Journal of Immunological Methods 446 (2017) 54–59

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Validation of a multi-analyte panel with cell-bound complement activation products for systemic lupus erythematosus

Thierry Dervieux [a,*], John Conklin [a], Jo-Anne Ligayon [a], Leilani Wolover [a], Tyler O'Malley [a], Roberta Vezza Alexander [a], Arthur Weinstein [b], Claudia A. Ibarra [a]

[a] Exagen Diagnostics, Vista, CA, United States
[b] Georgetown University, Washington, DC, United States

ARTICLE INFO

Article history:
Received 7 March 2017
Received in revised form 31 March 2017
Accepted 3 April 2017
Available online 5 April 2017

Keywords:
Biomarkers
Complement
Diagnostic immunology

ABSTRACT

Background: We describe the analytical validation of an assay panel intended to assist clinicians with the diagnosis of systemic lupus erythematosus (SLE). The multi-analyte panel includes quantitative assessment of complement activation and measurement of autoantibodies.
Methods: The levels of the complement split product C4d bound to erythrocytes (EC4d) and B-lymphocytes (BC4d) (expressed as mean fluorescence intensity [MFI]) are measured by quantitative flow cytometry, while autoantibodies (inclusive of antinuclear and anti-double stranded DNA antibodies) are determined by immunoassays. Results of the multi-analyte panel are reported as positive or negative based on a 2-tiered index score. Post-phlebotomy stability of EC4d and BC4d in EDTA-anticoagulated blood is determined using specimens collected from patients with SLE and normal donors. Three-level C4 coated positive beads are run daily as controls. Analytical validity is reported using intra-day and inter-day coefficient of variation (CV).
Results: EC4d and BC4d are stable for 2 days at ambient temperature and for 4 days at 4 °C post-phlebotomy. Median intra-day and inter-day CV range from 2.9% to 7.8% (n = 30) and 7.3% to 12.4% (n = 66), respectively. The 2-tiered index score is reproducible over 4 consecutive days upon storage of blood at 4 °C. A total of 2,888 three-level quality control data were collected from 6 flow cytometers with an overall failure rate below 3%. Median EC4d level is 6 net MFI (Interquartile [IQ] range 4-9 net MFI) and median BC4d is 18 net MFI (IQ range 13-27 net MFI) among 86,852 specimens submitted for testing. The incidence of 2-tiered positive test results is 13.4%.
Conclusion: We have established the analytical validity of a multi-analyte assay panel for SLE.
© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Diagnostic immunology laboratory tests provide important information to assist clinicians with the differential diagnosis of systemic lupus erythematosus (SLE), a prototypical systemic autoimmune rheumatic disease characterized by the presence of pathogenic autoantibodies, inflammation, and damage to multiple organs (Rahman and Isenberg, 2008). Because SLE has an order of magnitude lower prevalence than other rheumatic diseases such as rheumatoid arthritis (RA) or fibromyalgia (FM) (Somers et al., 2014; Helmick et al., 2008; Vincent et al., 2013), even experienced clinicians can have difficulty in distinguishing SLE from other conditions. As a result, they often rely not only on their clinical findings, but also on panels of autoantibodies to rule-in or rule-out SLE. Commonly measured lupus autoantibodies include antinuclear antibodies (ANA), a highly sensitive (~90%) but poorly specific marker for SLE, and anti-double stranded DNA (anti-dsDNA), whose presence helps confirm disease with high specificity (>95%) (Agmon-Levin et al., 2014). Other autoantibodies, such as anticitrullinated peptide autoantibodies (ACPA) and antibodies to extractable nuclear antigens (ENA), are also commonly used, and their utility in the differential diagnosis of other autoimmune rheumatic diseases is well established (Stinton and Fritzler, 2007; Mahler et al., 2014).

Immune complexes-mediated activation of the classical complement system is a hallmark of SLE (Sturfelt and Truedsson, 2012; Atkinson and Yu, 2010; Wener, 2010). More than a decade of basic and translational research has established that cell-bound complement activation products (CB-CAPs) and, in particular, C4d bound to erythrocytes (EC4d) and B-lymphocytes (BC4d), are valuable biomarkers of complement activation (Freysdottir and Sigfusson, 1991; Manzi et al., 2004; Liu et al., 2004; Liu et al., 2009; Yang et al., 2009; Kao et al., 2010). CB-CAPs have higher sensitivity for SLE diagnosis than serum complement levels while maintaining high specificity (Yang et al., 2009). Quantitative measurement of CB-CAPs was recently combined with autoantibody profiling to produce an integrated multi-analyte

* Corresponding author at: Exagen Diagnostics, Inc., 1261 Liberty Way, Vista, CA, United States.
E-mail address: tdervieux@exagen.com (T. Dervieux).

http://dx.doi.org/10.1016/j.jim.2017.04.001
0022-1759/© 2017 Elsevier B.V. All rights reserved.

diagnostic immunology assay panel (Kalunian et al., 2012; Putterman et al., 2014). This 10-analyte panel, which includes 8 autoantibodies (ANA, anti-dsDNA, ACPA, anti-Smith, anti-SSB, anti-Centromere [CENP], anti-Jo-1 and anti-Scl70) and the CB-CAPs EC4d and BC4d, is currently available from the centralized clinical laboratory. The performance characteristics of this panel have been established in a population of 794 subjects enrolled at various Lupus Center of Excellence in the USA (Putterman et al., 2014). This panel has 80% sensitivity and 86% specificity for SLE diagnosis, and has been validated prospectively in 2 consecutive cohorts of patients (Wallace et al., 2016; Askanase et al., 2015).

We describe the analytical validity of the multi-analyte assay panel, including the quality control (QC) systems in place to ensure that performance remains adequate over time. We also report data from a large database of specimens submitted for testing to the clinical laboratory.

2. Materials and methods

2.1. Donor specimens

The analytical performances (repeatability, reproducibility, and integrity of the pre-analytical phase) of EC4d and BC4d were evaluated using blood specimens collected from patients with SLE or normal healthy volunteers (n = 14 specimens from 11 NHV, median age 47 years, 55% females). Patients with SLE (n = 11, median age 41 years, 63% females, all fulfilling the American College of Rheumatology criteria of SLE) were treated with hydroxychloroquine and 5 of them also received immunosuppressants (azathioprine, mycophenolate or methotrexate). All patients were allowed to donate blood at consecutive times with a minimum of 1-week interval. Study protocols were approved by institutional review boards, and all donors provided informed consent.

Blood from NHV and from SLE patients was drawn in EDTA-containing tubes (10 ml) and processed within 2 h of collection. To evaluate EC4d and BC4d stability, blood was distributed into polypropylene tubes within 2 h of collection and stored at ambient room temperature (23 °C) for up to 2 days post phlebotomy. The stability of soluble C4d fragment was evaluated in the group of NHV only. On the day of collection, and for the following 2 days, plasma was isolated from whole blood stored at room temperature and soluble plasma C4d levels were determined by immunoassay (MicroVue C4d EIA, Quidel, San Diego, CA). The relationship between the level of the markers at baseline, day 1, and day 2 post phlebotomy was estimated using Deming regression.

Blood (5 ml) was also collected in serum separator tubes for determination of autoantibodies.

2.2. Quantitative assessment of complement activation by flow cytometry

The detailed protocol for the measurement of EC4d and BC4d levels by quantitative fluorescence activated cell sorting (FACS) is described in Supplementary materials. Briefly, erythrocytes and B-lymphocytes are isolated, washed, and labeled with a mouse monoclonal antibody against human C4d (specific antibody) or a non-specific antibody (isotype). A goat anti-mouse antibody conjugated to fluorescein isothiocyanate (FITC) is used as secondary antibody to detect C4d deposited on cells. The mean fluorescence intensity (MFI) of the isotype background control and C4d is collected from every specimen, and the net MFI is determined by subtracting the non-specific MFI from the total MFI.

To assess repeatability, EC4d and BC4d (and their post-analytical reduction in the CB-CAPs component in 2-tiered index score) were determined 10 consecutive times on the same day from a total of 30 specimens. Reproducibility of EC4d and BC4d levels was determined on 4 consecutive days (including day of receipt) upon storage of blood at 4 °C during the 4-day period. The population of specimens was separated into low, medium, and high EC4d or BC4d net MFI (based on their distribution as tertiles) to estimate the variability of the FACS method at different levels of C4d intensity.

2.3. Autoantibody profiling

ANA is determined using an enzyme linked immunosorbent assay (ELISA) with Hep-2 extracts as antigens (Quanta Lite, Inova Diagnostics, San Diego, CA), and manufacturer cutoffs are used in the multi-analyte assay panel (≥20 units, positive; ≥60 units, strong positive). All specimens received in the clinical laboratory are also tested for ANA by the gold standard indirect immunofluorescence (IIF) on the NOVAVIEW instrument (Nova-Lite, Inova Diagnostics), and positivity at 1:80 dilution is considered positive. ELISA and IIF have similar sensitivity for SLE (89% and 85%, respectively) (data not shown).

Anti-dsDNA titers are determined by ELISA (Quanta Lite, Inova Diagnostics). All positive serum samples (>301 IU/ml) are further evaluated using the *Crithidia luciliae* indirect immunofluorescence (IIF) assay (Nova-Lite, Inova Diagnostics) upon a 1:10 dilution.

Autoantibodies to ENA, (anti-Smith, anti-SSB, anti-CENP, anti-Jo-1, anti-Scl-70) and anti-cyclic citrullinated peptide antibodies (CCP) are determined using the EliA system on the Phadia 250 platform (Thermofisher, Uppsala, Sweden) with cutoff of 10 U/ml for all markers.

All the assays for autoantibody assessment are cleared by the US Food and Drug Administration as in vitro diagnostic assays, and analytes are stable in whole blood or serum for at least 72 h post phlebotomy.

2.4. Multi-analyte assay testing method

Levels of the 10 markers in the panel (EC4d, BC4d, ANA, anti-dsDNA, ACPA, anti-Smith, anti-SSB, anti-CENP, anti-Jo-1, and anti-Scl70) are utilized to calculate a multi-analyte testing method according to the standard operating procedures in place in the clinical laboratory and approved by the clinical evaluation program from the New York Department of Health (Fig. S1, Supplementary materials). Briefly, the method uses 2 consecutive tiers of analysis. In tier-1, specimens are tested for presence of highly specific markers of SLE: anti-dsDNA (>301 IU/ml confirmed using the *Crithidia luciliae* IIF assay), anti-Smith (>10 U/ml), and EC4d or BC4d above the 99th percentile of a group of non-SLE subjects (>75 net MFI and >200 net MFI, respectively) (Putterman et al., 2014). Specimens negative for all tier-1 analytes are subsequently evaluated in a second tier of analysis (tier-2) that consists of an index score combining an ANA component (using ANA with cutoffs of 20 and 60 units, as determined by ELISA), a CB-CAPs component (the sum of log normalized EC4d and BC4d net MFIs) and an antibody specificity component as negative discriminator (corresponding to positivity to either anti-CCP, anti-SSB, anti-CENP, anti-Scl-70, or anti-Jo-1). The mathematical equation used to derive the index score is presented in Fig. S1. For each specimen, an overall assessment is reported. Assessment is reported as positive (tier-1 positive or tier-2 index score positive), negative (tier-2 index score negative), indeterminate (if tier-2 index score ranges from −0.1 to 0.1), or equivocal if certain criteria are not met (see Supplementary materials).

2.5. QC and implementation in clinical laboratory practice

Various daily QC systems are in place to ensure that EC4d and BC4d MFIs meet specifications. QC includes daily calibration and alignment of flow cytometry instruments (Flow-Check and Flow-Set [Beckman Coulter, Indianapolis, IN], PMT voltages, compensation); a daily control to establish the linearity of the assay (Rainbow Calibration Particles, Spherotech, Lake Forest, IL); and a negative control (ImmunoTrol, Beckman Coulter) for EC4d (<10 net MFI) and BC4d (<20 net MFI). In addition, 3-level positive C4 coated beads (biotinylated human C4 protein [Quidel] conjugated to streptavidin beads [Bangs Laboratories, Fishers, IN]) are run daily as controls, and are processed with the clinical specimens. For each lot of beads prepared, the mean ± 2 standard

Download English Version:

https://daneshyari.com/en/article/5522004

Download Persian Version:

https://daneshyari.com/article/5522004

Daneshyari.com