Autoimmunity Reviews 15 (2016) 776–780

Contents lists available at ScienceDirect

Autoimmunity Reviews

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

Review

Anti-peptidyl-arginine deaminase 3 (PAD3) antibodies as a promising marker to measure joint damage in patients with rheumatoid arthritis

Andrea Seaman [a], Erika Darrah [b], Maria Infantino [c], Francesca Meacci [d], Mariangela Manfredi [c], Maurizio Benucci [d], Michael Mahler [a,*]

[a] Inova Diagnostics, Inc., San Diego, CA, USA
[b] Rheumatology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c] Immunology and Allergology Laboratory Unit, Ospedale S. Giovanni di Dio, Florence, Italy
[d] Rheumatology Unit, Ospedale S. Giovanni di Dio, Florence, Italy

ARTICLE INFO

Article history:
Received 7 March 2016
Accepted 10 March 2016
Available online 18 March 2016

Keywords:
CCP
Citrullinated cyclic peptide
Rheumatoid arthritis
RA
Autoantibodies
PAD
Peptidyl arginine deiminase

ABSTRACT

Background: Recently, antibodies directed against peptidyl arginine deiminase 3 and 4 (anti-PAD3/PAD4 antibodies), calcium-dependent enzymes that catalyze the conversion from arginine to citrulline, have been described. Furthermore, antibodies that cross-react between PAD3 and PAD4 cause increased PAD4 activity and consequently correlate with joint damage. This study analyzes the correlation of anti-PAD3 antibodies with joint damage.

Methods: To validate the novel chemiluminescent immunoassay (CIA) for the detection of anti-PAD3 antibodies, 20 samples were tested by CIA and by immunoprecipitation (IP). Next, 39 RA patients with available joint erosion score (JES), Total Sharp Score (TSS) and Joint Space Narrowing Score (JSNS) were tested for anti-CCP (using different methods) and anti-PAD3 antibodies by CIA.

Results: Excellent correlation was observed between the CIA and IP for the detection of anti-PAD3 antibodies (rho = 0.85, $p < 0.0001$). The median JES of our 39 patients was 14.1 with a standard deviation of 11.5. Anti-PAD3 antibody levels (rho = 0.39, 95% CI = 0.1–0.6; $p = 0.0149$) were correlated with JES. No correlation was found with TSS and JSNS. In this cohort, ACPA measured using different anti-CCP assays did not correlate with the JES.

Conclusion: In our cohort, anti-PAD3 antibodies correlate with joint erosion score. Therefore, anti-PAD3 antibodies might represent promising markers to predict joint damage in RA patients.

Contents

1. Introduction

Rheumatoid arthritis (RA) is chronic autoinflammatory disorder affecting the joints of up to 1% of the general population. During the last decades, early diagnosis and thus early treatment became a new paradigm in the management of this prevalent disease. As reported in several studies, early treatment can prevent disease progression and

* Corresponding author at: Inova Diagnostics, 9900 Old Grove Road, San Diego, CA 92131-1638, USA. Tel.: +1 858 586 9900; fax: +1 858 586 9911.
E-mail addresses: mmahler@inovadx.com, m.mahler.job@web.de (M. Mahler).

http://dx.doi.org/10.1016/j.autrev.2016.03.016
1568-9972/

irreversible damage of the joints and depends on reliable biomarkers to make an early and accurate diagnosis. Although anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) are widely used as an aid in the diagnosis and classification of RA, both biomarkers have their limitations. Consequently, there is a strong need for new biomarkers to further improve the diagnosis of RA. Additionally, besides closing the serological gap in RA [1], novel biomarkers are needed that help in the prognosis of disease outcome and monitoring of disease activity as well as the treatment response prediction. Recently, several novel autoantibodies have been described in the serum of RA patients including antibodies targeting carbamylated (CarP) [1–4] and peptidyl-arginine deaminase (PAD) antigens [5,6]. PAD proteins are calcium-dependent enzymes that catalyze the conversion from arginine to citrulline which represents key antigenic targets in patients suffering from RA. Some studies provided further evidence that PAD enzymes have the intrinsic capacity to select unique protein targets which thereby may play a role in autoantigen selection in RA [7]. PAD enzymes require supraphysiologic calcium concentrations for activity *in vitro*, but they are active *in vivo* (for example, in RA synovial fluid) at much lower calcium concentrations. A recent study found that a subset of autoantibodies that cross-react between PAD3 and PAD4 markedly decrease the enzyme's calcium requirement into the physiologic range, thereby increasing the catalytic efficiency of PAD4 [8]. These particular antibodies are also associated with higher baseline radiographic damage and a higher risk of progression. These observations might provide important insights into the pathogenesis of RA and consequently present anti-PAD3 antibodies as an important marker to identify a subgroup of RA patients that would benefit from early and aggressive treatment or in the future the addition of PAD inhibitor therapy.

Prior to the identification of PAD3/PAD4 cross-reactive antibodies, several groups had identified PAD4 as an autoantigen in RA [6,9–13]. Using an unbiased proteomic approach, Auger et al. identified PAD4 as a target of autoantibodies and in a follow-up study, characterized the linear epitopes and effect of these antibodies on PAD4 enzyme function [5]. Autoantibodies to PAD4 preferentially recognized four peptide epitopes located both in the N-terminal domain (211–290) and the C-terminal domain (601–650) of PAD4, but recognition did not correlate with effects on PAD4 function [5]. Similar to ACPA, RF and anti-CarP antibodies [14,15], anti-PAD4 antibodies also precede the clinical onset of RA [16]. The present study aimed to evaluate a research-based chemiluminescence immunoassay (CIA) for the detection of anti-PAD3 antibodies for the assessment of joint erosions in patients with RA.

2. Methods

Patient samples—To validate the novel chemiluminescent CIA for the detection of anti-PAD3 antibodies, a total of 20 samples, 10 immunoprecipitation (IP) positive and 10 negative, were collected at Johns Hopkins University and tested on CIA. Next, 39 RA patients with available joint erosion score (JES), Total Sharp Score (TSS) and Joint Space Narrowing Score (JSNS) were selected. None of our patients had recorded lung disease. All patient samples were tested for anti-CCP (using different methods) and anti-PAD3 antibodies by CIA.

Recombinant PAD3 antigen—Recombinant PAD3 antigen was produced in *Escherichia coli* and purified using HIS tag affinity purification. Purity of the proteins was analyzed using SDS-PAGE and determined as >95%.

The QUANTA Flash® PAD3 assay is a novel CIA (research use only) that uses recombinant PAD3 coated onto paramagnetic beads and is designed for the BIO-FLASH® instrument (Biokit s.a., Barcelona, Spain). The principles and protocols of the assay system have been previously described [17]. In brief: the relative light units (RLUs) are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which in turn is proportional to the amount of anti-PAD3 antibodies bound to the antigen on the beads. Samples above the analytical measuring range were diluted to determine the exact concentration of anti-PAD3 antibodies. Antibodies to CCP were detected using QUANTA Flash CCP3 (Inova Diagnostics, San Diego, US) as previously described [18].

Epitope analysis of PAD3/PAD4—Synthetic peptides corresponding to sequences previously identified as targets of anti-PAD4 antibodies [5] were synthesized to study their potential correlation and cross-reactivity to anti-PAD3 antibodies (see Fig. 3). The peptides were synthesized as soluble, biotin-containing peptides to be used in a streptavidin ELISA method.

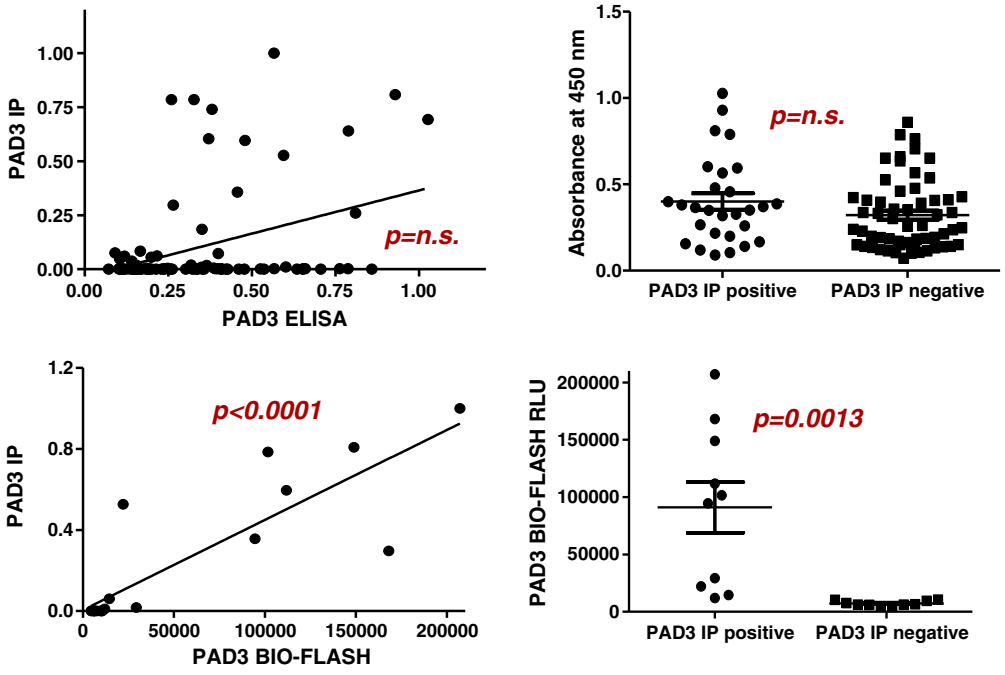

Fig. 1. Development of a chemiluminescence immunoassay (CIA) with good correlation to immunoprecipitation (IP) for the detection of anti-PAD3 antibodies. A significant correlation was found between the levels of anti-PAD3 antibodies measured by a novel CIA and by IP. No correlation was found between ELISA and IP.