

Etiopathogenic Role of Surfactant Protein D in the Clinical and Immunological Expression of Primary Sjögren Syndrome

María José Soto-Cárdenas, Myriam Gandía, Pilar Brito-Zerón, Maria Teresa Arias, Noelia Armiger, Albert Bové, Xavier Bosch, Soledad Retamozo, Miriam Akasbi, Marta Pérez-De-Lis, Hoda Gueitasi, Belchin Kostov, Roberto Pérez-Alvarez, Antoni Siso-Almirall, Francisco Lozano, and Manuel Ramos-Casals

ABSTRACT. Objective. To analyze the etiopathogenic role of genetic polymorphisms and serum levels of surfactant protein-D (SP-D) in primary Sjögren syndrome (pSS).

Methods. We analyzed 210 consecutive patients with pSS. *SFTPD* genotyping (M11T polymorphism rs721917) was analyzed by sequence-based typing and serum SP-D by ELISA.

Results. Thirty-two patients (15%) had the Thr11/Thr11 genotype, 80 (38%) the Met11/Met11 genotype, and 96 (46%) the Met11/Thr11 genotype; 2 patients could not be genotyped. Patients carrying the Thr11/Thr11 genotype had a higher prevalence of renal involvement (13% vs 1% and 4% in comparison with patients carrying the other genotypes, $p = 0.014$). Serum SP-D levels were analyzed in 119 patients (mean 733.94 ± 49.88 ng/ml). No significant association was found between serum SP-D levels and the SP-D genotypes. Higher mean values of serum SP-D were observed in patients with severe scintigraphic involvement (851.10 ± 685.69 vs 636.07 ± 315.93 ng/ml, $p = 0.038$), interstitial pulmonary disease (1053.60 ± 852.03 vs 700.36 ± 479.33 ng/ml, $p = 0.029$), renal involvement (1880.64 ± 1842.79 vs 716.42 ± 488.01 ng/ml, $p = 0.002$), leukopenia (899.83 ± 661.71 vs 673.13 ± 465.88 ng/ml, $p = 0.038$), positive anti-Ro/SS-A (927.26 ± 731.29 vs 642.75 ± 377.23 ng/ml, $p = 0.006$), and positive anti-La/SS-B (933.28 ± 689.63 vs 650.41 ± 428.14 ng/ml, $p = 0.007$), while lower mean values of serum SP-D were observed in patients with bronchiectasis (489.49 vs 788.81 ng/ml, $p = 0.019$).

Conclusion. In pSS, high SP-D levels were found in patients with severe glandular involvement, hypergammaglobulinemia, leukopenia, extraglandular manifestations, and positive anti-Ro/La antibodies. The specific association between SP-D levels and pulmonary and renal involvements may have pathophysiological implications. (First Release Nov 1 2014; J Rheumatol 2015;42:111–18; doi:10.3899/jrheum.140394)

Key Indexing Terms:

SJÖGREN SYNDROME

SURFACTANT

INNATE IMMUNITY

Sjögren syndrome (SS) is a systemic autoimmune disease that presents with sicca symptomatology of the main mucosal surfaces¹. The main sicca features (xerophthalmia

and xerostomia) are determined by specific ocular (Rose Bengal staining, Schirmer test) and oral (salivary flow measurement, parotid scintigraphy) tests. The histological

From the Sjögren Syndrome Research Group (AGAUR), Laboratory of Autoimmune Diseases Josep Font, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); Department of Autoimmune Diseases, Department of Internal Medicine, Instituto Clínic de Medicina y Dermatología, Immunology Department, Hospital Clínic; Primary Care Research Group, Centre d'Assistència Primària ABS Les Corts, GESCLINIC; Department of Cell Biology, Immunology and Neurosciences, School of Medicine, University of Barcelona, Barcelona; Group of Immunoreceptors, Department of Internal Medicine, Hospital Infanta Leonor, Madrid; Department of Internal Medicine, Hospital do Meixoeiro, Vigo; Department of Internal Medicine, University of Cadiz, Hospital Puerta del Mar, Cádiz, Spain.

Supported by Grants La Marató de TV3 (071810), Fondo de Investigaciones Sanitarias (080103/1201009), and "Ajut per a la Recerca Josep Font" from Hospital Clínic-Barcelona (PBZ, 2012).

M.J. Soto-Cárdenas, MD, PhD; M. Gandía, MD, PhD, AGAUR, Laboratory of Autoimmune Diseases Josep Font, IDIBAPS, Department of Autoimmune Diseases, and the Department of Internal Medicine, University of Cadiz, Hospital Puerta del Mar; P. Brito-Zerón, MD, PhD;

A. Bové, MD, PhD; S. Retamozo, MD; H. Gueitasi, MD; M. Ramos-Casals, MD, PhD, AGAUR, Laboratory of Autoimmune Diseases Josep Font, IDIBAPS, Department of Autoimmune Diseases; M.T. Arias, BSc, Immunology Department, Hospital Clínic; N. Armiger, BSc, Group of Immunoreceptors, IDIBAPS; X. Bosch, MD, PhD, Department of Internal Medicine, Instituto Clínic de Medicina y Dermatología; M. Akasbi, MD, PhD, Department of Internal Medicine, Hospital Infanta Leonor; M. Pérez-De-Lis, MD, PhD; R. Pérez-Alvarez, MD, PhD, Department of Internal Medicine, Hospital do Meixoeiro; B. Kostov, MSC; A. Siso-Almirall, MD, PhD, Primary Care Research Group, IDIBAPS, Centre d'Assistència Primària ABS Les Corts, GESCLINIC; F. Lozano, MD, PhD, Immunology Department, Hospital Clínic, Barcelona, and the Group of Immunoreceptors, IDIBAPS, and the Department of Cell Biology, Immunology and Neurosciences, School of Medicine, University of Barcelona.

Address correspondence to Dr. M. Ramos-Casals, Servei de Malalties Autoimmunes, Hospital Clínic, C/Villarreal 170, 08036-Barcelona, Spain. E-mail: mramos@clinic.ub.es

Accepted for publication September 12, 2014.

hallmark is focal lymphocytic infiltration of the exocrine glands, determined by biopsy of the minor labial salivary glands². The spectrum of the disease extends from sicca syndrome to systemic involvement (extraglandular manifestations) and may be complicated by the development of lymphoma. Patients with SS present a broad spectrum of analytical features (cytopenias, hypergammaglobulinemia) and autoantibodies, of which antinuclear antibodies are the most frequently detected, anti-Ro/SSA the most specific, and cryoglobulins and hypocomplementemia the main prognostic markers³.

The study of innate immunity has led to an increasing interest in autoimmune diseases. Innate immunity is an ancient and universal form of host defense against invading pathogens, but also an integral part of a broader system responsible for the homeostasis of the internal environment in multicellular organisms. Its main function is to detect non-self or modified-self molecules and eliminate them, whether directly or through the stimulation of an adaptive immune response. To do this, the innate immune system relies on a relatively small number of nonpolymorphic and broadly distributed receptors that have evolved to mainly recognize the so-called pathogen-associated molecular patterns, which are conserved pathogenic structures, essential for pathogen survival, and not shared by the host⁴.

Collectins are pattern-recognition receptors that preferentially bind to carbohydrate moieties expressed on a variety of pathogens, thereby enhancing aggregation, opsonization, or complement activation⁵. Surfactant protein-D (SP-D), a member of the collectin family, is a highly versatile innate immune molecule involved in a range of immune functions, including the neutralization and clearance of microorganisms and downregulation of allergic/inflammatory processes. Its receptor is located in a wide variety of cells, including endothelial cells, B lymphocytes, and antigen-presenting cells. In addition, the SP-D receptor can bind to the major IgG subclasses, suggesting it may play a role as a link between the innate and adaptive immune system⁶. SP-D is primarily synthesized by the respiratory epithelium (alveolar type II cells)⁷, but is also expressed by extrapulmonary epithelia^{8,9,10}. Serum levels of SP-D are genetically regulated, and a common polymorphism in the SP-D gene on chromosome 10 (Met11Thr) has been associated with serum levels and multimerization of SP-D^{11,12}. The Thr11 variant has been associated with reduced oligomerization, reduced binding capacity of microbes, and low serum levels in healthy subjects¹².

The recent detection of SP-D in epithelial cells and in luminal material from the ducts of glandular tissues, including lacrimal and salivary glands¹⁰, might suggest an etiopathogenic role in primary SS (pSS) which, pathogenically, is defined as an "autoimmune epithelitis"¹³. Our study investigated the relationship between genetic polymorphisms and serum levels of SP-D on the one hand, and

clinical and immunological disease expression on the other hand, in a large series of patients with pSS.

MATERIALS AND METHODS

We analyzed 210 consecutive patients who fulfilled the current classification criteria for pSS^{14,15}. In all patients, an exhaustive evaluation was made, discarding other causes of sicca syndrome (coexisting systemic autoimmune diseases, chronic viral infections, metabolic disorders, and preexisting lymphoma). Extraglandular involvement was evaluated according to the 2010 European League Against Rheumatism (EULAR) SS disease activity index at the time of blood extraction¹⁶; pulmonary involvement was defined by the presence of respiratory symptoms associated with altered pulmonary diagnostic tests (pulmonary function tests and/or computed tomography), although the EULAR Sjögren Syndrome Disease Activity Index (ESSDAI) definition also included asymptomatic patients with altered pulmonary imaging and those with persistent respiratory symptoms but normal imaging studies¹⁶. With respect to renal involvement, the ESSDAI includes both tubular and glomerular involvements¹⁶. Salivary scintigraphic results were classified according to the criteria proposed by Schall and Di Chiro¹⁷. Samples of blood donors ethnically matched were used for *SFTPD* genotyping in healthy controls. Clinical and laboratory data were collected and computerized according to our standard department protocol¹⁸. The study was approved by the Ethics Committee of the Hospital Clinic (Barcelona, Spain) and all patients gave informed, written consent. After blood extraction, all patients were followed up with regular visits at 6–12-month intervals, and the main outcomes (cardiovascular disease, infections, neoplasia, and death) were evaluated at the last visit.

SFTPD genotyping. Genomic DNA was extracted from EDTA-treated whole blood samples using the QIAamp DNA blood mini kit following the manufacturer's instructions (QIAGEN GmbH) and stored at –20°C until used. The *SFTPD* rs721917 polymorphism was genotyped using a typing technique based on PCR sequencing. In brief, a 317-bp fragment corresponding to exon 1 was obtained by PCR amplification using the sense 5'-AGC CCT AAA CCA TGT CCA TGA-3' and antisense 5'-AGG AAT GGT CAT TGG AAC TGT-3' primers and GoTaq DNA Polymerase (Promega). The cycling conditions were 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s; and finally, 1 cycle of 72°C for 7 min. Five ml of each PCR were treated with ExoSAP-IT (USB Corp.) and subjected to direct sequencing with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), and the sense and antisense gene-specific primers mentioned above following the manufacturer's instructions. Sequencing reactions were analyzed on an automated capillary DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems).

Determination of serum SP-D levels. Serum samples were available from 119 patients. SP-D was measured by sandwich ELISA using the Human SP-D ELISA kit (Hycult Biotech, Cat No Hk335) following the manufacturer's instructions. All analyses were done in duplicate. The measurement range was between 6.3 and 400 ng/ml. According to the specifications included in the ELISA kit, although the standard measurement range is between 6.3 and 400 ng/ml, basal SP-D levels are highly variable and may range in healthy individuals from 200 ng/ml to 5200 ng/ml.

Statistical analysis. Descriptive data are presented as the mean and SD for continuous variables or number and percentage for categorical variables. Qualitative variables were compared using the chi-square test and Fisher's exact test. Quantitative variables were analyzed with the Student t test. All significance tests were 2-tailed and values of $p < 0.05$ were considered significant. Bonferroni correction was applied for the variables that were statistically significant in the univariate analysis. The statistical analysis was performed using the SPSS program (IBM SPSS Statistics, version 20).

RESULTS

Prevalence and clinical relevance of Met11Thr SP-D

polymorphisms. Of the 210 patients with pSS, 96 (46%) carried the heterozygous Met11/Thr11 genotype, 80 (38%) the homozygous Met11/Met11 genotype, and 32 (16%) the homozygous Thr11/Thr11 genotype; DNA could not be genotyped in 2 patients. Genotype distribution was similar in healthy controls (42%, 40%, and 18% for the Met11/Thr11, Met11/Met11, and Thr11/Thr11 genotypes, respectively).

Analysis of the relationship between Met11Thr SP-D polymorphisms and the main epidemiological, clinical, and immunological characteristics of pSS (Table 1) showed statistically significant associations only for a higher prevalence of renal involvement in patients carrying the homozygous Thr11/Thr11 genotype (13% vs 1% and 4% in patients carrying the Met11/Thr11 and Met11/Met11 genotypes, respectively, $p = 0.014$). A significant trend was found in patients carrying the homozygous Thr11/Thr11 genotype for a lower frequency of vasculitis (0% vs 10% and 6%), and a higher frequency of severe parotid scintigraphy results (60% vs 48% and 46%), interstitial lung disease (ILD; 16% vs 8% and 8%), and positive anti-La antibodies (38% vs 21% and 30%) in comparison with patients carrying the other Met11Thr SP-D genotypes.

Serum SP-D levels and clinical/immunological expression. Serum SP-D levels were analyzed in 119 patients (mean 733.94 ± 49.88 ng/ml, median 588.72 ng/ml). No significant differences were found between serum SP-D levels and the SP-D genotypes (median serum levels of 577.59 ng/ml in Met11/Met11 genotype carriers, 633.49 ng/ml in Met11/Thr11 genotype carriers, and 519.62 ng/ml in Thr11/Thr11 genotype carriers, $p = 0.53$). Patients fulfilling the 2002 criteria¹⁴ for SS showed median higher levels in comparison with those fulfilling the American College of Rheumatology criteria, although the differences were not significant (627.79 ± 70.69 vs 558.32 ± 60 ng/ml, $p = 0.141$).

Table 2 and Table 3 summarize the mean values of serum SP-D according to the presence or absence of the main epidemiological, clinical, and laboratory features at the time of blood extraction. Epidemiologically, no correlation was found between age and SP-D serum levels (Pearson correlation coefficient -0.082 , bilateral p value = 0.377); males had higher mean values than females, although the difference was not significant ($p = 0.192$). Clinically, higher mean values of serum SP-D were observed in patients with severe involvement (grades III/IV) in parotid scintigraphy (851 ± 685.69 vs 636.07 ± 315.93 ng/ml, $p = 0.038$), renal

Table 1. Epidemiological, clinical, and immunological features of patients with pSS according to SP-D genotypes (Met11/Met11, Met11/Thr11, and Thr11/Thr11). Values are n (%) unless otherwise specified.

Characteristics	Met11/Met11, n = 80	Met11/Thr11, n = 96	Thr11/Thr11, n = 32	p
Male sex	5 (6)	7 (7)	1 (3)	0.837
Age, mean \pm SD	54.56 \pm 13.62	56.76 \pm 13.99	60.28 \pm 11.52	0.126
Dry mouth	79 (99)	93 (97)	32 (100)	0.46
Dry eyes	79 (99)	92 (96)	31 (97)	0.51
Altered ocular tests	70 (87)	89 (93)	32 (100)	0.47
Parotid scintigraphy grade III/IV	32/70 (46)	41/85 (48)	15/25 (60)	0.23
Positive salivary gland biopsy	27/39 (69)	33/47 (70)	12/14 (86)	0.46
Parotid enlargement	20 (25)	22 (23)	11 (34)	0.43
Fever	6 (8)	14 (15)	2 (6)	0.216
Arthralgia	49 (61)	50 (52)	21 (65)	0.29
Arthritis	8 (10)	12 (13)	8 (25)	0.22
Raynaud phenomenon	15 (18)	26 (27)	4 (13)	0.16
Vasculitis	5 (6)	10 (10)	0 (0)	0.13
Bronchiectasis	8 (10)	15 (16)	7 (22)	0.244
Pulmonary interstitial disease	6 (8)	8 (8)	5 (16)	0.374
Autoimmune liver disease	5 (6)	11 (11)	5 (16)	0.28
Renal involvement	3 (4)	1 (1)	4 (13)	0.014
Peripheral neuropathy	4 (5)	12 (13)	2 (6)	0.18
CNS involvement	5 (6)	11 (11)	3 (9)	0.49
ESR, mean \pm SD	39.24 \pm 30.53	35.77 \pm 32.27	37.47 \pm 29.54	0.504
Anemia, Hb < 10 g/l	13 (16)	15 (16)	4 (13)	0.880
Leukopenia, < $4 \times 10^9/l$	16 (20)	25 (26)	5 (16)	0.397
Thrombocytopenia, < $100 \times 10^9/l$	2 (2)	9 (9)	1 (3)	0.118
ANA+	64 (80)	86 (90)	28 (88)	0.186
RF+	35/79 (44)	36 (38)	13 (41)	0.660
Anti-Ro/SS-A+	28 (35)	30 (31)	12 (38)	0.769
Anti-La/SS-B+	24 (30)	20 (21)	12 (38)	0.134

pSS: primary Sjögren syndrome; SP-D: surfactant protein-D; CNS: central nervous system; ESR: erythrocyte sedimentation rate; ANA: antinuclear antibody; RF: rheumatoid factor.

Table 2. Epidemiological and clinical features of patients with pSS according to SP-D serum levels.

Characteristics	SP-D levels, ng/ml, mean ± SD	Bilateral p
Sex		0.192
Male	992.07 ± 654.26	
Female	719.98 ± 525.30	
Xerostomia		0.665
Yes	739.42 ± 540.06	
No	603.45 ± 212.32	
Xerophthalmia		0.319
Yes	745.13 ± 540.75	
No	473.13 ± 146.83	
Ocular tests		0.477
Altered	751.05 ± 543.57	
Not altered	525.95 ± 119.06	
Parotid scintigraphy		0.038
Severe dysfunction		
grades III/IV	851.10 ± 685.69	
Grades I/II	636.07 ± 315.93	
Salivary biopsy		0.745
Positive	727.98 ± 526.35	
Negative	675.71 ± 565.14	
Parotid enlargement		0.113
Yes	863.93 ± 534.82	
No	688.92 ± 529.40	
Fever		0.943
Yes	745.33 ± 446.41	
No	734.64 ± 547.67	
Arthralgia		0.954
Yes	738.39 ± 567.01	
No	732.66 ± 491.20	
Arthritis		0.727
Yes	774.27 ± 557.82	
No	728.25 ± 532.02	
Raynaud phenomenon		0.430
Yes	649.48 ± 409.40	
No	753.46 ± 556.24	
Vasculitis		0.657
Yes	801.39 ± 447.94	
No	728.65 ± 544.45	
Pulmonary bronchiectasis		0.019
Yes	489.49 ± 219.74	
No	788.81 ± 566.88	
Pulmonary interstitial disease		0.029
Yes	1053.60 ± 852.03	
No	700.36 ± 479.33	
Renal involvement		0.002*
Yes	1880.64 ± 1842.79	
No	716.42 ± 488.01	
Peripheral neuropathy		0.629
Yes	672.51 ± 441.56	
No	745.38 ± 548.56	
CNS involvement		0.525
Yes	656.26 ± 358.89	
No	740.62 ± 572.02	

* Statistically significant in the Bonferroni correction. pSS: primary Sjögren syndrome; SP-D: surfactant protein-D; CNS: central nervous system.

Table 3. Laboratory and immunological features of patients with pSS according to the SP-D serum levels.

Characteristics	SP-D levels, ng/ml, mean ± SD	Bilateral p
Haemoglobin levels		0.736
< 10 g/l	769.97 ± 495.17	
> 10 g/l	727.84 ± 545.40	
White blood cell count		0.038
< 4 × 10 ⁹ /l	899.83 ± 661.71	
> 4 × 10 ⁹ /l	673.12 ± 465.88	
Platelet count		0.924
< 100 × 10 ⁹ /l	719.45 ± 553.04	
> 100 × 10 ⁹ /l	737.34 ± 535.32	
ANA		0.695
Positive, > 1/40	743.04 ± 559.24	
Negative	683.05 ± 292.86	
RF		0.064
Positive, > 25 UI/l	851.23 ± 677.74	
Negative	663.94 ± 411.43	
Anti-Ro/SS-A		0.006*
Positive	927.26 ± 731.29	
Negative	642.75 ± 377.23	
Anti-La/SS-B		0.007*
Positive	933.28 ± 689.63	
Negative	650.41 ± 428.14	
Monoclonal band		0.778
Positive	776.65 ± 401.17	
Negative	736.48 ± 607.55	
Cryoglobulins		0.563
Positive	817.60 ± 620.96	
Negative	724.02 ± 517.99	
C3 levels		0.442
< 0.82 g/l	828.77 ± 510.82	
> 0.82 g/l	720.52 ± 538.98	
C4 levels		0.246
< 0.11 g/l	536.75 ± 322.26	
> 0.11 g/l	752.29 ± 545.78	

* Statistically significant in the Bonferroni correction. pSS: primary Sjögren syndrome; SP-D: surfactant protein-D; ANA: antinuclear antibody; RF: rheumatoid factor; C3: complement factors.

interstitial involvement (1880.64 ± 1842.79 vs 716.42 ± 488.01 ng/ml, $p = 0.002$), and interstitial pulmonary disease (1053.60 ± 852.03 vs 700.36 ± 479.33 ng/ml, $p = 0.029$) while patients with pulmonary bronchiectasis had lower SP-D levels (489.49 ± 219.74 vs 789 ± 566.88 ng/ml, $p = 0.019$). With respect to laboratory variables, higher SP-D levels were found in patients with leukopenia (899.83 ± 661.71 vs 673.12 ± 465.88 ng/ml, $p = 0.038$); we also found a positive correlation with erythrocyte sedimentation rate values (Pearson coefficient 0.163, $p = 0.076$) and with serum gammaglobulin levels (Pearson coefficient 0.26, $p = 0.005$). We retrospectively analyzed in a subset of patients the correlation between SP-D levels and serum immunological markers, including serum immunoglobulins IgG, IgM, and IgA, C-reactive protein, β_2 -microglobulin, soluble selectin, serum CD5 and CD6, and cytokines [interleukin (IL)-2, IL-6, IL-10, and tumor necrosis factor (TNF)- α]. A

significant correlation was only found for IgG levels ($p = 0.012$) and statistically significant trends with serum CD5 ($p = 0.113$), TNF- α ($p = 0.12$), and soluble selectin ($p = 0.112$) levels. With respect to autoantibodies, higher SP-D levels were found in patients with positive rheumatoid factor (RF; 851.23 ± 677.74 vs 663.94 ± 411.43 ng/ml, $p = 0.064$), anti-Ro/SS-A antibodies (927.26 ± 731.29 vs 642.75 ± 377.23 ng/ml, $p = 0.006$), and anti-La/SS-B antibodies (933.28 ± 689.63 vs 650.41 ± 428.14 ng/ml, $p = 0.007$). After correction for multiple comparisons, SP-D levels correlated with renal involvement and anti-Ro/La antibodies.

Table 4 summarizes the mean values of serum SP-D according to the development of the main adverse outcomes during the followup after blood extraction. Patients who developed hyperuricemia (1133.35 ± 928.96 vs 707.44 ± 481.66 ng/ml, $p = 0.047$) and infections (896.31 ± 736.24 vs 688.93 ± 453.25 ng/ml, $p = 0.076$) had higher serum SP-D levels.

DISCUSSION

To our knowledge, ours is the first study to evaluate the relationship between genetic polymorphisms and serum levels of SP-D in patients with pSS, one of the most prevalent systemic autoimmune diseases. SP-D is involved in innate immune responses and has been investigated in patients with other systemic autoimmune diseases, including systemic sclerosis (SSc), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA)^{19,20,21,22,23}. Our

results showed that SP-D serum levels had a stronger association with disease expression than Met11Thr polymorphisms, suggesting a poor correlation between SP-D polymorphisms and serum levels in pSS.

We found no significant differences in the prevalence of the Met11Thr polymorphisms of the SP-D gene between patients with pSS and controls, whose percentages were also similar to those found in healthy controls included in previous studies in European populations (Met11/Met11 35%, Thr11/Thr11 18%, Met11/Thr11 46%)²³. With respect to the clinical significance of the Met11Thr polymorphisms in patients with pSS, we found no correlation with most epidemiological, clinical, and immunological SS features, except for a higher frequency of renal involvement in patients carrying the Thr11/Thr11 genotype. Other studies in autoimmune diseases have also found a poor correlation between SP-D genotypes and clinical disease expression in patients with RA^{22,23} and SLE²¹. In contrast, some studies have correlated genetic variants with poor outcomes in patients with pulmonary diseases^{24,25}.

The mechanisms involved in the synthesis of SP-D are unknown, but seem to be genetically influenced. In healthy subjects, some studies have shown that the Thr11/Thr11 genotype is associated with SP-D deficiency^{11,12}. In our patients with pSS, we found no significant correlation between genetic polymorphisms and serum SP-D levels. Some authors have suggested that the association with serum levels from a single polymorphism may underestimate the genetic association, which could be represented by haplotype blocks²⁶. In addition, Sorensen, *et al*¹¹ found that serum SP-D is influenced not only by the effect of Met11Thr variations, but also by additive genetic effects and environmental factors while other studies have found that serum SP-D levels increased with male sex, age, smoking status, and physical activity²⁷. In addition, oligomerization of SP-D may be influenced by the Met11Thr polymorphism, and studies have suggested a differentiated role in the immune response of SP-D multimers (antiinflammatory) with respect to SP-D trimers (proinflammatory)^{28,29,30}. It could be hypothesized that, in pSS, genetic polymorphisms may have an influence on the etiopathogenic local epithelial process while serum SP-D levels may reflect the enhanced inflammatory systemic response seen in patients with greater clinical and immunological activity.

The clinical utility of measuring serum SP-D levels is centered on the correlation between high levels and chronic pulmonary diseases or cancer^{31,32,33}. Some studies have also evaluated SP-D levels in patients with autoimmune and rheumatic systemic diseases and have found lower levels of SP-D in patients with SLE (mean of 800 ng/ml)²¹ and RA (mean 693–878 ng/ml)^{22,23} in comparison with healthy subjects, who have a mean of > 900 ng/ml^{21,23}. Our study found lower levels of SP-D in patients with pSS (mean of 734 ng/ml) as reported in patients with SLE and RA. In

Table 4. Cardiovascular risk factors and outcomes of patients with pSS according to the SP-D serum levels.

Characteristics	SP-D levels, ng/ml, mean \pm SD	Bilateral p
Cardiovascular risk factors		0.729
Yes	744.92 \pm 552.41	
No	702.92 \pm 468.69	
Hyperuricemia		0.047
Yes	1133.35 \pm 928.96	
No	707.44 \pm 481.66	
Metabolic syndrome		0.896
Yes	747.62 \pm 466.72	
No	732.40 \pm 555.77	
Cardiovascular disease		0.169
Yes	581.21 \pm 286.81	
No	765.39 \pm 565.54	
Infections		0.076
Yes	896.31 \pm 736.24	
No	688.93 \pm 453.25	
Neoplasia		0.214
Yes	860.82 \pm 691.87	
No	706.08 \pm 488.93	
Death		0.588
Yes	803.64 \pm 513.97	
No	725.48 \pm 539.05	

pSS: primary Sjögren syndrome; SP-D: surfactant protein-D.

contrast, higher SP-D levels have been reported in patients with SSc^{19,20,34,35}, especially those with pulmonary involvement. Therefore, it could be postulated that SP-D levels are higher in autoimmune diseases with a higher frequency of interstitial pulmonary involvement, and lower in diseases in which the majority of patients have no pulmonary inflammatory involvement.

Thus, the rationale for correlating SP-D levels with chronic pulmonary diseases (even those with a different etiology) seems clear because, as mentioned, SP-D is synthesized primarily in the lungs. SP-D has been linked to the maintenance of the equilibrium of the pulmonary immune system, and plays a double role as both an anti-inflammatory agent in basal conditions and as a promoter of inflammation in clearance of pathogens^{6,36,37,38}. It has also been associated with a potential antitumoral effect³⁹ and with other spliced proteins involved in mucosal innate immunity, such as DMBT1⁴⁰. Other immune-related functions of SP-D include cytokine secretion, clearance of apoptotic cells, or reduction of autoantibody generation in mice models^{41,42,43}. An association has been observed between pulmonary diseases and increased serum levels of SP-D, which correlate with disease activity^{44,45} in patients with idiopathic ILD⁴⁶, hepatitis C virus infection treated with interferon⁴⁷, and in patients with SSc, especially those with pulmonary involvement^{19,20,21,36,37,48}. Our results show that, in spite of the low mean levels found in our patients with pSS, those with SS-related ILD had elevated serum levels of SP-D (> 1000 ng/ml) as reported in patients with SSc-related ILD, suggesting that measurement of serum SP-D could be a potentially useful soluble biomarker in clinical practice. Interestingly, we found an inverse correlation between SP-D levels and bronchiectasis, another frequent pulmonary disease in patients with pSS⁴⁹. A possible explanation could be the frequent asymptomatic presentation of bronchiectasis in pSS in contrast to ILD, and also the different etiopathogenic role of SP-D, which has been clearly shown to be involved in the remodeling of an animal model of lung fibrosis⁵⁰.

The finding that SP-D is expressed in other extrapulmonary tissues suggests that it might play a role in the development of autoimmune damage in other organs. Our results show that patients with SS-related renal involvement had higher serum SP-D levels. The presence of SP-D in glomeruli and renal tubules⁷ has been described. A study by Hu, *et al*⁵¹ found that SP-D functions as an antiinflammatory factor in renal tubular epithelial cells and may modulate tubulointerstitial fibrosis in the kidney. Xie, *et al*⁵² correlated serum SP-D levels with the prognosis in patients with chronic renal disease. These studies, together with our findings in pSS, suggest that SP-D could play a role in renal involvement because of its potential proinflammatory role in the tubular epithelial cells.

Gardai, *et al*³⁶ found that SP-D may induce either pro-

inflammatory or antiinflammatory responses, depending on the orientation of the molecule. This dual role of SP-D is important in maintaining homeostasis in the lungs through suppression of inflammation in naive healthy lungs and through induction of inflammation when active clearance of damaging pathogens, allergens, and apoptotic and necrotic cells is required³⁷. In systemic inflammation, it seems that serum SP-D may act as an acute-phase reactant. In systemic autoimmune diseases, it could be hypothesized that high serum SP-D levels may be associated to a nonspecific systemic inflammation (consequence of the immune system activation) or may be closely related to the pathogenesis of the disease. In our study, we found that high serum SP-D levels were more closely associated with B cell hyperactivity (hypergammaglobulinemia, raised IgG levels, and positive RF, anti-Ro, and anti-La antibodies) and less with acute reactant proteins.

The etiopathogenic role of SP-D in pSS seems to be centered on peripheral serum levels, with genetic polymorphisms having little influence. Patients with greater glandular and extraglandular involvement had higher SP-D serum levels, as did patients carrying anti-Ro/La autoantibodies. Serum SP-D levels may have a potential role as a soluble marker of systemic disease activity in patients with pSS, especially those with interstitial pulmonary and renal involvement.

REFERENCES

1. Ramos-Casals M, Brito-Zerón P, Sisó-Almirall A, Bosch X. Primary Sjögren syndrome. *BMJ* 2012;344:e3821.
2. Ramos-Casals M, Font J. Primary Sjögren's syndrome: current and emergent aetiopathogenic concepts. *Rheumatology* 2005;44:1354–67.
3. Ramos-Casals M, Tzioufas AG, Font J. Primary Sjögren's syndrome: new clinical and therapeutic concepts. *Ann Rheum Dis* 2005;64:347–54.
4. Medzhitov R, Janeway CA Jr. Advances in immunology: innate immunity. *N Engl J Med* 2000;343:338–44.
5. Holmskov UL. Collectins and collectin receptors in innate immunity. *APMIS Suppl* 2000;100:1–59.
6. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, et al. Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol Immunol* 2006;43:1293–315.
7. Stahlman MT, Gray ME, Hull WM, Whitsett JA. Immunolocalization of surfactant Protein-D (SP-D) in human fetal, newborn, and adult tissues. *J Histochem Cytochem* 2002;50:651–60.
8. Haagsman HP, van Golde LM. Synthesis and assembly of lung surfactant. *Annu Rev Physiol* 1991;53:441–64.
9. Wright JR, Dobbs LG. Regulation of pulmonary surfactant secretion and clearance. *Annu Rev Physiol* 1991;53:395–414.
10. Madsen J, Kliem A, Tørnøe I, Skjoldt K, Koch C, Holmskov U. Localization of lung surfactant protein D (SP-D) on mucosal surfaces in human tissues. *J Immunol* 2000;164:5866–70.
11. Sørensen GL, Hjelmberg Jv, Kyvik KO, Fenger M, Høj A, Bendixen C, et al. Genetic and environmental influences of surfactant protein D serum levels. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1010–7.
12. Leth-Larsen R, Garred P, Jensenius H, Meschi J, Hartshorn K, Madsen J, et al. A common polymorphism in the SFTPD gene influences assembly, function, and concentration of surfactant

- protein D. *J Immunol* 2005;174:1532-8.
13. Moutsopoulos HM. Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994;72:162-5.
 14. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. *Ann Rheum Dis* 2002;61:554-8.
 15. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res* 2012;64:475-87.
 16. Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T, et al. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). *Ann Rheum Dis* 2014 Mar 11 (E-pub ahead of print).
 17. Schall GL, Di Chiro G. Clinical usefulness of salivary gland scanning. *Semin Nucl Med* 1972;2:270-7.
 18. Ramos-Casals M, Font J, Garcia-Carrasco M, Brito MP, Rosas J, Calvo-Alen J, et al. Primary Sjögren syndrome: hematologic patterns of disease expression. *Medicine* 2002;81:281-92.
 19. Elhaj M, Charles J, Pedroza C, Liu X, Zhou X, Estrada-Y-Martin RM, et al. Can serum surfactant protein D or CC-chemokine ligand 18 predict outcome of interstitial lung disease in patients with early systemic sclerosis? *J Rheumatol* 2013;40:1114-20.
 20. Bonella F, Volpe A, Caramaschi P, Nava C, Ferrari P, Schenk K, et al. Surfactant protein D and KL-6 serum levels in systemic sclerosis: correlation with lung and systemic involvement. *Sarcoidosis Vasc Diffuse Lung Dis* 2011;28:27-33.
 21. Hoegh SV, Voss A, Sorensen GL, Høj A, Bendixen C, Junker P, et al. Circulating surfactant protein D is decreased in systemic lupus erythematosus. *J Rheumatol* 2009;36:2449-53.
 22. Hoegh SV, Lindegaard HM, Sorensen GL, Høj A, Bendixen C, Junker P, et al. Circulating surfactant protein D is decreased in early rheumatoid arthritis: a 1-year prospective study. *Scand J Immunol* 2008;67:71-6.
 23. Christensen AF, Sorensen GL, Hørslev-Petersen K, Holmskov U, Lindegaard HM, Junker K, et al. Circulating surfactant protein-D is low and correlates negatively with systemic inflammation in early, untreated rheumatoid arthritis. *Arthritis Res Ther* 2010;12:R39.
 24. Aramini B, Kim C, Diangelo S, Petersen E, Lederer DJ, Shah L, et al. Donor surfactant protein D (SP-D) polymorphisms are associated with lung transplant outcome. *Am J Transplant* 2013;13:2130-6.
 25. Ishii T, Hagiwara K, Ikeda S, Arai T, Mieno MN, Kumasaka T, et al. Association between genetic variations in surfactant protein d and emphysema, interstitial pneumonia, and lung cancer in a Japanese population. *COPD* 2012;9:409-16.
 26. Heidinger K, König IR, Bohnert A, Kleinsteinber A, Hilgendorff A, Gortner L, et al. Polymorphisms in the human surfactant protein-D (SFTPD) gene: strong evidence that serum levels of surfactant protein-D (SP-D) are genetically influenced. *Immunogenetics* 2005;57:1-7.
 27. Christensen AF, Hoegh SV, Lottenburger T, Holmskov U, Tornøe I, Hørslev-Petersen K, et al. Circadian rhythm and the influence of physical activity on circulating surfactant protein D in early and long-standing rheumatoid arthritis. *Rheumatol Int* 2011;31:1617-23.
 28. Kotecha S, Doull I, Davies P, McKenzie Z, Madsen J, Clark HW, et al. Functional heterogeneity of pulmonary surfactant protein-D in cystic fibrosis. *Biochim Biophys Acta* 2013;1832:2391-400.
 29. Atochina-Vasserman EN. S-nitrosylation of surfactant protein D as a modulator of pulmonary inflammation. *Biochim Biophys Acta* 2012;1820:763-9.
 30. Atochina-Vasserman EN, Beers MF, Gow AJ. Review: chemical and structural modifications of pulmonary collectins and their functional consequences. *Innate Immun* 2010;16:175-82.
 31. Shiels MS, Chaturvedi AK, Katki HA, Gochoico BR, Caporaso NE, Engels EA. Circulating markers of interstitial lung disease and subsequent risk of lung cancer. *Cancer Epidemiol Biomarkers Prev* 2011;20:2262-72.
 32. Barlo NP, van Moersel CH, Ruven HJ, Zanen P, van den Bosch JM, Grutters JC. Surfactant protein-D predicts survival in patients with idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2009;26:155-61.
 33. Bowler RP. Surfactant protein D as a biomarker for chronic obstructive pulmonary disease. *COPD* 2012;9:651-3.
 34. Hant FN, Ludwicka-Bradley A, Wang HJ, Li N, Elashoff R, Tashkin DP, et al. Scleroderma Lung Study Research Group. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol* 2009;36:773-80.
 35. Takahashi H, Kuroki Y, Tanaka H, Saito T, Kurokawa K, Chiba H, et al. Serum levels of surfactant proteins A and D are useful biomarkers for interstitial lung disease in patients with progressive systemic sclerosis. *Am J Respir Crit Care Med* 2000;162:258-63.
 36. Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, et al. By binding SIRP alpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 2003;115:13-23.
 37. Pastva AM, Wright JR, Williams KL. Immunomodulatory roles of surfactant proteins A and D: implications in lung disease. *Proc Am Thorac Soc* 2007;4:252-7.
 38. Schob S, Schicht M, Sel S, Stiller D, Kekulé AS, Paulsen F, et al. The detection of surfactant proteins A, B, C and D in the human brain and their regulation in cerebral infarction, autoimmune conditions and infections of the CNS. *PLoS One* 2013;8:e74412.
 39. Hasegawa Y, Takahashi M, Ariki S, Asakawa D, Tajiri M, Wada Y, et al. Surfactant protein D suppresses lung cancer progression by downregulation of epidermal growth factor signaling. *Oncogene* 2014 Mar 10 (E-pub ahead of print).
 40. Madsen J, Mollenhauer J, Holmskov U. Review: Gp-340/DMBT1 in mucosal innate immunity. *Innate Immun* 2010;16:160-7.
 41. Mahajan L, Pandit H, Madan T, Gautam P, Yadav AK, Warke H, et al. Human surfactant protein D alters oxidative stress and HMGA1 expression to induce p53 apoptotic pathway in eosinophil leukemic cell line. *PLoS One* 2013;8:e85046.
 42. Nayak A, Dodagatta-Marri E, Tsolaki AG, Kishore U. An insight into the diverse roles of surfactant proteins, SP-A and SP-D in innate and adaptive immunity. *Front Immunol* 2012;3:131.
 43. Palaniyar N, Clark H, Nadesalingam J, Shih MJ, Hawgood S, Reid KB. Innate immune collectin surfactant protein D enhances the clearance of DNA by macrophages and minimizes anti-DNA antibody generation. *J Immunol* 2005;174:7352-8.
 44. Takahashi H, Shiratori M, Kanai A, Chiba H, Kuroki Y, Abe S. Monitoring markers of disease activity for interstitial lung diseases with serum surfactant proteins A and D. *Respirology* 2006;11 Suppl:S51-4.
 45. Greene KE, King TE Jr, Kuroki Y, Bucher-Bartelson B, Hunninghake GW, Newman LS, et al. Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. *Eur Respir J* 2002;19:439-46.
 46. Ichiyasu H, Ichikado K, Yamashita A, Iyonaga K, Sakamoto O, Suga M, et al. Pneumocyte biomarkers KL-6 and surfactant protein D reflect the distinct findings of high-resolution computed tomography in nonspecific interstitial pneumonia. *Respiration* 2012;83:190-7.
 47. Ishikawa T, Kubota T, Abe H, Hirose K, Nagashima A, Togashi T, et al. Surfactant protein-D is more useful than Krebs von den Lungen 6 as a marker for the early diagnosis of interstitial

- pneumonitis during pegylated interferon treatment for chronic hepatitis C. *Hepatogastroenterology* 2012;59:2260-3.
48. Asano Y, Ihn H, Yamane K, Yazawa N, Kubo M, Fujimoto M, et al. Clinical significance of surfactant protein D as a serum marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. *Arthritis Rheum* 2001;44:1363-9.
 49. Soto-Cardenas MJ, Perez-De-Lis M, Bove A, Navarro C, Brito-Zeron P, Diaz-Lagares C, et al. Bronchiectasis in primary Sjögren's syndrome: prevalence and clinical significance. *Clin Exp Rheumatol* 2010;28:647-53.
 50. Aono Y, Ledford JG, Mukherjee S, Ogawa H, Nishioka Y, Sone S, et al. Surfactant protein-D regulates effector cell function and fibrotic lung remodeling in response to bleomycin injury. *Am J Respir Crit Care Med* 2012;185:525-36.
 51. Hu F, Liang W, Ren Z, Wang G, Ding G. Surfactant protein D inhibits lipopolysaccharide-induced monocyte chemoattractant protein-1 expression in human renal tubular epithelial cells: implication for tubulointerstitial fibrosis. *Clin Exp Immunol* 2012;167:514-22.
 52. Xie F, Wang X, Ding Z, Fan P, Fan L, Chen Z, et al. Serum surfactant protein D is associated with the prognosis in patients with chronic kidney disease. *J Cardiovasc Med* 2013;14:461-5.