Journal of Immunological Methods xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Review

Development and validation of a lateral flow assay (LFA) for the determination of IgG-antibodies to Pr3 (cANCA) and MPO (pANCA)

Offermann N.[a], Conrad K.[b], Fritzler M.J.[c], Fooke Achterrath M.[a,*]

[a] Dr. Fooke Laboratorien GmbH, Germany
[b] Technical University Dresden, Institute of Immunology, Dresden, Germany
[c] Faculty of Medicine, University of Calgary Calgary, Alberta, Canada

ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 18 November 2013
Accepted 19 November 2013
Available online xxxx

Keywords:
Lateral flow assay
Myeloperoxidase, MPO
Proteinase 3, Pr3
Autoantibodies
pANCA
cANCA

ABSTRACT

The timely diagnosis of vasculopathies, such as granulomatosis with polyangiitis, has important implications for the favorable clinical outcome of these diseases. In the clinical setting, autoantibodies to proteinase 3 (Pr3) and myeloperoxidase (MPO) have been shown to be valuable adjuncts to an early and accurate diagnosis. The sensitive and specific detection of anti-Pr3 and anti-MPO was shown using a point of care device that employed rapid Lateral Flow Technologies. The validation of the lateral flow assay (LFA) was performed with serum samples collected in two Reference Laboratories and showed excellent results that were comparable to widely accepted and used ELISA. The advantage of the LFA is the flexibility to be used as an economical, point of care diagnostic device, features that are especially important for an early and accurate diagnosis and the prompt initiation of appropriate treatment so as to avoid inevitable development of undue complications of these diseases such as disseminated organ involvement, e.g. renal failure.

Contents

* Corresponding author at: Dr. Fooke Laboratorien GmbH, Habichtweg 16, 41468 Neuss, Germany. Tel.: +49 2131 2984 135; fax: +49 2131 2984 184.

0022-1759/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2013.11.017

1. Introduction

Vasculitis is caused by inflammation of blood vessels and it manifests itself through a broad spectrum of clinical phenotypes. Anti-neutrophil cytoplasmic antibodies (ANCAs) are one of the most common serological features of small vessel vasculitis (SVV). ANCA associated vasculitis includes microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA) and granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis. (Wegener, 1987; Falk et al., 2011).

The sensitive and specific detection of proteinase 3 (Pr3, cANCA) and myeloperoxidase (MPO, pANCA) autoantibodies, occurring in about 80% of GPA cases, are time-proven serological biomarkers that can aid in the diagnosis of these conditions. Only timely and appropriate treatment can avoid the development of renal failure and/or other systemic complications of these diseases (Conrad et al., 2007a,b).

Our aim was to develop a sensitive and specific, semi-quantitative LFA together with a newly designed portable lateral flow assay reader as an approach to a point of care technology that would be an important adjunct to diagnosing and monitoring these diseases. Minimal laboratory equipment and an assay time of only 20 min result in an economic immunoassay. We compared the technical and diagnostic performance to conventional diagnostic assays such as indirect immunofluorescence (IIF) measuring cANCA and pANCA as well as the latest generation ELISA and a new chemiluminescence immunoassay (CLIA, BioFlash, INOVA Diagnostics, San Diego, CA, USA) (Lucassen et al., 2011; Schulte-Pelkum et al., 2012).

2. Material and methods

2.1. Serum samples

The assay was developed by utilizing 87 commercially available serum samples representing 40 healthy donors, 5 unrelated disease controls, 20 cANCA positive and 22 pANCA positive individuals. ELISA and IIF ANCA results were available for these samples as supplied by several commercial institutions. International reference sera for anti-Pr3 (Pr3-ANCA human reference serum #16, Product Package Insert Catalog #IS2721 Lot #07-0002) and anti-MPO antibodies (MPO–ANCA human reference serum #15, Product Package Insert Catalog #IS2720 Lot #07-0001) were obtained from the Center for Disease Control and Prevention (CDC, Atlanta, GA, USA). The quantitative content of anti-Pr3 and anti-MPO antibodies in the reference sera had been determined as 100 IU/ml by different laboratories.

For validation of the LFA, a different set of serum samples was collected in two different reference laboratories: Technical University Dresden, Institute of Immunology ($n = 40$, cANCA positive $n = 20$ and pANCA positive $n = 20$) and the University of Calgary, Calgary, Alberta, Canada ($n = 64$, healthy donors $n = 34$ and ANCA positive $n = 30$). This study was approved by respective institutional human ethics review boards or was part of quality assurance and quality control processes. All patient identities remained anonymous in keeping with the latest version of the Helsinki Declaration of Human Research Ethics.

The serum samples were tested by commercially available Pr3 and MPO ELISA tests and a new CLIA technology. IFA results were available for 40 sera collected in Dresden (pANCA 37/40; cANCA 39/40). Results for IFA are given in titers. The serum titer is defined as the highest serial dilution of a serum sample (expressed as the dilution factor) that still yields a positive result. Titers above 1/40 were considered as positive. All serum samples were tested for anti-Pr3 and anti-MPO antibodies in the new AI-LFA assays, measured with the newly developed lateral flow assay reader and compared to the results obtained with the other methods.

2.2. Assay system

The autoimmune lateral flow assay system (AI-LFA) is based on a universal test cassette which can be used for all available IgG-antibody AI-parameters (Fig. 1). Purified native Pr3 and MPO antigens, both isolated from human neutrophils were biotinylated and used as liquid sources.

The assay was performed as follows: 10 µl of undiluted serum was applied onto the sample application point (S) of the Basis Set module (Fig. 1b). Immediately after, the antigen solution was applied. The serum antibody to antigen binding occurs during the 20 min incubation while the mixture of antigen solution, conjugate and serum is driven through the device by capillary flow. The antigen specific IgG of the sample binds specifically to its corresponding antigen in the solution and these soluble antibody-(sIgG)-bound antigens are retained at the test line (T) by a capture molecule (Fig. 1). At the same time, the sIgG bound to the antigen is bound by an antibody coupled to gold particles (conjugate). The intensity of the color reaction at the test line is directly proportional to the amount of immune complexes consisting of ligand tagged antigen, sIgG, and anti-IgG specific conjugate. The signal intensity ranges from faintly pink color (low titer of sIgG) to dark ruby red (high titer of sIgG). Excess conjugate, which is not bound at the test line, forms a dark ruby control line (C) after 20 min of incubation. Interpretation of the test results is done by densitometry using a portable, proprietary lateral flow assay reader (Fig. 1c). Semi-quantitative results were expressed as relative units (RU), whereas the RU value corresponds to the color intensity of the test line (measured as grayscale level by a CCD camera) and, hence, to the concentration of autoantibody in the serum sample.

2.3. Technical performance analysis

For Pr3 and MPO AI-LFA, the inter-assay variation was analyzed with 3 different lots of antigen and 3 different lots

Please cite this article as: N., O., et al., Development and validation of a lateral flow assay (LFA) for the determination of IgG-antibodies to Pr3 (cANCA) and MPO (pANCA), J. Immunol. Methods (2013), http://dx.doi.org/10.1016/j.jim.2013.11.017