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Review

PM1-Alpha ELISA: The assay of choice for the detection of anti-PM/Scl autoantibodies?

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ABSTRACT

A characteristic serological feature of patients suffering from the overlap polymyositis and scleroderma (PM/Scl) syndrome are antibodies to the human counterpart of the yeast exosome referred to as the PM/Scl complex. Historically, the detection of anti-PM/Scl antibodies was laborious and relied largely on indirect immunofluorescence and immunodiffusion techniques. In 1992 the major autoantigen PM/Scl-100 was identified and cloned. Subsequently, the major epitopes were mapped and one of these, termed PM1-Alpha, became the antigen for a novel ELISA exhibiting high sensitivity and specificity for the detection of anti-PM/Scl antibodies. Comparative studies with other methods using other PM/Scl autoantigens have shown that the PM1-Alpha ELISA has higher sensitivity and specificity than assays that employed recombinant PM/Scl-75c and PM/Scl-100. Anti-PM1-Alpha antibodies were identified in 55.0% of sera from PM/Scl overlap syndrome patients, but were also seen in 7.9% of SSc and in 7.5% of PM patients. The frequency in other systemic autoimmune diseases and in infectious diseases was significant lower. In summary, the data derived from individual studies suggest that PM1-Alpha may become the "gold standard" for the detection of anti-PM/Scl antibodies.

Contents

1. Introduction

Anti-nucleolar antibodies (ANoA), a subset of anti-nuclear antibodies (ANA), are directed against a number of nucleolar antigens, which include the PM/Scl complex, an antigen target that has been historically associated with a polymyositis (PM), scleroderma (Scl, or systemic sclerosis; SSc) overlap syndrome [1]. The PM/Scl autoantigen was initially described in 1977 in sera of PM patients and some years later the antigen was named the 'PM/Scl antigen' when two groups reported that these autoantibodies (aab) were most prevalent in PM/Scl patients [1–4]. In the 1990s, the major antigens PM/Scl-75 and PM/Scl-100 were cloned and the PM/Scl antigen system was identified as the human counterpart of the yeast exosome, a

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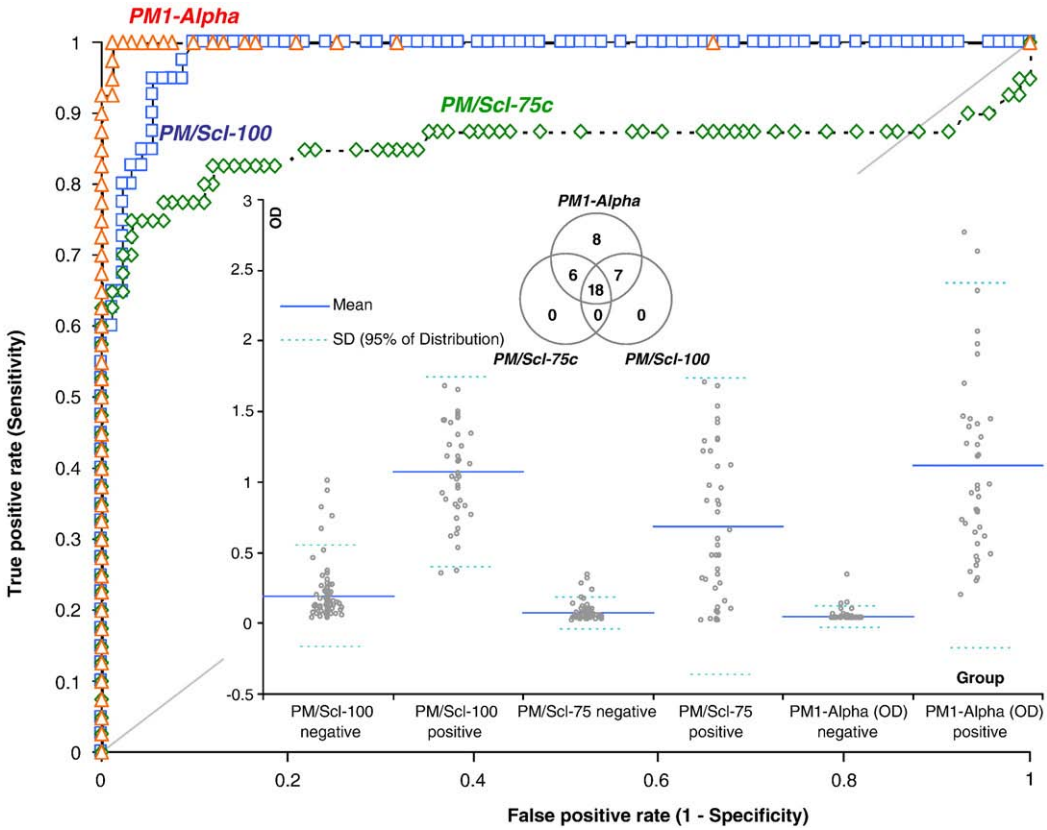

Fig. 1. Reactivity to the major PM/Scl autoantigens PM/Scl-75c, PM/Scl-100, PM1-Alpha. 40 samples with predefined anti-PM/Scl reactivity as defined by indirect immunofluorescence and confirmation methods and 40 controls were tested by ELISA. Receiver operating (ROC) characteristic and comparative descriptive analysis show better discrimination between PM/Scl positive and negative samples using the PM1-Alpha ELISA compared to ELISAs with recombinant PM/Scl-75c or PM/Scl-100. At cut-off values that results in 100% specificity for all three ELISAs 18 samples were positive by all three methods, 7 by PM1-Alpha and PM/Scl-100 and 6 by PM1-Alpha and PM/Scl-75c. Eight samples demonstrated exclusive reactivity to PM1-Alpha. None of the samples negative for anti-PM1-Alpha were positive for antibodies to one or both of the recombinant antigens. [33]. Mean values and standard deviation (SD) of 95% distribution are shown for each group.

macromolecular complex involved in RNA degradation and processing [4–7]. This discovery allowed the purification of the complex and enabled the identification of other subunits [8]. Although not as often recognized by patient sera as PM/Scl-75 and PM/Scl-100, some of these other exosome subunit proteins also proved to be target autoantigens [1].

The ring shaped complex of nine core exosome proteins has been localized to the cytoplasm and nucleoplasm, but is most abundant in the nucleolus [9,10]. The most clinically relevant protein is believed to be PM/Scl-100, which is stably associated with a fraction of the core exosome and also has ribonuclease activity [1].

A variety of techniques have been used to detect anti-PM/Scl antibodies in CTD including the identification of a characteristic ANoA staining pattern as detected by indirect immunofluorescence (IIF) on HEp-2 cells followed by confirmation using double immunodiffusion (ID), immunoprecipitation (IP), immunoblotting (IB) with extractable nuclear antigens, or enzyme linked immunosorbent assay (ELISA) [1] employing purified native or recombinant proteins. The detection of anti-PM/Scl antibodies by IB and IIF, however, is difficult, due to weak reactivity on IB and potential confusion with a number of other ANoA (e.g. anti-fibrillarin, anti-RNA polymerase I, B23/nucleophosphmin) in IIF screening tests. The majority of the anti-PM/Scl ELISA tests use recombinant PM/Scl-100 protein expressed in *E. coli* or in insect cells, because this is the best known autoantigenic component of the PM/Scl complex. Although the reactivity of several other PM/Scl components were tested, aab against these proteins were most commonly found in patients who were also positive for PM/Scl-100 [1,11]. In 2005, however, a new isoform of the PM/Scl-75 protein, termed PM/Scl-75c was shown to have a slightly higher sensitivity than PM/Scl-100 when used in ELISA. The aab reactivity of many other components associated with the exosome has yet to be tested. Combined or multiplexed testing for these and other exosome related proteins might further increase the sensitivity of protein based ELISA assays for anti-PM/Scl reactivity in the future.

Anti-PM/Scl antibodies were primarily found in patients with PM, SSc or DM, with the highest occurrence in overlap syndromes of SSc with PM or DM (polymyositis or dermatomyositis respectively; both referred to as PM/Scl here). A meta-analysis of all studies that used ID, IIF and/or IP to determine anti-PM/Scl reactivity shows that this aab reactivity is found in 31% of all PM/Scl patients, compared to 8% of patients with PM alone, 11% of DM patients and 2% of SSc patients (data not shown). Due to the low prevalence of anti-PM/Scl aab in CTD, the relationship to clinical features is derived from a relatively small number of patients, although multiple studies have addressed the issue in various cohorts of patients and, as described above, anti-PM/Scl aab have