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Review

Autoantibody profiling of patients with antiphospholipid syndrome using an automated multiplexed immunoassay system

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ABSTRACT

The antiphospholipid syndrome (APS) is an autoimmune disease defined by the co-occurrence of clinical and serological symptoms [presence of at least one of the antiphospholipid autoantibodies (aPL), such as anti-cardiolipin (aCL) IgG/IgM and anti-β2glycoprotein I (aβ2GPI) IgG/IgM]. The measurement of these autoantibodies constitutes the first-line approach for the diagnosis of APS. Recently the advent of multiplex proteomic technologies seems to be an optimal solution for the parallel detection of autoantibodies (IgG, IgA, IgM) related to APS. The BioPlex 2200 is an automated commercial platform based on the multi-analyte profiling technology that allows the detection of different types of autoantibodies, particularly ANA, ENA, dsDNA, PR3, MPO, GBM. We performed firstly a study to evaluate the diagnostic accuracy of this analytical system in a group of APS patients. The BioPlex system showed a good diagnostic accuracy for all test evaluated, very similar to that of the other established commercial singleplex immunoassays. In our study, the simultaneous detection of aCL and aβ2GPI of IgA isotype in addition to IgG and IgM isotypes did not increase the diagnostic sensitivity for APS. The good diagnostic accuracy, the high level of automation, and the high throughput make this multiplex platform a very useful and practical tool for the laboratory diagnosis of aPL in daily practice.

Contents

1. Introduction

The antiphospholipid syndrome (APS) is an autoimmune disease defined by the co-occurrence of clinical (vascular thrombosis and/or pregnancy morbidity) and serological symptoms (persistent presence of at least one of the following antiphospholipid antibodies (aPL), such as lupus anticoagulant (LA), anti-cardiolipin (aCL) and anti-β2glycoprotein I (aβ2GPI)], that are defined as Sydney criteria [1]. The APS can occur in isolation (primary APS) or in association with other autoimmune syndromes, especially with systemic lupus erythematosus (secondary APS).

Appropriate laboratory testing is required to detect the isotypes of the two autoantibodies (aAbs): they fall into 2 categories: a) immunological assays that detect aPL as protein molecules, and b) clotting assays, that detect aPL indirectly by measuring their effect on the coagulation system [2].

All laboratory tests considered for the diagnosis of APS have some limitations related to robustness, variability, standardization, and clinical relevance [2,3]. However, although the diagnostic value of single aPL is currently under debate, mainly for pathophysiological and analytical

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conditions [4–7], the measurement of aCL and aβ2GPI IgG and IgM antibodies constitutes the first-line approach for the diagnosis of APS. The laboratory diagnosis of APS requires the parallel measurement of these four aAbs with singleplex immunoassays, mainly of the solid-phase ELISA or CLIA type, recently implemented in automated platforms [8,9]. This technical solution can improve the reproducibility of the results and reduce interlaboratory variability, but requires running several immunoassays simultaneously, which generates time expenditure and costs [10].

Clearly a different analytical approach is needed. Given the low standardization and harmonization of aPL testing, as stressed recently [10,11], the advent of multiplex proteomic technologies (that allow the autoantibody profiling of patients' sera) seems to be an optimal solution for the simultaneous detection of different aAbs related to APS: some of these technologies (microbead-based multiplex immunoassays, multiline immunodot assays, lab-on-a-chips, biosensors) may contribute to overcome these drawbacks [10,12].

Moreover, there are other significant benefits in proteomic technologies [13–16]: indeed the 'multiple markers strategy' may be useful for disease screening and assessing pathophysiological pathways that contribute to disease activity and prognosis [15,16].

Between the different proteomic solutions, the technology based on beads or microspheres as the solid phase, in combination with flow cytometry, has been widely used for the detection of aAbs in different autoimmune diseases, such as autoimmune rheumatic diseases, autoimmune thyroid diseases and vasculitides [17–19]. In the last 10 years advances in immunoassay technology resulted in the development of multiplex platforms at several level of automation, used as a first or second line screening assay for aAbs.

2. The BioPLex™ 2200 as the state of the art of automated technology for multiplex detection of aAbs

The BioPlex 2200, developed by BioRad Laboratories (Hercules, USA), is a unique fully-automated multiplex immunoassay platform that allows the qualitative and quantitative measurements of several aAbs and antibodies. The instrument combines the multi-analyte profiling technology with antigen-coated fluoromagnetic beads as solid phase, in an automated platform where sampling, processing and data reduction are performed automatically. Unlike other methods, the beads are washed after both the incubation and the labeling steps; magnetic beads are used to automate the washing steps and support random access testing [20]. Samples are run on random access design rather than in batches, which permits the analysis of single samples, at any time, everyday: the consequence is that it is now possible to generate a full profile of the significant aAbs for each specific autoimmune disease.

In the last 8 years several publications highlighted the diagnostic accuracy of this automated platform for the detection of different types of aAbs, particularly ANA, ENA, dsDNA, PR3, MPO, GBM aAbs [21–32]. In Table 1 we summarized the most relevant studies: the cumulative results of these studies demonstrated the benefits of simultaneously measuring ANA/ENA/ANCA in systemic rheumatic diseases and suggested high agreement between the detection of aAbs by multiplex and conventional singleplex (ELISA, CLIA) methods.

3. Parallel detection of APL aAbs in APS patients using the BioPLex™ 2200 system

Recently the parallel detection of aCL and aβ2GPI antibodies of IgG, IgA and IgM isotypes was developed in the BioPlex system: we performed firstly a study to evaluate the diagnostic accuracy of these tests in a group of APS patients and to compare the results obtained by other widely used singleplex immunoassays.

Table 1
The most relevant published studies of autoantibody detection with BioPLEx 2200 system.

AAbs	Year	Author	Journal	Ref
ANA/ENA	2005	Shovman O, et al.	Ann N Y Acad Sci	[21]
ANA/ENA	2007	Moder KG, et al.	J Rheumatol	[22]
ANA/ENA	2007	Desplat-Jego S, et al.	Ann N Y Acad Sci	[23]
ANCA/GBM	2009	Kaul R, et al.	Autoimmun Rev	[24]
dsDNA/ANuA	2009	Bardin N, et al.	Autoimmunity	[25]
ANA/ENA	2010	Hanly JG, et al.	J Immunol Methods	[26]
ANA/ENA	2011	Shanmugam VK, et al.	Clin Rheumatol	[27]
ANA/ENA	2012	Kim Y, et al.	Clin Chim Acta	[28]
ANA/ENA	2012	Op De Beéck K, et al.	Autoimmun Rev	[29]
ANuA	2012	Bose N, et al.	Int J Clin Exp Med	[30]
ANA/ENA	2012	Bruner BF, et al.	Arthritis Rheum	[31]
dsDNA	2013	Venner AA, et al.	Clin Biochem	[32]

3.1. Material and methods

Sera from 50 patients with APS (38 females and 12 males; mean age: 45.5; range: 22–69 years) diagnosed according to the Sydney criteria (36 pAPS and 14 sAPS), and from 81 patients with infectious diseases as controls (20 syphilis, 32 Lyme disease and 29 HCV infection and cryoglobulinemia) were tested for aCL and anti-β2GPI of IgG, IgM and IgA isotype using the BioPLex™ 2200 multiplex immunoassay system and APLS reagents.

Briefly, paramagnetic particles died with two fluorophores, which emit at distinct wavelength and adsorb significantly at 635 nm; a third fluorophore, B-phycoerytrin functions as a reporter. The detector of the system simultaneously measures the light at 4 wavelengths: the two classification dyes, the reporter dye and the scatter of incident light. When the assays are performed by the instrument, magnetic beads coated with antigens are mixed with 5 μl of sample and sample diluents. The mixture is allowed to incubate at 37 °C for 20 min. After wash cycles, anti-human IgG (IgA, IgM) conjugated to phycoerytrin is added and incubated at 37 °C for 10 min. Following removal of excess conjugate, the magnetic beads are passed through the detector: the analyte concentration is proportional to the fluorescence intensity. The antigens coated in the magnetic beads are: a. synthetic (tetraoleyl–succynil) cardiolipin, and b) β2GPI (purified from human plasma by western blot and SDS-PAGE). Reference values proposed by the manufacturer were 20 GPL/MPL for aCL IgG and IgM, respectively and 20 AU/mL for anti-β2GPI of IgG, IgM, corresponding to the 99th percentile levels determined in a series of healthy subjects.

All sera were tested also for aCL and aβ2GPI antibodies (IgG and IgM) with other three singleplex commercial methods: a) the EliA automated immunoassay (Phadia, Freiburg, Germany), b) the Orgentec assay (Orgentec Diagnostics, Mainz, Germany), and c) the Quanta Lite (IL, San Diego, USA).

3.2. Statistical analysis

Diagnostic sensitivity and specificity of aPL were calculated using the manufacturer cut-off. For aCL sensitivity and specificity were also calculated using the alternative cut-off proposed by the Sydney consensus (40 GPL or GPM units). To compare the diagnostic efficiency of BioPLex™ 2200 method with EliA, Orgentec and Quanta Lite methods, comparison of areas under independent ROC curves was performed ($p < 0.05$ was considered statistically significant).

3.3. Results

The interassay precision of the system ranged from 2.8% to 6.5%, from 4.3% to 9.3% and from 5.4% to 8.2% for aCL IgG, IgA, and IgM, and from 3.1% to 6.0%, from 4.0% to 8.8%, and from 3.7% to 8.0%, for aβ2GPI, IgA, IgA, and IgM, respectively.