

Do We Need Immunofluorescence Tests For PM/SCL Antibody Detection?

V. Ramsperger, M. Lindemann, V. Kumar. IMMCO Diagnostics, Inc. Buffalo, NY

Abstract

Objective: To determine if it is necessary to perform immunofluorescence tests on HEp-2 cells to detect PM-Scl autoantibodies.

Background: PM/Scl overlap syndrome is an autoimmune disease. Patients with antibodies to PM/Scl have symptoms of both systemic sclerosis and either polymyositis or dermatomyositis. A characteristic serological feature of PM/Scl and scleroderma is the presence of antinuclear antibodies (ANA) of a nucleolar reaction pattern; however, the limitation of this method is that it is subjective and requires a skilled reader for the identification of a specific nucleolar reaction pattern associated with PM/Scl antibodies. The antigens to which PM/Scl antibodies react have been identified, resulting in the availability of specific immunoassays that are easy to perform. The objective of our study was to determine if ELISA is sufficient for detecting PM/Scl antibodies or if testing on HEp-2 may be desired for confirmation, especially when the ELISA results exhibit weak positive results.

Method: Serum samples were examined on HEp-2 for ANA and for PM/Scl antibodies by ELISA using an in-house kit and two commercially available kits. The test results were read blinded and an association of the ANA reaction pattern to the ELISA was determined.

Results: Of the 111 samples tested for PM/Scl antibodies, seventeen were positive for ANA with nucleolar pattern. Twelve of these seventeen were positive and five negative for PM/Scl antibodies on in-house PM/Scl immunoassay. Of the nucleolar negative samples only four were positive for PM/Scl antibodies. All four samples had low positive results on the ELISA.

Conclusions: The use of ELISA in combination with nucleolar antibody detection on HEp-2, especially when the ELISA results are low positive provide a reliable combination for detecting PM/Scl antibodies.

Introduction

Anti-nuclear antibodies (ANA) are a characteristic feature of systemic connective tissue diseases (CTD) such as polymyositis (PM), dermatomyositis (DM), systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). A subset of ANAs are directed against autoantigens located in the nucleolar compartment of the cell. This includes antibodies to the PM-Scl complex, also known as the human exosome complex, which are found in patients with polymyositis-scleroderma (PM/Scl) overlap syndrome and related diseases. Anti-PM-Scl antibodies represent a specific serological marker for a subset of patients with scleroderma (Scl) and polymyositis (PM), and especially with PM-Scl overlap syndrome. Anti-PM-Scl reactivity is found in 24% of PM-Scl overlap patients and is found in 3-10% of Scl and PM patients. Scleromyositis or PM/Scl overlap syndrome is a complex autoimmune disease with symptoms of both polymyositis and systemic sclerosis, and therefore it is considered an overlap syndrome. The incidence of the PM/Scl is estimated in the range of 0.5-8.4 cases per million in the U.S. population. Polymyositis is an idiopathic autoimmune inflammatory joint and muscle disorder. In polymyositis, the inflammatory cells of the immune system directly attack muscles and related tissue, causing weakness in the hips, thighs, upper arms, shoulders, back and neck. Scleroderma affects the connective tissue of the body, skin, and internal organs, and also attacks muscles, joints and blood vessels. Sclerosis or "hardening" of the organs may occur, causing dysfunction.

Several studies have used a variety of techniques to detect anti-PM/Scl antibodies in CTD. Most studies have used immunofluorescence (IFA) staining on HEp-2 as a screening test and then follow subjects with a nucleolar pattern with additional tests such as immunodiffusion, immunoprecipitation, western immunoblot or enzyme linked immunosorbent assay (ELISA) to confirm anti-PM/Scl reactivity. The detection of anti-PM/Scl antibodies by immunoblot and by IFA are subjective and sometimes weak reactivity on immunoblot and interference of other ANA reactions on IFA makes interpretation difficult. In addition IFA test is subjective and requires significant skills for the identification of a specific reaction pattern. The intent of this study is to determine if it is possible to detect PM/Scl specific antibodies by ELISA alone, or is it necessary to also perform IFA tests on HEp-2 for confirmation.

Materials and Methods

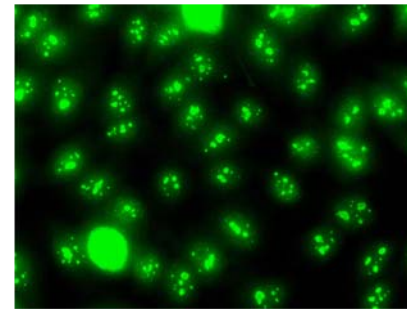
Patient Population: 111 serum samples submitted to the laboratory were tested for PM/Scl antibodies by ELISA and for ANA by IFA on HEp-2 cells. Of these 51 samples were requested for testing for PM/Scl antibodies. Of these 51 samples 24 were positive by IFA on HEp-2: 17 with a nucleolar; four speckled, one each with mitochondrial, homogenous/speckled and centromere pattern. Of the remaining 60 samples, 32 were normal human sera and 28 were disease controls positive for antibodies to Sm, La (SS-B), Ro (SS-A), and RNP. Twenty six of these normal and disease control serum samples were positive for ANA by IFA on HEp-2 (24 speckled, 2 homogenous).

Immunofluorescence: ANA were determined by indirect immunofluorescence on HEp-2 (IMMCO) at dilutions of 1:40 and 1:160. IFA reactions were read blindly.

PM/Scl Antibody ELISA: Microtiter plates coated with PM/Scl antigen utilizing optimal coating and blocking conditions were incubated with diluted sera, washed, and incubated with anti-human IgG HRP conjugate. Plates were washed with the wash diluent and the presence of PM-Scl antibodies were detected by a color change produced by the conversion of substrate to a colored reactant. Absorbance was read at 450nm. In addition to the IMMCO test, two other commercially available assays were run simultaneously.

Results

Of the 111 samples tested for PM/Scl antibodies, seventeen were positive for ANA with nucleolar reaction pattern. Twelve of these seventeen were positive and five negative for PM/Scl antibodies on the IMMCO ELISA. Of the nucleolar negative samples only four were positive for PM/Scl antibodies on IMMCO ELISA. The ELISA results on these four samples were less than 40EU/ml. There was a higher percentage of PM/Scl positive results on ANA positive samples with nucleolar staining on the two other assays. Similarly a larger percentage of samples that were nucleolar negative were also positive for PM/Scl antibodies on these two assays.



Indirect immunofluorescence staining of nucleolar patterns on HEp-2 cells.

Correlation Between Nucleolar Pattern and PM-Scl ELISA

| HEp-2 Positive for Nucleolar Antibodies | | | |
|---|-------|---------|---------|
| | IMMCO | Comp. A | Comp. B |
| ELISA Positive | 12 | 17 | 14 |
| Positive Predictive Agreement | 71% | 100% | 82% |
| Nucleolar Positive = 17 | | | |

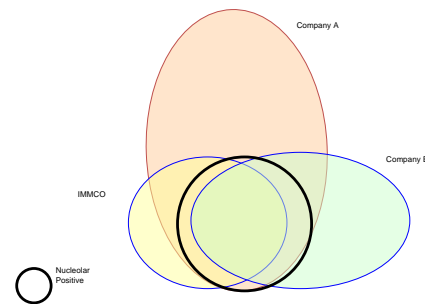
Incidence of PM/Scl ELISA positive samples with a positive Nucleolar staining pattern on HEp-2 cells.

| HEp-2 Negative for Nucleolar Antibodies | | | |
|---|-------|---------|---------|
| | IMMCO | Comp. A | Comp. B |
| ELISA Positive | 4 | 20 | 10 |
| Negative Predictive Agreement | 92% | 62% | 81% |
| Overall Agreement | 93% | 71% | 81% |
| Nucleolar Negative = 53 | | | |

Incidence of PM/Scl ELISA positive samples with a negative Nucleolar staining pattern on HEp-2 cells.

| HEp-2 Negative with Low Positive ELISA (< 2x Cutoff) | | | |
|--|-------|---------|---------|
| | IMMCO | Comp. A | Comp. B |
| ELISA Positive | 0 | 6 | 6 |
| Percent Positive | 0% | 11% | 11% |
| Nucleolar Negative = 53 | | | |

Incidence of Nucleolar negative patients that have ELISA PM/Scl values that are less than twice the cutoff.



Correlation of ANA positivity with nucleolar pattern on HEp-2 with ELISA for PM/Scl antibodies. The black circle in the center is representative of the 17 nucleolar positive samples. Twelve samples with a nucleolar pattern and 4 samples with negative nucleolar pattern are positive for PM/Scl Antibodies on IMMCO ELISA. Seventeen samples with a nucleolar pattern and 20 nucleolar antibody negative are positive on Company A. Fourteen samples with nucleolar pattern and 10 nucleolar negative are positive for PM-Scl antibody on Company B.

Conclusions

1. IMMCO PM-Scl peptide ELISA is a sensitive and specific assay for detection of PM-Scl antibodies.
2. Detection of a nucleolar staining pattern by HEp-2 is helpful for cases with low positive ELISA values (i.e. <40 EU/ml).
3. There are fewer samples on the IMMCO ELISA that are PM-Scl positive and Nucleolar HEp-2 negative.

References

- Behan A, Peter JB. Polymyositis and dermatomyositis 292:344-347;1975.
 Limaye VS, Blumbergs P, Roberts-Thompson PJ, Idiopathic inflammatory myopathies. Intern. Med J 39:179-190;2009.
 Mahler M, Fritzier MJ. PM1-Alpha ELISA: The assay of choice for the detection of anti-PM/Scl autoantibodies? Autoimmunity Rev 8:373-378; 2009.
 Mahler M, Rajmakers R, Novel aspects of autoantibodies to the PM/Scl complex: Clinical, genetic and diagnostic in sights. Autoimmunity Rev 6:432-437; 2007
 Mahler M Rajmakers R, Dahnrich C, et al. Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen. Arthritis Res Ther 7:R704-R713, 2005
 Von Muhlen CA, Tan EM, Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 24:323-358;1995..
 Reichlin M, Maddison PJ, Targoff I, et al. Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes J Clin Immunol 4:40-44, 1984.