Evaluation of the BioPlex™ 2200 ANA Screen

Analysis of 510 Healthy Subjects: Incidence of Natural/Predictive Autoantibodies

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ABSTRACT: The BioPlex™ 2200 ANA Screen is a fully automated system that determines levels for 13 different autoimmune antibodies of established clinical significance. The objective of this study was to determine the specificity of the BioPlex™ 2200 ANA Screen assay and to analyze the antibody profile samples collected from healthy subjects against comparative ELISA and IIF screening methods. A total of 510 specimens were randomly selected from a cohort of apparently healthy blood bank donors. Samples were distributed to five age brackets. All samples were tested using Bio-Rad's ANA Screen kit. Specificity was compared to IIF and ELISA results. Most of the samples were found negative in all ANA screening systems (84.5% by IIF, 92.5% by BioPlex™ 2200 ANA Screen kit, and 94.5% by ELISA). The frequency of positive results was highest (15.5%) using IIF, in comparison to almost similar results (5.5% vs. 7.5%) achieved by ANA ELISA and BioPlex™ 2200 ANA Screen kits. The positive rate of autoantibodies was significantly reduced when analyzed by different combinations of ANA screen assays (from 2.35% using IIF + BioPlex ANA Screen tests to 0.98% by using all three tests). Using the BioPlex™ 2200 ANA Screen system, we were able to identify samples with high levels of individual antibodies: anti-dsDNA at 20–63 IU/mL, antichromatin at 4–8 AI, anti-SmRNP at 2–6 AI, and anti-RNPA at 2–4.5 AI. Importantly, from 7 IIF and ELISA positive sera, 5 of these were also BioPlex 2200 positive, suggesting that the BioPlex is seeing the samples that are of the greatest interest, using the established techniques. The specificity of the BioPlex 2200 ANA Screen analysis of 13 different analytes (dsDNA, centromere B, chromatin, Jo1, ribosomal P, RNP 68, RNP A, Scl-70, Sm, SmPNP, SS-A52, SS-A60, SS-B) is comparable ($P < 0.252$) to the ELISA ANA screening test. Like the ELISA, the BioPlex 2200 has a lower ($P < 0.001$) positive rate than IIF for the autoantibody screening.

KEYWORDS: ANA; autoantibodies; autoimmunity; predictive value; healthy; multiplexed assay

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INTRODUCTION

Measurement of autoantibodies provides supporting evidence in the diagnosis and monitoring of systemic rheumatic diseases. The presence of autoantibodies in the sera of healthy subjects in low–medium titers is reported frequently.[1] The role and predictive value of this incidental finding of autoantibodies is uncertain.

In the last several years, a different constellation has been raised, namely, that the presence of autoantibodies in healthy populations might be a marker of future autoimmune disease.[2,3] Initially, this notion has been supported by data showing increased concentration of anti-DNA antibodies before the clinical onset of SLE[4] and autoantibodies against IgM rheumatoid factor[5] or anti–cyclic citrullinated peptide (anti-CCP)[5,6] prior to the clinical onset of rheumatoid arthritis. Pregnancy-triggered autoimmune conditions such as postpartum rheumatoid arthritis[7] and thyroid autoimmune disease[8] have been predicted by the existence of respective autoantibodies: rheumatoid factor and antithyroid-peroxidase antibodies (anti-TPO) detected in previously healthy pregnant women. Therefore, an identification of specific antibodies might allow the proper selection of patients with preclinical natural history, risk stratification of potential autoimmune diseases, follow-up of specific populations, and possibly preventive trials in the future. Hence, high-throughput technology in which hundreds of autoantibodies can be analyzed simultaneously is necessary.

The multiplexed assay plays an important role in laboratory work. Considerable data confirm the advantage of the multiplexed technology and its applications in diverse fields of medicine, including cancer research, cytokines, gene expression, and genetic and infectious diseases.[9–14] An application of multiplexed technology in the field of autoimmunity suggests that this assay may be suitable as a sensitive screening method for detection of numerous autoantibodies in patients with autoimmune diseases.[15–18] It has been demonstrated that multiplexed technology offers a useful tool for the detection of ANA and extractable nuclear antigens in autoimmune diseases.[15,16] Furthermore, an assessment of the multiplexed system in patients with Sjögren's syndrome[17] and SLE[18] confirmed its specificity, sensitivity, and reproducibility for measuring autoantibodies.

The BioPlex 2200 multiplexed system was developed for high-throughput analysis of 13 autoimmune analytes simultaneously in a single tube. The current evaluation of the BioPlex 2200 system for assessment of Epstein-Barr immunologic status[19] or IgM *Toxoplasma gondii* antibodies[20] proposed its potential for the diagnosis and surveillance of infectious diseases.

The objective of this study was to determine the specificity of the BioPlex 2200 ANA Screen assay in the analysis of an antibody profile in 510 samples collected from healthy subjects against comparative enzyme immunoassay (ELISA) and indirect immunofluorescence (IIF) screening methods.

METHODS

A total of 510 specimens were randomly selected from a cohort of apparently healthy blood bank donors. Samples were distributed to five age brackets, as shown in TABLE 1. All samples were tested using the BioPlex™ 2200 system (BioPlex) (Bio-Rad Laboratories, Hercules, CA). Specificity was compared to IIF (Kallestad

TABLE 1. Characterization of healthy subjects

Age (years)	Male (no.)	Female (no.)	Total
20–30	24	101	125
30–40	25	98	123
40–50	25	95	120
50–60	25	99	124
60–70	5	13	18
Total	104	406	510

TABLE 2. Frequency of autoantibodies in healthy subjects evaluated by different ANA screening methods

Method	Positive rate
IIF	15.5% (79)
ELISA	5.5% (28)
BioPlex ANA Screen	7.5% (38)

HEp-2 cell line substrate, Bio-Rad Laboratories, Redmond, WA) and ELISA (Autoimmune EIA ANA Screening Test, Bio-Rad Laboratories, Hercules, CA) results determined at the Chaim Sheba Medical Center.

The BioPlex™ 2200 system employs multiplexed bead technology to simultaneously perform measurements of 13 autoantibodies in a single tube as previously described.[21] The overall specificity of the BioPlex 2200 ANA Screen kit was calculated by considering each of the 13 analytes as a separate test performed on each sample (e.g., 510 samples × 13 autoantibodies = 6630 results). Fisher's exact test was used for comparison of frequency of positive autoantibody rate.

RESULTS

Five hundred ten samples from healthy subjects were evaluated for presence of autoantibodies by three different assays (IIF, ELISA, and BioPlex 2200 ANA Screen kit). The majority of the samples were found negative in all ANA screening systems (84.5% by IIF, 92.5% by BioPlex 2200 ANA Screen kit, and 94.5% by ELISA). The frequency of positive results (TABLE 2) was highest (15.5%) using IIF, in comparison to almost similar results (5.5% vs. 7.5%) obtained by using ANA ELISA and BioPlex 2200 ANA Screen kits. It is remarkable that the positivity rate of the BioPlex is similar to the ELISA ANA kit, given that it is based on 13 times as many determinations. The positive rate of autoantibodies (TABLE 3) was significantly reduced after analysis with different combinations of ANA screening assays (from 2.35% using IIF + BioPlex ANA Screen tests to 0.98% by using all three tests). Importantly, from 7 IIF and ELISA positive sera, 5 of these were also BioPlex positive, suggesting that the BioPlex is seeing the samples that are of the greatest interest, using the established techniques. Low positive rates of all 13 autoantibodies were found in the

TABLE 3. Frequency of elevated autoantibody titers determined by different combinations of ANA screening methods

Methodology	Positive rate
IIF + BioPlex ANA Screen	2.35% (12)
ELISA + BioPlex ANA Screen	1.96% (10)
IFA + ELISA	1.37% (7)
IIF + ELISA + BioPlex ANA Screen	0.98% (5)

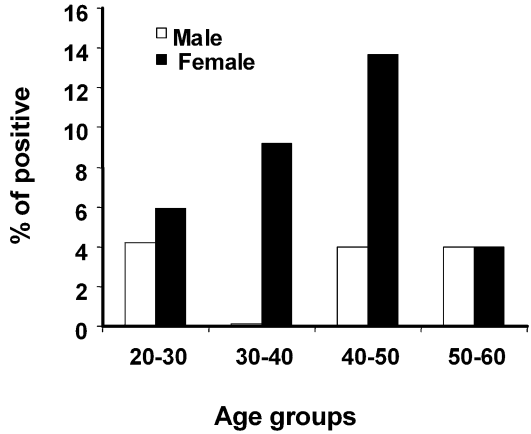

FIGURE 1. The prevalence of positive rate of autoantibodies in different age groups as evaluated by the BioPlex 2200 ANA Screen kit.

BioPlex 2200 ANA Screen kit. For 9 of 13 analytes, the positivity rate was <1.0%. These 9 include all the analytes that are associated with scleroderma and myositis (centromere, Scl-70, Jo-1) as well as 2 markers that are most specific for SLE (ribosomal P and Sm). This confirms that a positive finding by BioPlex for these analytes is likely to have a very high positive likelihood ratio (TABLE 4). The 40 positive results (0.6%) obtained with the BioPlex are out of a total of 6630 (510 × 13) determinations, indicating that the specificity by analyte is >99.4%. The significantly higher positive rate (FIG. 1) of autoantibodies was detected in females by the BioPlex ANA Screen test (30–40 and 40–50 year-groups). Using the BioPlex 2200 ANA Screen kit, we were able to identify samples with high levels of individual antibodies: anti-DNA, 20–63 IU/mL (FIG. 2A); antichromatin, 4–8 AI (FIG. 2B); anti-SmRNP, 2–6 AI (FIG. 2C); and anti-RNP A, 2–4.5 AI (FIG. 2D).

DISCUSSION

The capability of multiplexed technology to analyze several parameters simultaneously might permit large-scale prospective studies of healthy individuals.

TABLE 4. Frequency of the elevated titers of individual antibodies in healthy subjects

BioPlex ANA Screen individual analyte	Positive rate
dsDNA	1.0% (5)
Centromere B	0.2% (1)
Chromatin	1.4% (7)
Jo1	0.0% (0)
Ribosomal P	0.2% (1)
RNP 68	0.2% (1)
RNP A	1.8% (9)
Scl-70	0.2% (1)
Sm	0.2% (1)
SmRNP	1.2% (6)
SS-A52	0.2% (1)
SS-A60	0.6% (3)
SS-B	0.8% (4)
Overall (6630)	0.6% (40)

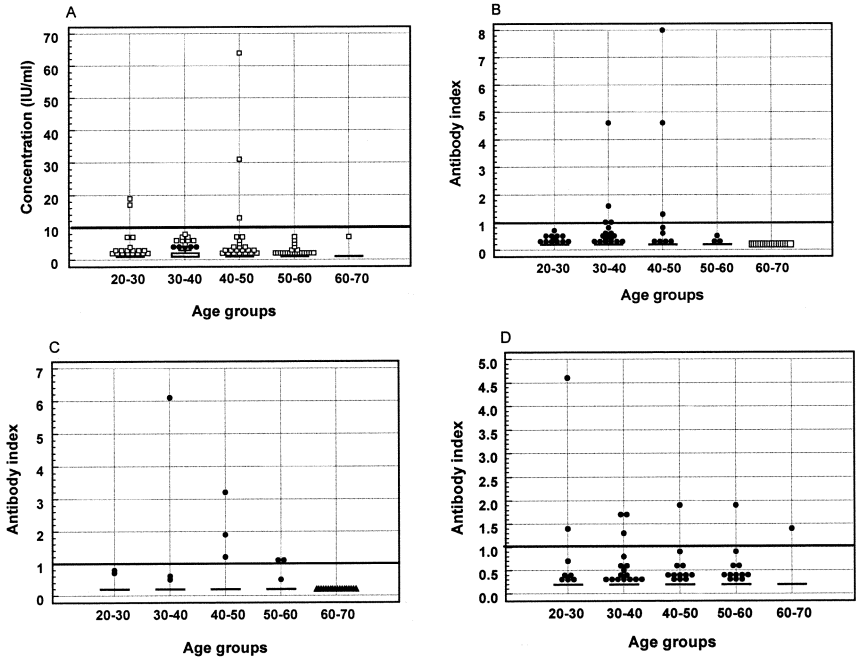

FIGURE 2. Elevated level of individual antibodies determined by the BioPlex 2200 Screen kit: **(A)** anti-dsDNA; **(B)** antichromatin; **(C)** anti-SmRNP; and **(D)** anti-RNP A.

In the present study, we evaluated a new method using a panel of multiplexed flow cytometer–based immunoassays combined with computer-assisted pattern recognition. A number of important lessons are clear from these observations: BioPlex 2200 ANA Screen analysis of 510 sera from well-distributed healthy blood donors for 13 different analytes (dsDNA, centromere B, chromatin, Jo1, ribosomal P, RNP 68, RNP A, Scl-70, Sm, SmPNP, SS-A52, SS-A60, SS-B autoantibodies) yielded low "false-positive" results of less than 2% (e.g., anti-dsDNA, 1%; antichromatin, 1.6%; anti-RNP A, 1.8%; others, <0.2%). These observations were comparable to autoantibody screening obtained by ELISA and IIF. We found that the positive rate of autoantibodies was significantly reduced after analysis with different combinations of ANA screening assays (from 2.35% using IIF + BioPlex ANA Screen tests to 0.98% by using all three tests).

The prevalence of the positive rate of autoantibodies evaluated by the BioPlex 2200 ANA Screen kit was in correlation with the reported prevalence of different autoantibodies in healthy subjects.[1]

Therefore, our results allowed us to conclude that the BioPlex ANA Screen test is suitable as a sensitive screening test to confirm or to exclude the presence of large numbers of autoantibodies simultaneously. Based on these observations, several questions should be raised: What is the significance of positive results in the screening of a healthy patient and could these findings be of a predictive value? Do the 5, 10, or 12 "false-positive" results obtained by 2 or 3 methods or the high levels of antibodies (anti-DNA, chromatin, SmRNP, or RNP A) detected in sera of healthy patients predict development of an overt disease (e.g., SLE or other autoimmune diseases), or is the existence of these antibodies epiphenomenal and thus lacking in diagnostic importance? Will the findings of sequential antibody formation in asymptomatic persons improve future clinical outcomes and, if so, how?

Recently, the presence of specific serum antibodies was shown to precede the clinical onset of a variety of autoimmune diseases by many years. Hence, the existence of autoantibodies may be considered to be a marker for future development of these diseases in presently healthy individuals. The concept of a crescendo autoimmunity culminating in clinical illness was proposed by Arbuckle *et al.*,[4] who reported about progressive accumulation of specific autoantibodies, especially anti-DNA, prior to the onset of SLE. Nielen *et al.* found antibodies against IgM rheumatoid factor and anti-CCP in serum samples of patients with rheumatoid arthritis taken at a median of 4–5 years before disease onset.[5] The negative predictive value of these tests was 75% and the positive predictive value was 100%. Several pregnancy-related conditions have been studied, such as postpartum rheumatoid arthritis,[7] thyroid autoimmune disease,[8] and type 1 diabetes after gestational diabetes.[22] It has been demonstrated that future autoimmune disease can be predicted with respective autoantibody assay at delivery. In our recent study, we proved that anti–*Saccharomyces cerevisiae* (ASCA) and antineutrophil cytoplasmic antibodies (ANCA) may predict the development of inflammatory bowel disease (IBD) years before the disease is clinically diagnosed and may even forecast the clinical course.[23] Furthermore, it was suggested that an immunological screening strategy and treatment follow-up can aid in the prevention of disease development or delay disease progression. For instance, detection of the highly specific diagnostic anti-pyruvate-dehydrogenase (PDH)[24] autoantibodies may precede primary biliary cirrhosis. An early initiation of a relatively benign therapy of ursodeoxycholic acid may delay the development of the

fatal autoimmune disease.[25] Several large-scale trials are under way in which individuals with two or more diabetes-associated antibodies are receiving immunomodulating therapy, such as nasal insulin in the Type 1 Diabetes Prediction and Prevention Project in Finland.[26]

Thus, on the one hand, an identification of patients that are prone to develop autoimmune disease may be important for follow-up, early diagnosis, and promising prevention by utilization of immune-modulating therapy. On the other hand, most patients who were tested positive for different autoantibodies never developed a disease. A positive result might have several meanings: It might be a clinical false-positive result, the patient may harbor an autoimmune condition at the time of testing, or it might predict a future disease. Perhaps, testing and follow-up of specific populations such as pregnant women or those with organ-specific autoimmune disease cannot be extrapolated to the general population, which is at lower risk of disease.[2] Additionally, autoimmune diseases may be characterized by dissimilar pattern of autoantibodies—for instance, more than 100 different autoantibodies found in SLE patients.[27] In general, the question related to the clinical importance of the presence of antibodies in asymptomatic subjects may only be resolved by additional prospective studies, with the follow-up of autoantibody titers as well as clinical symptoms in evaluated individuals. In this way, an application of BioPlex 2200 ANA Screen multiplexed technology is a useful tool for high-throughput screening of healthy populations.

CONCLUSIONS

The specificity of BioPlex 2200 ANA Screen analysis of 13 different analytes (dsDNA, centromere B, chromatin, Jo1, ribosomal P, RNP 68, RNP A, Scl-70, Sm, SmPNP, SS-A52, SS-A60, SS-B) is comparable ($P < 0.252$) to the ELISA ANA screening test. Like the ELISA, the BioPlex 2200 has a lower ($P < 0.001$) positive rate than IIF for the autoantibody screening.

For 9 of 13 analytes, the positivity rate was <1.0%. These 9 include all the analytes that are associated with scleroderma and myositis (centromere, Scl-70, Jo-1) as well as 2 markers that are most specific for SLE (ribosomal P and Sm). This confirms that a positive finding by BioPlex for these analytes is likely to have a very high positive likelihood ratio.

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O. Shovman and B. Gilburd contributed equally to this work.

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