

Novel aspects of autoantibodies to the PM/Scl complex: Clinical, genetic and diagnostic insights

Michael Mahler^{a,*}, Reinout Raijmakers^b

^a *Dr. Fooke Laboratorien GmbH, Mainstr.85, 41469 Neuss, Germany*

^b *Radboud University Nijmegen, Nijmegen, The Netherlands*

Received 17 December 2006; accepted 21 January 2007

Available online 20 February 2007

Abstract

A characteristic feature of patients suffering from connective tissue diseases such as polymyositis (PM), dermatomyositis, systemic sclerosis (scleroderma, SSc, Scl), systemic lupus erythematosus or overlap syndromes thereof are anti-nuclear antibodies and anti-nucleolar antibodies. Antibodies to the PM/Scl complex, also known as the human exosome complex, belong to the anti-nucleolar antibodies and are mainly found in patients with PM/SSc overlap syndrome and related diseases. Until recently, the detection of anti-PM/Scl antibodies was laborious and relied largely on indirect immunofluorescence and immunodiffusion techniques. With the identification and characterization of the autoantigens, especially PM/Scl-75, PM/Scl-100 and a synthetic peptide (PM1-Alpha) thereof, newly developed assays based on recombinant proteins and peptides have allowed the development of a new generation of anti-PM/Scl tests with high sensitivity and specificity. These novel assays (i.e. ELISA, line immunoassays and protein arrays) enable testing for anti-PM/Scl in modern, automated, multi-parametric assay settings. The present review focuses on recent insights on anti-PM/Scl autoantibodies with special emphasis on clinical, genetic and diagnostic aspects.

© 2007 Elsevier B.V. All rights reserved.

Keywords: PM/Scl; Autoantibody; Systemic sclerosis; Polymyositis; Exosome

Contents

1. Introduction	433
2. History and biochemical aspects of the PM/Scl autoantigen	433
3. Clinical and genetic features of patients with anti-PM/Scl antibodies	434
4. Novel approaches for the detection of anti-PM/Scl antibodies	435
5. Conclusions	436
Take-home messages	436
References	436

* Corresponding author. Tel.: +49 2131 4742709; fax: +49 1212 6 466266866.

E-mail address: m.mahler.job@web.de (M. Mahler).

1. Introduction

Anti-nuclear antibodies (ANA) are a characteristic feature in the serum of patients suffering from connective tissue diseases (CTD) such as polymyositis (PM), dermatomyositis (DM), systemic sclerosis (scleroderma, Scl; SSc) or systemic lupus erythematosus (SLE) [1]. Many of these ANAs are known as diagnostic tool and have also been reported to predict disease onset many years in advance of the clinical diagnosis [1,2]. A subset of ANAs, namely anti-nucleolar antibodies (ANoA), is directed against autoantigens located in the nucleolar compartment of the cell [1,3]. This includes antibodies to the PM/Scl complex, also known as the human exosome complex, which are found in patients with PM/SSc overlap syndrome and related diseases [3].

2. History and biochemical aspects of the PM/Scl autoantigen

The PM/Scl autoantigen, which is nowadays known as the human exosome, was first described as a target of autoantibodies in patients suffering from the systemic autoimmune disorders PM and SSc. The first mention of these autoantibodies in literature was in 1977, when Wolfe et al. found that sera of patients suffering from PM could precipitate an antigen (labeled ‘PM-1’ at the time) from calf thymus extract in an immunodiffusion (ID) assay [4]. In 1984, the antigen was first named the ‘PM/Scl antigen’ when two research groups described that these autoantibodies were most prevalent in patients with an overlap syndrome that included features of PM and SSc [5,6]. In the following years, the PM/Scl antigen was shown to be a nucleolar macromolecular complex of more than 10 proteins. In the early 1990s, two autoantigenic protein components of the complex were identified and cloned. These proteins were named PM/Scl-75 and PM/Scl-100 based on their apparent molecular weights [7–11]. In 1999 it was shown that the PM/Scl complex was the human counterpart of the yeast exosome, a complex involved in RNA degradation and processing, which was discovered 2 years earlier. This discovery allowed the purification of what was now called the ‘human exosome’ and enabled the identification of other subunits. Although not as often recognized by patient sera as PM/Scl-75 and PM/Scl-100, many of the other exosome proteins also proved to be target autoantigens [12].

The human exosome consists of a core complex of nine proteins and several proteins that associate with the exosome in particular subcellular locations or during

certain processes (Fig. 1A). The core complex is present in many cellular compartments, including the cytoplasm and nucleoplasm, but is most abundant in the nucleolus [7]. The core complex is a ring structure comprised of six proteins, all containing an RNase PH domain (including PM/Scl-75) and on top of which are three proteins with S1 and/or KH RNA binding domains [7,13]. The most important of the proteins that associate with the core of the human exosome complex is PM/Scl-100, which is stably associated with a significant pool of the core exosome and also has ribonuclease activity [7]. The exosome complex involved a large variety of functions, which includes ribosomal RNA processing and messenger RNA degradation [7].

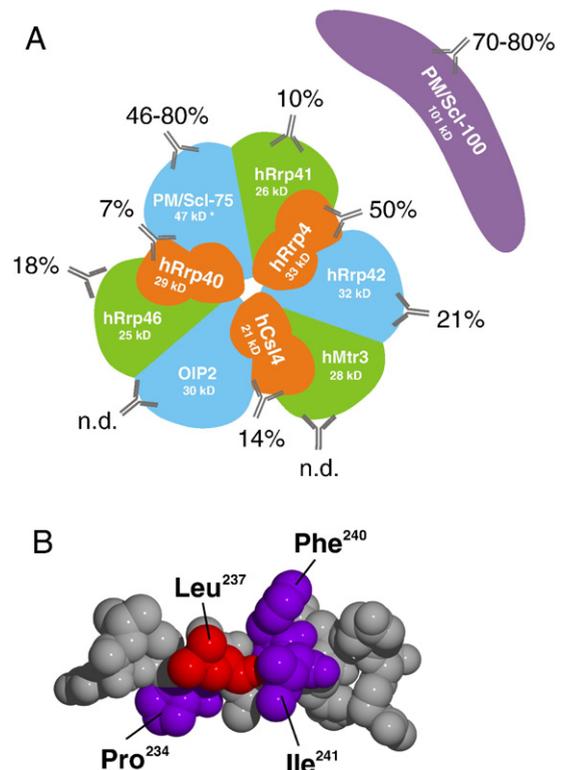


Fig. 1. Composition and autoantigenicity of the human PM/Scl complex and structural model of PM1-Alpha. Figure A) shows the composition of the human PM/Scl complex (human exosome) and the frequency with which the individual components (PM/Scl-75, PM/Scl-100, hRrp41, hRrp4, hRrp42, hMtr3, hCsl4, OIP2, hRrp46, hRrp40) are targeted by autoantibodies reacting with the PM/Scl complex. Note that PM/Scl-75 has a molecular weight of only 47 kD (marked with an asterisk) but runs at approximately 75 kD in SDS-PAGE due to a highly charged C-terminal domain. Values are based on references Brouwer et al. [12] and Raijmakers et al. [19] and are given in percent. Figure B) shows a structural model of PM1-Alpha based on structure prediction using the homologue amino acid stretch on the heterochromatin p25beta autoantigen [11,33].

3. Clinical and genetic features of patients with anti-PM/Scl antibodies

Several studies have used a variety of techniques to address the occurrence of anti-PM/Scl antibodies in CTD [3]. Most studies have used nucleolar staining in indirect immunofluorescence (IIF) on HEp-2 cells as a screening test and then follow this with an assay such as double ID, immunoprecipitation (IP) or enzyme linked immunosorbent assay (ELISA) to confirm anti-PM/Scl reactivity [5–17]. Anti-PM/Scl antibodies are generally only found in patients with PM, DM or SSc, with the highest occurrence in overlap syndromes of SSc with PM or DM (scleromyositis or sclerodermatomyositis respectively; both referred to as PM/SSc here). A meta-analysis of all studies that used ID, IIF and/or IP to determine anti-PM/Scl reactivity shows that the reactivity is found in 31% of all PM/SSc patients, compared to 8% of patients with PM alone, 11% of DM patients and 2% of SSc patients (data not shown). These data are corroborated by studies that have applied protein ELISA assays to test for reactivity [18,19], although the recently developed peptide ELISA for anti-PM/Scl antibodies is more sensitive and allows detection of the antibodies in 55% of PM/SSc patients [20].

Due to the low prevalence of anti-PM/Scl antibodies in CTD, the relationship to clinical features comes 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 antibodies have been associated with the PM/SSc overlap syndrome. Table 1 shows the clinical features and diagnoses associated with anti-PM/Scl antibodies. Depending on the original cohort of patients, 25–95% of the positive patients were thought to have the PM/SSc overlap syndrome, but when combining all these studies, 59% (80/139) of all PM/Scl positive patients are diagnosed with an overlap syndrome. The majority of the remaining patients in all studies had either SSc, PM or DM. In sporadic cases, anti-PM/Scl-antibodies studies have been reported in patients with inclusion body myositis [21], Sjögren syndrome [22], systemic lupus erythematosus (SLE) with dysphagia [23,24], and even in a case of acquired haemophilia [25]. The most frequently observed symptoms are muscle weakness and inflammation (myositis), Raynaud's phenomenon (RP) and arthritis. Other often reported clinical manifestations include swallowing problems (deglutition), lung or kidney involvement and mechanic's hands (Table 1). The overlap

Table 1
Overview of clinical associations of anti-PM/Scl antibodies

Study	Reichlin	Reimer	Genth	Oddis	Marguerie	Hausmanowa	Vandergheynst	Selva-O'callaghan
Reference	[6]	[16]	[36]	[17]	[27]	[30]	[22]	[21]
Year	1984	1988	1990	1992	1992	1997	2006	2006
Patient selection	PM	SSc	SSc/ANA	IIM/SSc/RP	SSc/IIM	IIM	ANoA	IIM
Anti-PM/Scl detection	ID	IP	IIF / ID	IIF/ID	ID	ID	IIF/ID	IP
Number of patients	20	8	12	23	32	20	14	10
Percentage females	–	88	92	–	–	–	93	40
<i>Diagnosis (%)</i>								
PM	23	–	–	4	3	–	7	10
DM	32	–	–	22	–	–	29	80
PM/SSc ^a	41	25	(42)	43	84	95	36	(40)
Scleroderma	5	75	83	30	13	–	21	–
Other	–	–	17	–	–	5	7	10
<i>Symptoms^b (%)</i>								
Raynaud's phenomenon	50	88	92	65	100	70	71	50
Arthritis	50	50	58	83	97	95	86	80
Muscle involvement	95	43	50	78	88	100	79	–
Skin involvement	20	100	50	–	47	45	–	30
Lung involvement	35	67	42	30	78	60	86	60
Esophageal involvement	–	43	36	–	78	–	21	50
Kidney involvement	–	25	0	–	–	5	21	–
Dry mouth/eyes	–	–	25	–	34	35	43	11

^a A percentage between brackets indicates these patients are a subset of the other diagnoses.

^b Muscle involvement includes muscle weakness and inflammation; skin involvement includes rash, digital ulcers, telangiectasia, calcinosis and mechanic's hands; lung involvement includes dyspnea, interstitial lung disease and pulmonary infiltrates; esophageal involvement includes dysphagia and deglutition abnormalities.

syndrome of PM/SSc is often reported as being a more benign and chronic form of the disease and anti-PM/Scl antibodies have been shown to predict limited cutaneous involvement [3]. With respect to therapeutic modalities, the inflammatory features in PM/SSc patients respond to a low to moderate dose of corticosteroids [3,26,27]. Autoantibodies co-occurring with anti-PM/Scl include rheumatoid factor (66%) [26], anti-SS-B/La (9%), anti-Ro52 (13%), anti-SS-A/Ro60 (6%), anti-Mi-2 (9%) and anti-Ku [20,28].

The frequency of anti-PM/Scl also appears to vary between different ethnic groups, as it was not found in a large series of 275 Japanese patients with SSc [29]. The PM/SSc overlap syndrome and anti-PM/Scl antibodies are strongly linked to MHC alleles HLA-DRB1*0301 (DR3), HLA-DQA1*0501 and HLA-DQB1*02 [30,31] whereas HLA-DRB1*15/*16 and HLA-DQA1*0101 appear to have a protective effect against developing anti-PM/Scl reactivity [32].

4. Novel approaches for the detection of anti-PM/Scl antibodies

Historically, the presence of anti-PM/Scl antibodies was monitored by IIF on HEp-2 cells, ID with calf thymus

extract, immunoblotting (IB) using extractable nuclear antigens and/or by IP from radioactively labelled cell extracts [6,16]. The detection of anti-PM/Scl antibodies by IB and IIF, however, is difficult, due to weak reactivity on IB and interference of other ANoA (e.g. anti-fibrillarlin or anti-RNA polymerase I) in IIF [3,20]. More recently, recombinant proteins, mainly the PM/Scl-100 autoantigen, have been used as antigenic targets in immunoassays such as ELISA or line immunoassay (LIA). Especially during the last few years, the profiling of autoantibody repertoires in patients has attracted much attention, mainly using LIA, protein chips or addressable laser bead assays (ALBIA). With an increasing number of techniques to test anti-PM/Scl reactivity, the availability of an international anti-PM/Scl standard serum provided by the Serology Committee of the IUIS/WHO/Centre of Disease Control (CDC) (<http://www.autoab.org/>) through the auspices of CDC is an important step forward. An overview of the currently available tests with CE-certification to detect anti-PM/Scl reactivity is given in Table 2.

The majority of the anti-PM/Scl ELISA tests use recombinant PM/Scl-100 protein either 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

Table 2
Overview of CE-certified antibody assays that allow for the detection of anti-PM/Scl antibodies

Name and product number	Features of the test/other antigens	Company	Antigen (PM/Scl)	Assay type	Format
PM/Scl ELISA (EA 1584-9601 G)	PM/Scl	Euroimmun	PM/Scl-100 <i>E.coli</i>	SPA	ELISA
Vareliisa® ANA CTD Screen (130 96)	dsDNA, U1-snRNP, Sm (B, B', D), SS-A/Ro (52 kD and 60 kD), SS-B/La, Scl-70, CENP, Jo-1, Histone, PM/Scl, Rib-P	Phadia	PM/Scl-100 Baculo	SA	ELISA
IMTEC-PM-Scl-Antibodies (ITC70035)	PM/Scl	Human	PM/Scl-100 recombinant	SPA	ELISA
PM1-Alpha ELISA (25001)	PM/Scl	Dr. Fooke Laboratorien	PM1-Alpha peptide	SPA	ELISA
Myositis profile (DL 1530–1601 G)	Mi-2, Ku, PM/Scl, Jo-1, PL-7, PL-12, Ro-52	Euroimmun	PM/Scl-100 <i>E.coli</i>	MPP	LIA
DA 1590-1003-30 G	Scl-70, Jo-1, PM-Scl	Euroimmun	PM/Scl-100 <i>E.coli</i>	MPP	LIA
Anti-ENA ProfilPlus with PM-Scl (DA 1590-1003-25 G)	nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, PM/Scl	Euroimmun	PM/Scl-100 <i>E.coli</i>	MPP	LIA
RecomLine Scleroderma (6372)	Scl-70, CENP-B, Scl-34 (Fibrillarlin), PM/Scl-100	Mikrogen	PM/Scl-100 Baculo	MPP	LIA
IMTEC-Myositis-LIA (ITC60200)	Jo1, Mi2, PM-Scl-100, U1-snRNP and Ku70/80	Human	PM/Scl-100 recombinant	MPP	LIA
PMSclpuls DOT (4049)	Jo-1, PL-7, PL-12, Ku, PM/Scl and Scl-70	Generic assays	n.p.	MPP	DB
Whatman® CombiChip Autoimmune	SS-A Ro52+ Ro60, SS-B La, PR3, MPO, dsDNA, Mi-2, CENP-B, U1-70K, Jo-1, Sm, Scl-70, PM/Scl-75c and-100	Whatman®	PM/Scl-100 PM/Scl- 75c recombinant	MMA	Protein Chip

Abbreviations: SPA = single parametric assay; MPP = multi parametric profile; SA = single assay; MMA = multi parametric array; ELISA = enzyme linked immunosorbent assay; LIA = line immunoassay; DB = dot blot; n.p. = not provided.

were tested, autoantibodies against these proteins were mostly found in patients who were also positive for PM/Scl-100 [12]. 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 the ELISA based on PM/Scl-100. In a cohort of 36 PM/Scl patients, 31% were positive for at least one of the two proteins (compared to 25% for PM/Scl-100 alone) [19]. The majority of the anti-PM/Scl reactivity is directed against the PM/Scl-75 and PM/Scl-100 proteins, but many of the other components of the exosome do contribute slightly to the autoantigenicity of the complex (Fig. 1A). The autoantibody reactivity of many exosome associated components has yet to be tested. Therefore, 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.

LIAs can be considered a variant of immuno(dot)blots and as precursor of today's more sophisticated multiplex assays such as protein arrays or ALBIA. Due to their simplicity and ease of implementation, LIAs have become a popular technique for the simultaneous detection of several autoantibody reactivities. Several LIAs that allow for the detection of anti-PM/Scl antibodies are available on the market and the majority include a variety of myositis and/or SSc associated autoantigens. In all commercially available LIAs, the PM/Scl-100 protein is the antigen used to detect anti-PM/Scl reactivity.

In 2000, the prime reactivity of anti-PM/Scl-100 positive sera was localized to amino acids 231–245 of PM/Scl-100. Based on this study, a local alpha-helical structure was proposed as the major PM/Scl-100 epitope [11,33] (Fig. 1B). This epitope, called PM1-Alpha, was used to develop an ELISA to detect anti-PM/Scl antibodies. Reactivity to this peptide was found in 55% of PM/Scl, 13.2% of SSc, 7.5% of PM and 1.7% of unrelated controls, and was in good agreement with recombinant PM/Scl-ELISA data [20]. In a recent international multi-centre study, anti-PM1-Alpha reactivity was found in 7.1% (35/495) of SSc patients without significant differences among the three centres [34]. No statistically relevant correlation could be observed between anti-PM1-Alpha and clinical features such as muscle, skin, heart or lung involvement. The most prevalent clinical features of anti-PM1-Alpha seropositive SSc patients are RP (100%), telangiectasias (52.2%), oesophageal involvement (39.1%) and digital ulcers (39.1%) (unpublished data). Anti-PM1-Alpha antibodies were found in 15.7% of the samples with a nucleolar IIF pattern, very similar to PM/Scl-100 reactivity, which has been reported to be found in 14–26% of sera with nucleolar IIF patterns [34,35]. In another study, sera mainly from SLE patients were reactive with both dsDNA

(tested by CLIFT) and PM/Scl (tested by ID) [23], findings which could only partly be confirmed in a recent study using the PM1-Alpha ELISA (unpublished data).

In 2006, a membrane based protein array (Combi-Chip, Whatman®, New Jersey, USA) with 14 autoantigens became available. This CE-certified assay is the first to implement more than one of the PM/Scl components in a single assay, as it includes both PM/Scl-75c and PM/Scl-100, next to 12 other CTD associated autoantigens.

5. Conclusions

After 30 years of research on anti-PM/Scl antibodies and their relation with clinical symptoms, there are still some important unsolved issues. These include the predictive value of anti-PM/Scl antibodies and the best method to detect anti-PM/Scl antibodies. Although no international standard method has been defined for the detection of anti-PM/Scl antibodies, it is likely that, due to automation of diagnostic assays and an increase in sensitivity and specificity, novel assay systems with highly purified autoantigens in the form of either recombinant proteins or synthetic peptides will become the golden standard for the detection of anti-PM/Scl antibodies.

Take-home messages

- Anti-PM/Scl antibodies are associated with polymyositis/scleroderma overlap syndrome but are also found in other connective tissue diseases.
- Anti-PM-Scl antibodies are strongly linked to HLA-DQA1*0501, HLA-DQB1*02 and HLA-DRB1*0301.
- Traditionally, anti-PM/Scl antibodies were detected by double immunodiffusion (ID) using calf thymus extract. State of the art methods include line immunoassays (LIA), ELISA or autoantigen arrays with (multiple) recombinant proteins.
- A single PM/Scl peptide termed PM1-Alpha can be used as sensitive and specific marker for the detection of anti-PM/Scl antibodies.
- An international reference serum for anti-PM/Scl antibodies is available from the Serology Committee of the IUIS/WHO/Center of Disease control (CDC).
- The precise clinical association of anti-PM/Scl antibodies remains a matter of further research.

References

- [1] von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum* 1995;24: 323–58.

- [2] Arbucl MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349:1526–33.
- [3] Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003;5:80–93.
- [4] Wolfe JF, Adelstein E, Sharp GC. Antinuclear antibody with distinct specificity for polymyositis. *J Clin Invest* 1977;59: 176–8.
- [5] Treadwell EL, Alspaugh MA, Wolfe JF, Sharp GC. Clinical relevance of PM-1 antibody and physicochemical characterization of PM-1 antigen. *J Rheumatol* 1984;11:658–62.
- [6] Reichlin M, Maddison PJ, Targoff I, et al. Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes. *J Clin Immunol* 1984;4:40–4.
- [7] Schilders G, van Dijk E, Raijmakers R, Pruijn GJ. Cell and molecular biology of the exosome: how to make or break an RNA. *Int Rev Cytol* 2006;251:159–208.
- [8] Alderuccio F, Chan EK, Tan EM. Molecular characterization of an autoantigen of PM-Scl in the polymyositis/scleroderma overlap syndrome: a unique and complete human cDNA encoding an apparent 75-kD acidic protein of the nucleolar complex. *J Exp Med* 1991;173:941–52.
- [9] Blüthner M, Bautz FA. Cloning and characterization of the cDNA coding for a polymyositis-scleroderma overlap syndrome-related nucleolar 100-kD protein. *J Exp Med* 1992;176:973–80.
- [10] Ge Q, Frank MB, O'Brien C, Targoff IN. Cloning of a complementary DNA coding for the 100-kD antigenic protein of the PM-Scl autoantigen. *J Clin Invest* 1992;90:559–70.
- [11] Mahler M, Blüthner M, Pollard KM. Advances in B-cell epitope analysis of autoantigens in connective tissue diseases. *Clin Immunol* 2003;107:65–79.
- [12] Brouwer R, Vree Egberts WT, Hengstman GJ, et al. Autoantibodies directed to novel components of the PM/Scl complex, the human exosome. *Arthritis Res* 2002;4:134–8.
- [13] Raijmakers R, Egberts WV, van Venrooij WJ, Pruijn GJ. The association of the human PM/Scl-75 autoantigen with the exosome is dependent on a newly identified N terminus. *J Biol Chem* 2003;278:30698–704.
- [14] Targoff IN, Reichlin M. Nucleolar localization of the PM-Scl antigen. *Arthritis Rheum* 1985;28:226–30.
- [15] Gelpi C, Alguero A, Angeles Martinez M, Vidal S, Juarez C, Rodriguez-Sanchez JL. Identification of protein components reactive with anti-PM/Scl autoantibodies. *Clin Exp Immunol* 1990;81:59–64.
- [16] Reimer G, Scheer U, Peters JM, Tan EM. Immunolocalization and partial characterization of a nucleolar autoantigen (PM-Scl) associated with polymyositis/scleroderma overlap syndromes. *J Immunol* 1986;137:3802–8.
- [17] Oddis CV, Okano Y, Rudert WA, Trucco M, Duquesnoy RJ, Medsger Jr TA. Serum autoantibody to the nucleolar antigen PM-Scl. Clinical and immunogenetic associations. *Arthritis Rheum* 1992;35:1211–7.
- [18] Brouwer R, Hengstman GJ, Vree Egberts W, et al. Autoantibody profiles in the sera of European patients with myositis. *Ann Rheum Dis* 2001;60:116–23.
- [19] Raijmakers R, Renz M, Wiemann C, et al. PM-Scl-75 is the main autoantigen in patients with the polymyositis/scleroderma overlap syndrome. *Arthritis Rheum* 2004;50:565–9.
- [20] Mahler M, Raijmakers R, Dahnrich C, Blüthner M, Fritzler MJ. Clinical evaluation of autoantibodies to a novel PM/Scl peptide antigen. *Arthritis Res Ther* 2005;7:R704–13.
- [21] Selva-O'callaghan A, Labrador-Horrillo M, Solans-Laqué R, Simeon-Aznar CP, Martinez-Gomez X, Vilardell-Tarres M. Myositis-specific and myositis-associated antibodies in a series of eighty-eight Mediterranean patients with idiopathic inflammatory myopathy. *Arthritis Rheum* 2006;55:791–8.
- [22] Vanderghenst F, Ocmant A, Sordet C, et al. Anti-pm/scl antibodies in connective tissue disease: clinical and biological assessment of 14 patients. *Clin Exp Rheumatol* 2006;24: 129–33.
- [23] Warner NZ, Greidinger EL. Patients with antibodies to both PmScl and dsDNA. *J Rheumatol* 2004;31:2169–74.
- [24] Borrows R, Chapel H, Steuer A, Maidment G. Dysphagia associated with anti-PM-Scl antibodies in systemic lupus erythematosus. *Scand J Rheumatol* 2006;35:156–7.
- [25] Beyne-Rauzy O, Fortenfant F, Adoue D. Acquired haemophilia and PM-Scl antibodies. *Rheumatology (Oxford)* 2000;39: 927–8.
- [26] Jablonska S, Blaszyk M. Scleromyositis (scleroderma/polymyositis overlap) is an entity. *J Eur Acad Dermatol Venereol* 2004;18: 265–6.
- [27] Marguerie C, Bunn CC, Copier J, et al. The clinical and immunogenetic features of patients with autoantibodies to the nucleolar antigen PM-Scl. *Medicine* 1992;71:327–36.
- [28] Franceschini F, Cavazzana I, Generali D, et al. Anti-Ku antibodies in connective tissue diseases: clinical and serological evaluation of 14 patients. *J Rheumatol* 2002;29:1393–7.
- [29] Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M. Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. *Arthritis Rheum* 1994;37:75–83.
- [30] Hausmanowa-Petrusewicz I, Kowalska-Oledzka E, Miller FW, et al. Clinical, serologic, and immunogenetic features in Polish patients with idiopathic inflammatory myopathies. *Arthritis Rheum* 1997;40:1257–66.
- [31] Chinoy H, Salway F, Fertig N, et al. In adult onset myositis, the presence of interstitial lung disease and myositis specific/associated antibodies are governed by HLA class II haplotype, rather than by myositis subtype. *Arthritis Res Ther* 2006;8:R13.
- [32] O'Hanlon TP, Carrick DM, Targoff IN, et al. Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. *Medicine (Baltimore)* 2006;85:111–27.
- [33] Blüthner M, Mahler M, Müller DB, Dunzl H, Bautz FA. Identification of an alpha-helical epitope region on the PM/Scl-100 autoantigen with structural homology to a region on the heterochromatin p25beta autoantigen using immobilized overlapping synthetic peptides. *J Mol Med* 2000;78:47–54.
- [34] Mahler M, Koenig M, Sénécal JL, et al. Sero-clinical aspects of autoantibodies to a PM/Scl peptide with special emphasis on a cohort of 495 systemic sclerosis patients. In: Shoenfeld Y, Gershwin ME, editors. *Abstracts of 5th international congress on Autoimmunity* Amsterdam, Boston: Elsevier; 2006. p. 124.
- [35] Van Eenennaam H, Vogelzangs JH, Bisschops L, et al. Autoantibodies against small nucleolar ribonucleoprotein complexes and their clinical associations. *Clin Exp Immunol* 2002;130: 532–40.
- [36] Genth E, Mierau R, Genetzky P, et al. Immunogenetic associations of scleroderma-related antinuclear antibodies. *Arthritis Rheum* 1990;33:657–65.