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Endostatin is higher and associated with pulmonary involvement in primary Sjögren's syndrome

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ABSTRACT

Objective. *To investigate serum levels of endostatin in a well characterised cohort of patients with primary Sjögren's syndrome (pSS) and healthy controls (HC) and assess associations between these mediators and clinical parameters.*

Methods. *All patients (n=144) were recruited from the Norwegian systemic connective tissue disease and vasculitis registry (NOSVAR) and fulfilled American-European classification criteria for pSS. Pulmonary involvement was based on clinical symptoms and abnormal findings on high-resolution computed tomography of the lungs. The controls were 100 healthy blood donors. Serum levels of endostatin was determined by enzyme immunoassay.*

Results. *We found higher mean levels of serum endostatin in patients with pSS compared with the controls ($p<0.001$). The patients with interstitial lung disease (ILD) had higher levels compared with those without pulmonary involvement.*

Conclusion. *Our results indicate that endostatin is increased in patients with pSS and particularly in those with ILD.*

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune, connective tissue disease (CTD) of unknown aetiology, most often affecting middle age women. Clinically, the hallmark of pSS is progressive dryness of eyes, mouth and genitalia; caused by chronic inflammatory processes that target and destroy exocrine glands. Non-glandular tissues, including lungs, skin, joints, blood vessels, and the nervous systems are also regularly affected; often with severe clinical consequences. Indeed, it appears that extraglandular features, particularly intestinal lung disease (ILD), are major determinants for morbidity and mortality in pSS (1, 2).

The pathogenesis of pSS is complex and not completely understood, but appears to follow a sequential program with target organ infiltration by immune competent cells, neo-angiogenesis and finally, tissue fibrosis (3-5). The process of neo-angiogenesis in pSS is not fully elucidated, but an altered balance between anti-angiogenic factors, like endostatin and pro-angiogenic mediators seems to be of importance (3, 4, 6-8).

Endostatin is a natural fragment of collagen XVIII and released by tissue-activated fibroblasts (9). Growing evidence indicates that endostatin has effects beyond inhibition of angiogenesis such as potent anti-fibrotic properties (10). These data support that endostatin may represent a link between neo-vascularisation and fibrosis, and provides a rationale for studying endostatin in CTDs and other systemic diseases characterised by persistent inflammation, vasculopathy and fibrotic end-stages.

The available data on endostatin in CTDs are limited, but small-scale studies in systemic sclerosis (SSc) and mixed connective tissue disease (MCTD) have shown elevated serum levels (8, 9, 11-13), associated with distinct fibrotic and/or vasculopathic features, including lung fibrosis, scleroderma renal crisis, or pulmonary hypertension (PH) in SSc (9, 12, 13) and PH or digital ulcera in MCTD (8, 13). There are, however, no data exploring levels of circulating endostatin in patients with pSS.

Based on the potential role of endostatin in the pathogenesis of pSS, we measured serum levels of endostatin in a well characterised cohort of patients with pSS, and tested the possibility that endostatin could be associated with distinct extra-glandular disease features.

Patients and methods

Patient cohort and controls

All pSS patients (n=144) were recruited from The Norwegian systemic connective tissue disease and vasculitis registry (NOSVAR) fulfilling the American-European Consensus Group (AECG) criteria for pSS (14). Cases with antibodies- or clinical findings consistent with other inflammatory rheumatic diseases were excluded. NOSVAR is a research registry, owned and funded by Oslo University Hospital (OUH), managed by the Department of Rheumatology and has no commercial interests or bindings. Registry data include age, gender and items of disease classification criteria, disease duration and complications. The pSS patients in the current study were included in

Table I. Characteristic of the patients with primary Sjögren's syndrome (n=144).

Females n (%)	134	(93)
Age at diagnosis, yrs, mean (SD)	52	(14)
Age at sampling, yrs, mean (SD)	58	(14)
Disease duration yrs, median (25^{th} ,75^{th})	4.5	(1.6, 8.2)
Daily dryness of eyes n (%)	139/143*	(97)
Daily oral dryness n (%)	137/140*	(98)
Schirmer's test ≤ 5mm n (%)	119/138*	(86)
Sialometry, unstimulated ≤ 1.5ml/15 min n (%)	114/131*	(87)
anti-Ro/SSA antibodies, n (%)	119/144	(82)
anti-La/SSB antibodies, n (%)	66/144	(45)
anti-Ro/SSA and anti-La/SSB antibodies, n (%)	66/144	(45)
Histology/biopsy with Focus score ≥1	74/78*	(95)
Death, n (%)	14/144	(9.7)

*Incomplete, due to missing data.

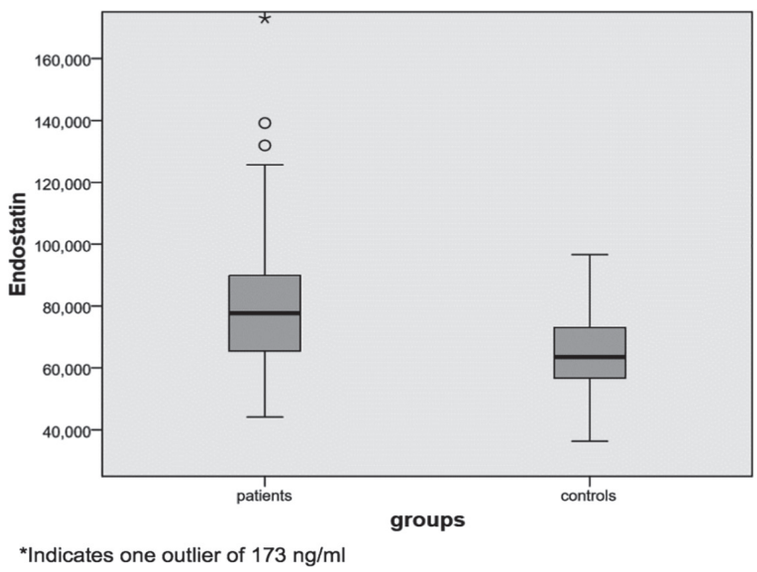

Fig. 1. Endostatinlevels (ng/ml) in serum of patients with pSS (Plot 1) and controls (plot 2).

NOSVAR between 1999 and December 2011 and followed until December 2014 or death. Inclusion in NOSVAR and sampling of serum followed shortly after the pSS diagnosis or at the first appointment in our department. Most patients were subsequently followed up by at least one annual control at our outpatient clinic. Supplementary clinical data were collected by reviewing the patient's hospital records.

In this study, disease duration was defined as time from diagnosis to serum sampling. The clinical parameters represent data assessed cumulatively by the last clinical control. Mortality was registered by linkage of data with the Norwegian National Registry (http://www.skatteetaten.no), which contains information of every Norwegian citizen. For comparison of endostatin levels, serum samples were collected from 100 healthy individuals (51 female, 49 male, mean age 42.8 years) drawn at random from the OUH blood bank.

Pulmonary involvement

Baseline items of pSS registered in the NOSVAR registry include pulmonary symptoms (dry cough or dyspnea at exertion). Patients with suspected pulmonary involvement were further investigated by systemic retrieval of pulmonary function tests and imaging data (2). In the current study, the diagnosis of ILD was based on abnormal lung findings on High-resolution computed tomography (HRCT), including reticular pattern (*i.e.* fibrosis less coarse than honeycombing), lung cysts, ground glass opacities and honeycombing (15). All HRCTs were evaluated in consensus by two experienced radiologists. Abnormal findings were systematically discussed with the rheumatologists at our department.

Quantification of endostatin levels in serum samples of patients and controls

Blood was drawn into pyrogen-free tubes without any additives and allowed to clot in room temperature before centrifugation at 3000*g* for 10 minutes. Serum was then stored in multiple aliquots at -70°C until assayed. Samples were thawed < three times. Circulating endostatin were determined by enzyme immunoassays from R&D Systems (Stillwater, MN, USA). Intra-

Table II. Association between clinical parameters and circulating endostatin in patients with Sjögren's syndrome.

Clinical parameters	Unadjusted					Adjusted*		
	Mean (SD) ng/L Yes	Mean (SD) ng/L No	β	95 % CL	*p*-value	β	95 % CL	*p*-value
Pulmonary involvement	88.9 (25)	76.8 (17)	12.1	5.1,19.0	0.001	10.5	3.7, 17.2	0.003
Raynaud's phenomenon	89.5 (24)	78.9 (19)	10.6	1.4, 19.8	0.024	7.9	-1.0, 16.9	0.082
Palpable purpura	84.4 (23)	80.2 (20)	4.2	-11.1,19.5	0.587	4.2	-10.4, 18.9	0.572
Lymphoma	79.3 (18)	80.5 (20)	-1.1	-14.8,12.5	0.868	0.077	-13.0, 13.1	0.991
Anti-Ro/SSA or Anti-La/SSB antibodies	83.1 (19)	79.8 (20)	-3.2	-12,0, 5.4	0.457	1.024	-7.6, 9.6	0.0816
Death	96.7 (29)	78.7 (18)	18.0	7.3, 28.8	0.037	10.1	-0.87, 21.09	0.071**

*Adjusted for age at sampling. **Adjusted for age at sampling and lung involvement.

and inter-assay coefficients of variation were <10%. All samples were analysed at the same period of time in patients and controls, using identical kits for laboratory analysis.

Statistical analysis

Descriptive data were reported as mean and standard deviation (SD) in normal distributed variables and median with interquartile range in non-normal distribution. Differences in continuous variables between patients and controls were tested with Student's *t*-test for normally distributed variables and the Mann-Whitney U-test for non-normally distributed data. The Chi-square test was used to detect association between categorical variables. Univariate linear regression analysis was performed to study the association between patients and controls and between patients with and without lung involvement, Raynaud's phenomenon, palpable purpura and mortality. Age at sampling and lung involvement was identified as possible confounders and included in multivariable analysis. A two-tailed p-value <0.05 were considered as statistically significant. SPSS, version 21.0 (SPSS) was used for statistical analyses.

Ethics

The registry (NOSVAR) is approved by the Norwegian authorities including the Norwegian Data Inspectorate and by the local ethics committee. Each included patient had given written consent to register in the database, accepteance for use of their registered data, information obtained from their hospital charts and the use of the serum samples.

Results

Out of 216 patients registered in NOSVAR and fulfilling the AECG criteria for pSS (14), 144 (67%) had serum samples available for endostatin analyses and were included in the study (Table I).

The mean (SD) serum levels of endostatin was higher in patients [80.4 ng/L (20)] compared with the healthy controls [65.0 ng/L (12)] (p<0.001) (Fig. 1). The difference remained significant after adjustment for age at sampling (p<0.001).

Pulmonary HRCT abnormalities were identified in 36 patients (25%). The most common pulmonary irregularities were reticular pattern, seen in 14/36 (39%), cysts 9/36 (25%), ground glass attenuation 7/36 (19%) and small nodules 5/36 (14%). Only 2/36 (6%) had end stage lung disease with honeycombing. Mean endostatin levels were higher in patients with ILD compared with those without (p=0.001), also when adjusting for age (p=0.003) (Table II). The presence of Raynaud's phenomenon (p=0.024), but not the presence of lymphoma, palpable purpura or the of anti-Ro/SSA and/or anti-La/SSB antibodies, was associated with higher endostatin levels, but not after adjusting for age (p=0.082) (Table II). During follow-up 14 patients died, and these patients had higher endostatin levels than the other patients (p=0.037), but this association with mortality was not seen when adjusting for age and lung involvement (p=0.071) (Table II). The endostatin levels were higher among the patients with lung involvement who died, compared with those surviving with ILD, but the difference did not reach statistical significance.

Discussion

In this controlled-transversal study, we show that patients with pSS have higher serum levels of endostatin compared with healthy controls, and that elevated endostatin is associated with occurrence of ILD.

The current study is, to the best of our knowledge, the first to assess circulating endostatin levels in pSS. A previous study has shown enhanced endostatin expression in pSS glandular tissues (7). Elevated serum levels of endostatin are not unique to pSS. Comparable findings have been reported in other CTDs, including SSc (8, 9, 12, 13) and MCTD (8, 13) and in idiopathic pulmonary fibrosis (11). Indeed, the observation that endostatin levels are elevated in pSS patients with ILD are in line with previous data showing associations between endostatin and pulmonary involvement in SSc (12).

Based on the possible anti-angiogenic and anti-fibrotic role of endostatin, our findings, may seem somewhat surprising. However, in addition to be a potential inhibitor of angiogenesis and fibrogenesis, endostatin may also be marker of enhanced extracellular matrix and vascular remodeling as a counteracting mechanism, and the high levels may in fact reflect enhanced activation of these pathways (10). Nonetheless, the role of endostatin in the pathogenesis of pSS will have to be further elucidated in forthcoming studies.

Our study has limitations. Serum sampling was not performed at the same time in the disease course in all patients. Hence, this study was not designed to assess possible associations between endostatin and disease duration. We cannot exclude that during disease progression a shift in the balance between the examined markers may occur. Lung HRCT data were only retrieved from pSS patients who reported respiratory symptoms. Obviously, this strategy missed pre-symptomatic ILD cases that would have been detected by systematic screening. Finally, our patients were recruited from a university clinic; with the possibility of selection against milder cases.

In conclusion, we report that serum endostatin is higher in patients with pSS compared with healthy controls. We demonstrated an association between endostatin levels and ILD among the patients. Future studies should elucidate endostatin as a potential marker for pulmonary involvement in pSS.

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