm, Sm-RNP complex, RNP-A, RNP-68, Scl-70, centromere B and Jo-1. The primary antibody isotype detected by this assay is IgG. The BioPlex ANA assay reports an antibody index (AI) value (range 0–8) depending on the fluorescence intensity of the antibody-bound magnetic beads. Antibody concentration is proportional to the fluorescence intensity. All cutoffs were established based on the 99% percentile value for the non-disease population in Spain. The cutoff for autoantibodies detected by BioPlex was 1.0 AI as recommended by the manufacturer. Except for anti-RNP-A, anti-RNP-68 antibodies and anti-dsDNA antibodies (cutoff: 2.0 AI, 2.0 AI and 20 AI, respectively). BioPlex ANA Screen positive serum (BP+) was considered when there was a serum with at least one positive result for the antibodies detected by this assay.

Statistical analysis

Data from the subjects and the BioPlex calibration group were included in two different randomized databases that were processed and analyzed using MedCalc for Windows version 14.12 (MedCalc Software, Ostend, Belgium).

Results

1. Prevalence of autoantibodies detected by BioPlex ANA Screen in ANA-IIF negative subjects.

   Anti-Ro60 (40.6%) and anti-Ro52 (27.3%) antibodies were the most frequently found in the BP+/IIF-sera samples. Antibodies against RNP-68 (1.5%) and Jo-1 (1.2%) were the least prevalent. The remaining nine antibodies detected by the BioPlex ANA Screen were also frequently found in this cohort (Table 1).
2. Multiplex technology (BioPlex ANA Screen) is more sensitive than IIF in the early detection of autoantibodies.

   After a 3-year follow-up of the 411 subjects with BP+/IIF–, 312 turned into IIF positive (BP+/IIF+) and the remaining 99 continued to be BP+/IIF– (Figure 1). Sixty-five of the subjects that did not fulfill the criteria of IIF+ had at least one or more positive determinations over the 3 years. Thirty-four subjects were permanent ANA negative by IIF and positive by BP. We can conclude that the BioPlex ANA Screen has more sensitivity than the IIF on Hep-2 cells for the detection of autoantibodies that are clinically relevant, as it can detect "pathological" antibodies at least 3 years before IIF.

   The relation between the specific antibodies detected by the BioPlex and the IIF+ sero-conversion

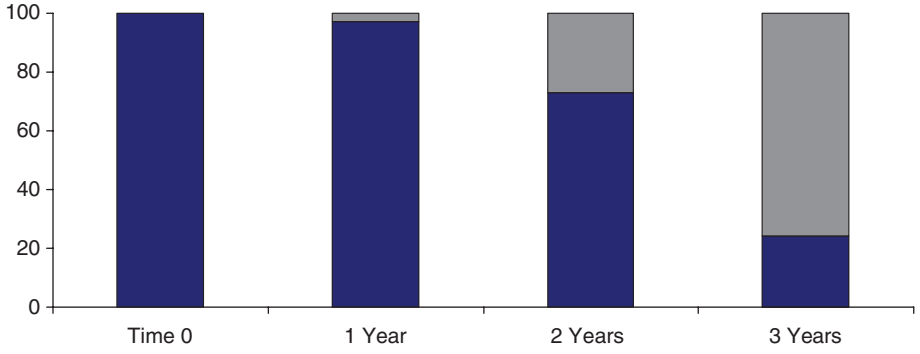

Figure 1: The proportion of subjects BP+/IIF– (blue bars) and the conversion to BP+/IIF+ (gray bars) in the 3 years of follow-up. At the beginning of the study all the subjects were BP+/IIF– and after the 3 years of follow-up, 76% of the subjects turned into BP+ and IIF+.

was calculated. Different frequencies of ANA-IIF seroconversion were observed depending on the existence of autoantibodies as detected by the BioPlex (Table 2).

3. Early detection of autoantibodies by the BioPlex can predict autoimmune disease development.

After a 3-year follow-up, 87% (358/411) of the subjects were diagnosed with an autoimmune disease. The most prevalent diagnosis was systemic lupus erythematous (37%) followed by Sjögren syndrome (23%) (Table 3A). Fifty-three subjects were not diagnosed with an autoimmune disease, yet 19 of them had clinical manifestations of autoimmune diseases as arthralgia and Raynaud's phenomenon. The remaining 34 had no clinical manifestations of autoimmunity (Table 3B). Predictive value for the BioPlex ANA Screen was 77.1% (70.1%–82.8% confidence interval). Positive predictive value (PPV) for the diagnosis of autoimmune disease for isolated antibodies detected by BioPlex was 65%–100% (Table 4).

Anti-Sm, anti-chromatin, anti-dsDNA and anti-ribosomal P antibodies had high predictive values for the diagnosis of SLE. The other autoantibodies detected by the BioPlex were also involved as predictors for clinical diagnosis of SLE (Table 5A). The anti-centromere B and anti-Scl70 antibodies could predict development of SS (Table 5B). Anti-SSA (Ro52 and R60) and SSB (La) appear at least 3 years before the diagnosis of Sjögren syndrome (Table 5C). Anti-RNP-A antibodies were associated with development of SLE and RA (Table 5A, D). Anti-RNP-A antibodies were the most prevalent antibodies detected in subjects without any external clinical manifestation for autoimmune disease after the 3 years of follow-up. The anti-Ro60 antibodies were detected in the serum of 22% of subjects without external clinical manifestations of autoimmunity (Table 6).

The probability of developing an autoimmune disease is directly proportional to the number of antibodies detected by BioPlex prior to the diagnosis. There is a higher predictive value of BioPlex ANA Screen when there are two positive antibodies, and it is almost 100% when three or more autoantibodies are detected by the BioPlex (Table 7).

Table 2: The relation between specific antibodies detected by BioPlex ANA Screen and the grade of IIF+ conversion after 3 years of follow-up.

Autoantibodies (BioPlex ANA Screen)	IIF positive
RNP-68	100%
Centromere B	91%
Chromatin	88%
Sm	88%
Ro52	86%
Sm-RNP	83%
La	82%
Ro60	81%
Ribonucleoprotein P	81%
Jo 1	80%
dsDNA	72%
Topo I/Scl 70	66%
RNP-A	62%

Table 3: The clinical characteristics of the 411 subjects after 3 years of follow-up.

(A) With autoimmune diseases	
Subjects with autoimmune diseases (n = 358)	
Rheumatoid arthritis	33
Sjögren syndrome	81
Systemic sclerosis	46
Systemic lupus erythematosus	134
Overlap syndrome	27
Inflammatory bowel disease	15
Myositis	8
Others[a]	14

[a]UCTD (n = 2), MCTD (n = 5), PBD (n = 1), AH (n = 3), celiac disease (n = 1), APS (n = 2).

(B) Without autoimmune diseases	
Subjects without autoimmune diseases (n = 53)	
Subjects with clinical manifestations of autoimmunity	n = 19
Arthralgies	63%
Raynaud's phenomenon	21%
Sicca symptoms	10%
Others[b]	16%
Subjects without clinical manifestations of autoimmunity	n = 34

[b]Swelling in hands, pain in musculotendinous insertions.

Table 4: The predictive value of isolated autoantibodies detected by BioPlex ANA Screen.

Autoantibodies	PPV, %	95% CI
RNP-A	65.3	51.3–77.1
Topo I/Scl-70	69.6	49.1–84.4
RIB-P	66.7	20.8–93.9
La	80	49.0–94.3
Sm	100	20.7–100
Sm-RNP	87.5	52.9–97.8
Jo-1	100	34.2–100
Chromatine	75	40.9–92.9
Cen B	87.8	74.5–94.7
dsDNA	85.7	65.4–95
Total	77.1	70.1–82.8

Table 5: The positive predictive value (PPV) of the antibodies detected by BioPlex ANA Screen for the diagnosis of autoimmune diseases.

	PPV, %	95% CI
(A) SLE		
RNP-A	37.0	27.73–47.29
Ro60	37.7	30.45–45.58
Ro52	24.1	16.75–33.28
RIB-P	75	47.41–91.67
La	23.1	14.60–34.25
Sm	84.6	64.27–94.95
Sm-RNP	66.7	51.49–79.19
Chrom	79.7	67.42–88.33
dsDNA	68.1	52.75–80.48
(B) SS		
Topo I/Scl-70	37.9	21.30–57.64
CenB	57.4	43.27–70.50
(C) SSj		
Ro60	37.1	29.88–44.97
Ro52	24.1	16.75–33.28
La	42.5	32.14–53.58
(D) RA		
RNP-A	15	8.91–23.85

PPV for the diagnosis as (A) systemic lupus erythematous, (B) systemic sclerosis, (C) Sjögren's syndrome and (D) rheumatoid arthritis.

Table 6: The frequency of antibodies detected by BioPlex ANA Screen in subjects without any external clinical manifestations of autoimmune diseases after 3 years of follow-up.

Autoantibodies	%
RNP-A	37.7
Ro60	22.6
Topo I/Scl-70	13.2
Others	26.5

Table 7: The predictive value of BioPlex ANA Screen when more than one antibody is positive.

Number of positive autoantibodies BioPlex ANA Screen	Autoimmune diseases after 3 years of follow-up (%)
One	77 (128/166)
Two	88.6 (78/88)
Three or more	96.8 (152/157)

Discussion

The multiplex technology (BioPlex 2200) has many advantages in the detection of autoantibodies, among them the simultaneous detection of 13 autoantibodies of established clinical significance [12, 22–25]. Several comparative studies exist between BioPlex ANA Screen and ANA-IIF with discrepant results [2, 13, 14, 26, 27]. We have performed a follow-up study for the first time on subjects without a diagnosis of autoimmune disease and with discrepant results between BP and IIF for autoantibodies detection.

BioPlex ANA Screen sensitivity in comparison to IIF was reported in healthy subjects [13] at the time of diagnosis [2] or in treated patients with autoimmune diseases [27]. We have demonstrated that the BioPlex ANA Screen detects autoantibodies years earlier than the IIF assay. Seventy-six percent of BP+/IIF− subjects became IIF+ over the 3-year follow-up period. It is mandatory to perform a long-term follow-up to confirm how many of the IIF-samples would be sero-converted to IIF+.

The most relevant consequence of the higher sensitivity of the BioPlex ANA Screen is its ability to predict autoimmune diseases by detecting early autoantibodies. Several prospective studies have demonstrated that autoantibodies can be detected in the sera of asymptomatic or pauci-symptomatic subjects even years before the development of the autoimmune diseases [19, 28]. After the decisive confirmation of the presence of autoantibodies years before the diagnosis of SLE [15], many studies have reported the presence of other specific autoantibodies that precede the clinical onset of autoimmune diseases [4, 16, 17, 29–31].

This study confirms the presence of autoantibodies at least 3 years before the diagnosis of an autoimmune disease. The most prevalent diseases diagnosed in this study were SLE, SSj and SS. SLE was mostly associated with predictive autoantibodies detected by BioPlex ANA Screen. Patients with SLE were found to be positive for anti-ds-DNA antibodies, anti-Sm and anti-chromatin antibodies at least 3 years before the diagnosis. Other autoantibodies as anti-ribonucleoprotein P, anti-Ro52 and anti-Ro60 also had a PPV for the eventual development of SLE. In other studies, the detection of anti-chromatin and anti-ds-DNA antibodies was reported as early as 10 years and 2 years, respectively, before the first emergence of SLE [15]. Anti-Ro, anti Sm, anti-ribonucleoprotein P were detected in subjects years before the diagnosis of SLE [4, 15, 21]. The presence of these antibodies in asymptomatic subjects may be an incentive to perform a follow-up study on these patients as they would be considered high-risk subjects [21]. Anti-CenB antibodies had a predictive positive value of 57% to develop SS. Similarly, anti-Topo I/Scl70 antibodies are also predictive of this disease. It has been reported that these antibodies are important as

diagnostic and prognostic biomarkers [32]. The results of this study confirm that BioPlex ANA Screen can detect the autoantibodies before the clinical diagnosis of SS. Anti-Ro52, anti-Ro60 and anti-La antibodies have a PPV for the development of SSj. The presence of these antibodies as early as 4 years before the diagnosis of SSj was reported previously [31].

Anti-RNP-A antibodies are the most prevalent antibodies in subjects without diagnosis of SARD after 3 years of follow-up. Shovman et al. [13] found that the anti-RNP-A were the most prevalent antibodies detected by the BioPlex ANA Screen in healthy subjects. Other studies confirm the high frequency of anti-RNP-A antibodies [2]. Association between antibodies against RNP-A and the diagnosis of SLE, RA and other autoimmune diseases was reported in other studies as well [2, 33].

Our results confirm that the number of types of autoantibodies increases towards the time of diagnosis [15]. In fact, the presence of three or more positive autoantibodies detected by the BioPlex ANA Screen had a PPV of 97% for the developing of an autoimmune disease. Our findings demonstrate that the BioPlex ANA Screen is useful for early detection of autoantibodies.

Early detection of autoantibodies has a significant role in the mosaic of autoimmunity as an important risk factor for the development of autoimmune disease [28, 34]. There are other genetic [35–38] and environmental factors (such as; female gender [39–41], low levels of vitamin D [42–44], vaccines [38, 45, 46], infections [38, 47, 48] and hormones [49] that help to evoke a pro-inflammatory condition that triggers the pathogenesis of autoimmune diseases.

In summary, autoimmune diseases can be predicted by early detection of the antibodies, their titer, and the different number of the autoantibodies present. The high sensitivity of the BioPlex ANA Screen can perform this task, thus predicting the emergence of autoimmune diseases and, consequently, reducing the severity impact of the diseases.

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References

1. Scholz J, Grossmann K, Knutter I, Hiemann R, Sowa M, Rober N, et al. Second generation analysis of antinuclear antibody (ANA) by combination of screening and confirmatory testing. Clin Chem Lab Med 2015;53:1991–2002.
2. Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Marien G, Blockmans D, et al. Antinuclear antibody detection by automated multiplex immunoassay in untreated patients at the time of diagnosis. Autoimmun Rev 2012;12:137–43.
3. Zebryk P, Puszczewicz M. Autoantibodies in systemic sclerosis. Postepy Hig Med Dosw 2015;69:654–60.
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. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014;73:17–23.
6. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010;69:1420–2.
7. Satoh M, Chan EK, Sobel ES, Kimpel DL, Yamasaki Y, Narain S, et al. Clinical implication of autoantibodies in patients with systemic rheumatic diseases. Expert Rev Clin Immunol 2007;3:721–38.
8. Tan EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. Adv Immunol 1982;33:167–240.
9. Hiemann R, Buttner T, Krieger T, Roggenbuck D, Sack U, Conrad K. Challenges of automated screening and differentiation of non-organ specific autoantibodies on HEp-2 cells. Autoimmun Rev 2009;9:17–22.
10. Narciso-Schiavon JL, Freire FC, Suarez MM, Ferrari MV, Scanhola GQ, Schiavon Lde L, et al. Antinuclear antibody positivity in patients with chronic hepatitis C: clinically relevant or an epiphenomenon? Eur J Gastroenterol Hepatol 2009;21:440–6.
11. Satoh M, Chan EK, Ho LA, Rose KM, Parks CG, Cohn RD, et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012;64:2319–27.
12. Gilburd B, Abu-Shakra M, Shoenfeld Y, Giordano A, Bocci EB, delle Monache F, et al. Autoantibodies profile in the sera of patients with Sjogren's syndrome: the ANA evaluation--a homogeneous, multiplexed system. Clin Dev Immunol 2004;11:53–6.
13. Shovman O, Gilburd B, Barzilai O, Shinar E, Larida B, Zandman-Goddard G, et al. Evaluation of the BioPlex 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. Ann N Y Acad Sci 2005;1050:380–8.

14. Desplat-Jego S, Bardin N, Larida B, Sanmarco M. Evaluation of the BioPlex 2200 ANA screen for the detection of antinuclear antibodies and comparison with conventional methods. Ann N Y Acad Sci 2007;1109:245–55.
15. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33.
16. Agmon-Levin N, Shoenfeld Y. Prediction and prevention of autoimmune skin disorders. Arch Dermatol Res 2009;301:57–64.
17. Israeli E, Grotto I, Gilburd B, Balicer RD, Goldin E, Wiik A, et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut 2005;54:1232–6.
18. Shoenfeld Y, Carp HJ, Molina V, Blank M, Cervera R, Balasch J, et al. Autoantibodies and prediction of reproductive failure. Am J Reprod Immunol 2006;56:337–44.
19. Meroni PL, Shoenfeld Y. Predictive, protective, orphan autoantibodies: the example of anti-phospholipid antibodies. Autoimmun Rev 2008;7:585–7.
20. Shepshelovich D, Shoenfeld Y. Prediction and prevention of autoimmune diseases: additional aspects of the mosaic of autoimmunity. Lupus 2006;15:183–90.
21. Harel M, Shoenfeld Y. Predicting and preventing autoimmunity, myth or reality? Ann N Y Acad Sci 2006;1069:322–45.
22. Buliard A, Fortenfant F, Ghillani-Dalbin P, Musset L, Oksman F, Olsson NO. Analysis of nine autoantibodies associated with systemic autoimmune diseases using the Luminex technology. Results of a multicenter study. Ann Biol Clin (Paris) 2005;63:51–8.
23. Bose N, Wang X, Gupta M, Yao Q. The clinical utility of anti-chromatin antibodies as measured by BioPlex 2200 in the diagnosis of systemic lupus erythematosus versus other rheumatic diseases. Int J Clin Exp Med 2012;5:316–20.
24. Hanly JG, Thompson K, McCurdy G, Fougere L, Theriault C, Wilton K. Measurement of autoantibodies using multiplex methodology in patients with systemic lupus erythematosus. J Immunol Methods 2010;352:147–52.
25. Martins TB, Burlingame R, von Muhlen CA, Jaskowski TD, Litwin CM, Hill HR. Evaluation of multiplexed fluorescent microsphere immunoassay for detection of autoantibodies to nuclear antigens. Clin Diagn Lab Immunol 2004;11:1054–9.
26. Shanmugam VK, Swistowski DR, Saddic N, Wang H, Steen VD. Comparison of indirect immunofluorescence and multiplex antinuclear antibody screening in systemic sclerosis. Clin Rheumatol 2011;30:1363–8.
27. Hanly JG, Su L, Farewell V, Fritzler MJ. Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus. J Immunol Methods 2010;358:75–80.
28. Bizzaro N, Tozzoli R, Shoenfeld Y. Are we at a stage to predict autoimmune rheumatic diseases? Arthritis Rheum 2007;56:1736–44.
29. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741–9.
30. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380–6.
31. Jonsson R, Theander E, Sjostrom B, Brokstad K, Henriksson G. Autoantibodies present before symptom onset in primary Sjogren syndrome. JAMA 2013;310:1854–5.
32. Koenig M, Dieude M, Senecal JL. Predictive value of antinuclear autoantibodies: the lessons of the systemic sclerosis autoantibodies. Autoimmun Rev 2008;7:588–93.
33. Abu-Shakra M, Krup M, Slor H, Shoenfeld Y. Anti-Sm-RNP activity in sera of patients with rheumatic and autoimmune diseases. Clin Rheumatol 1990;9:346–55.
34. Shoenfeld Y, Blank M, Abu-Shakra M, Amital H, Barzilai O, Berkun Y, et al. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune diseases – 2008. Isr Med Assoc J 2008;10:13–9.
35. Shoenfeld Y, Gilburd B, Abu-Shakra M, Amital H, Barzilai O, Berkun Y, et al. The mosaic of autoimmunity: genetic factors involved in autoimmune diseases--2008. Isr Med Assoc J 2008;10:3–7.
36. Rahamim-Cohen D, Shoenfeld Y. The mosaic of autoimmunity. A classical case of inhalation of a polyclonal activating factor in a genetically and hormonally susceptible patient leading to multiple autoimmune diseases. Isr Med Assoc J 2001;3:381–2.
37. Arango MT, Perricone C, Kivity S, Cipriano E, Ceccarelli F, Valesini G, et al. HLA-DRB1 the notorious gene in the mosaic of autoimmunity. Immunol Res 2017;65:82–98.
38. Arango MT, Kivity S, Chapman J, Shoenfeld Y. Narcolepsy – genes, infections and vaccines: the clues for a new autoimmune disease. Isr Med Assoc J 2014;16:636–7.
39. Ortona E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. Sex-based differences in autoimmune diseases. Ann Ist Super Sanita 2016;52:205–12.
40. Shoenfeld Y, Tincani A, Gershwin ME. Sex gender and autoimmunity. J Autoimmun 2012;38:J71–3.
41. Nussinovitch U, Shoenfeld Y. The role of gender and organ specific autoimmunity. Autoimmun Rev 2012;11:A377–85.
42. Borella E, Nesher G, Israeli E, Shoenfeld Y. Vitamin D: a new anti-infective agent? Ann N Y Acad Sci 2014;1317:76–83.
43. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. Autoimmun Rev 2013;12:976–89.
44. Rosen Y, Daich J, Soliman I, Brathwaite E, Shoenfeld Y. Vitamin D and autoimmunity. Scand J Rheumatol 2016;45:439–47.
45. Shoenfeld Y, Aharon-Maor A, Sherer Y. Vaccination as an additional player in the mosaic of autoimmunity. Clin Exp Rheumatol 2000;18:181–4.
46. Kivity S, Arango MT, Molano-Gonzalez N, Blank M, Shoenfeld Y. Phospholipid supplementation can attenuate vaccine-induced depressive-like behavior in mice. Immunol Res 2017;65:99–105.
47. Bogdanos DP, Smyk DS, Rigopoulou EI, Sakkas LI, Shoenfeld Y. Infectomics and autoinfectomics: a tool to study infectious-induced autoimmunity. Lupus 2015;24:364–73.
48. Kivity S, Arango MT, Ehrenfeld M, Tehori O, Shoenfeld Y, Anaya JM, et al. Infection and autoimmunity in Sjogren's syndrome: a clinical study and comprehensive review. J Autoimmun 2014;51:17–22.
49. Shoenfeld Y, Zandman-Goddard G, Stojanovich L, Cutolo M, Amital H, Levy Y, et al. The mosaic of autoimmunity: hormonal and environmental factors involved in autoimmune diseases--2008. Isr Med Assoc J 2008;10:8–12.