The novel Anti-PR3-hn-hr ELISA using a mixture of human native and in human cells expressed authentic recombinant PR3

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Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) with either cANCA or pANCA fluorescent patterns are detectable in ANCA-associated vasculitis (AAV). The cANCA pattern is mainly produced by antibodies against proteinase-3 (PR3), but some cANCA positive sera do not react with PR3 in different conventional ELISA systems which use inappropriate antigen substrates and thus lack sensitivity. A new PR3 substrate was created consisting of a recombinant human PR3 designer antigen mixed with a native antigen, isolated from human neutrophils.

Methods

An enzymatically inactive PR3 variant was constructed and, for the first time, expressed in human cells. By substitution of serine with alanine at position 176, the enzymatic activity could be totally blocked, which made an efficient, large-scale production of the authentic PR3 antigen in a human (!) cell line possible. A mixture of this recombinant (hr) PR3 with human native (hn) PR3 was used as coating antigen.

The resulting ELISA (Anti-PR3-hn-hr) was compared with ELISA systems, which apply directly coated substrates, either containing hn-PR3 or hr-PR3, as well as a capture ELISA for PR3-ANCA. Assay characteristics were determined in AAV patients (n = 248), with special attention to those patients with cANCA (n = 132), as well as a large cohort of disease controls (n = 586) and healthy blood donors (n = 429). Additionally, for prediction of relapses, serial samples of 46 PR3-AAV patients were analyzed.

Results

At a predefined specificity of 99%, the sensitivity for AAV was increased to 94.7% in both ELISA systems containing the hr-PR3 antigen. For prediction of relapses by rises in antibody titers against PR3, the capture ELISA showed even slightly better results (odds ratio 12.5), closely followed by the novel Anti-PR3-hn-hr ELISA (odds ratio 8.9).

Discussion

Proteolytic damage of the transfected cell line as well as of the molecule itself is a major problem in providing the target antigen PR3 for immunoassays. In order to stabilize the protein, we created the hr-PR3, which lacks enzymatic activity but exhibits all necessary conformational properties for antibody detection as well as a His-tag for easy and reliable microplate coating. As a consequence of the modern protein design, the test performance of the novel Anti-PR3-hn-hr ELISA became excellent, the sensitivity even comparable to IIF, by far superior to conventional ELISA systems. The new test system is not compromised by some disadvantages of the capture ELISA, i.e. potential masking of PR3 epitopes by the murine monoclonal antibody or reporting false positive reactions due to the presence of human anti-mouse antibodies. Together with the good predictability of clinical relapses, the novel Anti-PR3-hn-hr ELISA therefore serves as an ideal tool for the identification and follow-up of PR3-positive AAV patients.

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