Anti-PM/Scl-antibodies in systemic sclerosis: Comparison between PM1-Alpha ELISA and a line assay with recombinant PM/Scl-75c and PM/Scl-100

M. Mahler[1], M. Siegemund[2], W. Meyer[2], M. Petschinka[1], M.J. Fritzler[3]
[1]Dr. Fooke Laboratorien GmbH, Neuss, Germany; [2]Euroimmun AG, Lübeck, Germany;
[3]Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

Background: Autoantibodies (aab) to the exosome complex are a characteristic feature of patients with scleroderma (Scl or SSc), polymyositis (PM) and especially a PM/Scl overlap syndrome. Historically, anti-PM/Scl antibodies were detected by the presence of a nucleolar staining pattern in indirect immunofluorescence on HEp-2 cells and confirmed by immunodiffusion or immunoblot. More recently, ELISA and line immunoassays (LIA) using either recombinant proteins (PM/Scl-75c or PM/Scl-100) or a PM/Scl-100 derived peptide (PM1-Alpha) have become popular methods for the detection of those aabs. The aim of this study was to compare the PM1-Alpha ELISA with a novel LIA that allows for the parallel detection of anti-PM/Scl-75c and PM/Scl-100 aabs.

Methods: Sera were collected from SSc patients (n=153) and tested by PM1-Alpha ELISA (Dr. Fooke Laboratorien GmbH, Neuss) and LIA (Euroimmun, Lübeck). Statistics were done with Analyse-it for Microsoft-Excel.

Results and findings: The point prevalence of anti-PM/Scl aabs in 153 SSc sera was 12 (7.8%, PM1-Alpha), 10 (6.5%, PM/Scl-100) and 10 (6.5%, PM/Scl-75c), respectively. 8 sera were positive for all three antigens, two for PM/Scl-75c only, two for PM/Scl-100 only and four for PM1-Alpha only. According to the *kappa* method the agreements were 0.79 (PM/Scl-100 vs. PM/Scl-75c), 0.71 (PM/Scl-75c vs. PM1-Alpha) and 0.71 (PM/Scl-100 vs. PM1-Alpha). When PM/Scl-75c and PM/Scl-100 were combined the sensitivity was equal to PM1-Alpha ELISA (7.8%).

Table 1 Comparison of LIA and PM1-Alpha ELISA.

kappa = 0.71	PM/Scl-100		
PM1-Alpha	pos	neg	Total
pos	8	4	12
neg	2	139	141
Total	10	143	153

kappa = 0.71	PM1-Alpha		
PM/Scl-75	pos	neg	Total
pos	8	2	10
neg	4	139	143
Total	12	141	153

kappa = 0.79	PM/Scl-100		
PM/Scl-75	pos	neg	Total
pos	8	2	10
neg	2	141	143
Total	10	143	153

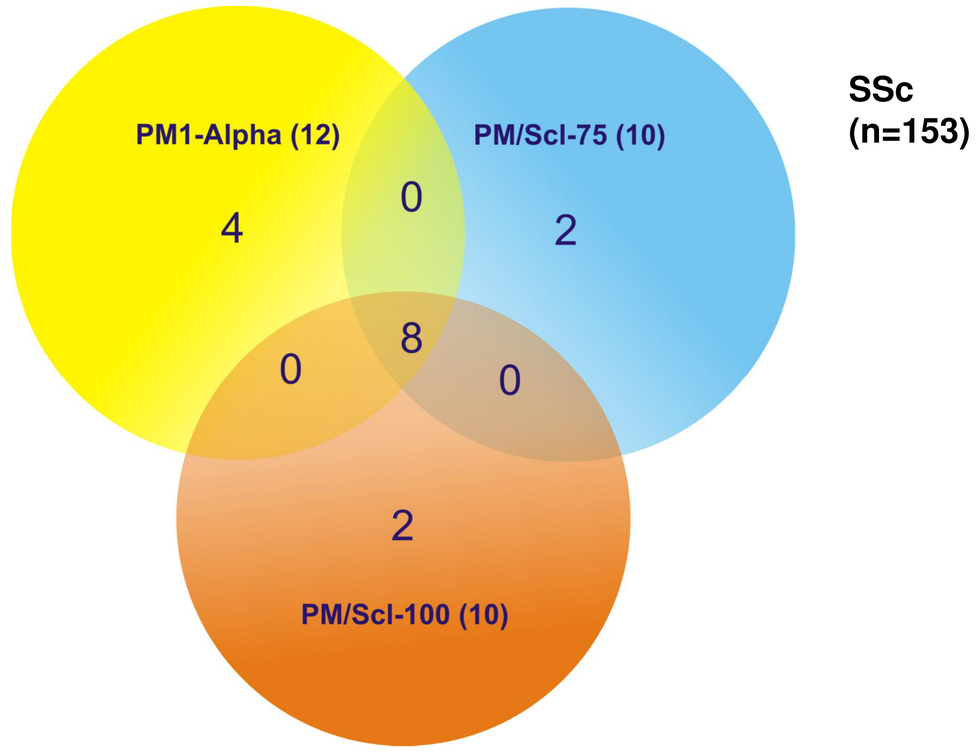

Figure 1 Venn-Diagram. Reactivity to PM/Scl antigens (PM1-Alpha, PM/Scl-75c and PM/Scl-100) in sera from patients with SSc (n=153). 16/153 showed reactivity to at least one antigen, 4 isolated to PM1-Alpha, 2 isolated to PM/Scl-100, 2 isolated to PM/Scl-75c and 8/153 to all three antigens.

Table 2 Reactivity profile of anti-PM/Scl positive samples.

ID	ANA IIF HEp-2	ENA	PM1-Alpha	PM/Scl-100	PM/Scl-75c
1	NLR, Sp	Ro52	+	+	+
2	NLR, Sp	neg	+	+	+
3	NLR, Sp	topo I	+	+	+
4	Sp	Ro52	+	+	+
5	CENP	CENP	+	+	+
6	NLR, Sp	RNP weak	+	+	+
7	NLR, Sp	neg	+	+	+
8	NLR, Sp, H	neg	+	+	+
9	CENP	CENP, RNAP	-	+	-
10	Sp	neg	-	+	-
11	NLR, Sp, CENP	Ro52, Ro60, CENP	-	-	+
12	Sp	RNAP	-	-	+
13	NLR, Sp	topo I	+	-	-
14	NLR, Sp	topo I, SS-B/La	+	-	-
15	NLR, Sp, H	topo I	+	-	-
16	H, Sp	topo I	+	-	-

NLR=nucleolar; Sp=nuclear speckled; H=homogeneous; CENP=centromere; RNAP=RNA polymerase III

Conclusion: We conclude that the prevalence of anti-PM/Scl aabs in SSc sera varies between 6.5% by LIA and 7.8% by PM1-Alpha ELISA, with good qualitative agreement between the methods. Further studies with larger and clinically characterized cohorts that analyze the additional value of more than one PM/Scl antigen are underway.

References:

1. Hanke K et al., Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients. *Arthritis Res Ther* 2009, **11**:R22.
2. Mahler M, Fritzler MJ. The changing landscape of the clinical value of the PM/Scl autoantibody system. *Arthritis Res Ther* 2009, **11**:106.
3. Mahler M, Fritzler MJ. PM1-Alpha ELISA: the assay of choice for the detection of anti-PM/Scl autoantibodies? *Autoimmun Rev* 2009, **8**:373-8.

9th Dresden Symposium on Autoantibodies; Dresden, September 2-5, 2009