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Elevated serum osteopontin levels in idiopathic retroperitoneal fibrosis

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ABSTRACT

Objective. *To investigate the role of serum osteopontin concentrations for monitoring idiopathic retroperitoneal fibrosis.*

Methods. *In 22 patients with idiopathic retroperitoneal fibrosis serum concentrations of osteopontin were measured by an enzyme-linked immunosorbant assay and related to retrospectively gathered clinical data, contrast enhanced magnetic resonance imaging studies, and laboratory parameters. Patients with secondary causes, an inflammatory abdominal aortic aneurysm, and immunoglobulin G4-associated idiopathic retroperitoneal fibrosis were excluded. Twenty-two healthy volunteers served as controls.*

Results. *Serum osteopontin concentrations of patients with idiopathic retroperitoneal fibrosis were elevated compared to healthy controls ($p=0.017$) and correlated with the transverse diameter of the periaortic cuff as determined by imaging studies ($\varrho=0.549$; $p=0.008$). Patients presenting with a diameter greater than 10mm had higher osteopontin concentrations than patients with smaller diameters ($p=0.004$). Increased inflammatory activity as determined by the presence of contrast enhancement in imaging studies ($p<0.001$) and the presence of typical symptoms ($p=0.013$) were associated with higher osteopontin concentrations.*

Conclusion. *Serum osteopontin concentrations were elevated in patients with idiopathic retroperitoneal fibrosis. Increased concentrations correlated with the presence of clinical symptoms and extended disease or activity parameters on magnetic resonance imaging.*

Introduction

Idiopathic retroperitoneal fibrosis (IRF) is a fibro-inflammatory disease affecting the abdominal aorta, the retroperitoneum, and adjacent structures. Its pathogenesis remains mostly unclear, but is believed to be of autoimmune origin (1-2). Different laboratory parameters such as the erythrocyte sedimentation rate (ESR) and the serum C-reactive protein (CRP) concentrations were evaluated for their suitability in monitoring the course of disease, but none has proved to be generally useful (2-5).

Osteopontin (OPN) is a cytokine which is highly expressed in fibrotic tissue and stimulates fibroblast proliferation and extracellular matrix formation (6). It is widely expressed by a variety of inflammatory cells, including T cells, macrophages, and natural killer cells (7). Moreover, OPN is involved in the reconfiguration of tissue integrity during inflammatory processes by regulating inflammatory cell accumulation and function, macrophage recruitment to sites of inflammation, and collagen deposition (7).

OPN has been implicated in the development of a variety of fibrotic diseases such as idiopathic pulmonary fibrosis and systemic sclerosis (8-10). Furthermore, it has been shown that OPN contributes to fibrogenesis in wound healing and that its inhibition can reduce scarring (11).

We therefore considered OPN a promising candidate and analysed the hitherto unknown value of its serum concentrations for the assessment of disease extent and severity in IRF patients.

Patients and methods

Clinical data

For this retrospective study, the electronic patient records of the University Medical Center Freiburg were searched and patients with a confirmed diagnosis of IRF were identified by evaluation of clinical data and imaging findings. In order to confirm the diagnosis and to rule out disease secondary to an underlying condition (inflammatory abdominal aortic aneurysm, malignancy, documented use of ergot derivates), a comprehensive evaluation of all patients was performed at the time of initial presentation (including detailed history-taking, complete review of all available patient records, thorough physical examination, and laboratory testing). In 4 patients with equivocal imaging findings, the diagnosis was confirmed histologically. Patients with serum IgG4 concentrations above 135mg/dl were classified as IgG4-related (5) and excluded from the study. Past medical history and cardiovascular risk factors (body mass index, diabetes mellitus,

Table I. Serum OPN concentrations in patients and controls.

Variable	Patients (n=22)	Controls (n=22)	*p*-value
Sex	11 females, 11 males	11 females, 11 males	1.000
Age (years)	59 (33-76)	56 (34-88)	0.319
OPN (ng/ml)	339 (67-967)	198 (0-638)	0.017

OPN: osteopontin; data are presented as median (range).

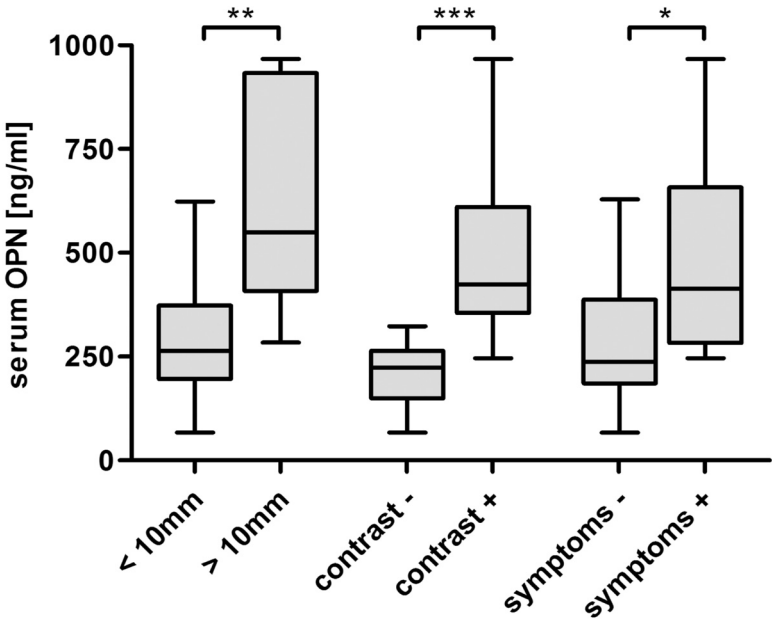

Fig. 1. Serum OPN concentrations in different subgroups. Serum OPN concentrations were different between patients with smaller and larger transverse diameters of the periaortic cuff, between patients with and without contrast enhancement in imaging studies, and between patients with and without typical symptoms of IRF; *p<0.05, **p<0.01, ***p<0.001; OPN: osteopontin, IRF: idiopathic retroperitoneal fibrosis.

hypercholesterolemia, hypertension, and smoking history), symptoms, and laboratory measurements were collected retrospectively from patient charts. The study was carried out in accordance with the Declaration of Helsinki and approved by the local ethics committee.

Imaging studies

Magnetic resonance imaging was performed in all patients using dedicated abdominal coil. In one patient, imaging was performed without contrast enhancement because of renal failure. Spin echo T_1 weighted, transversal slices were obtained before and after administration of a gadolinium-based contrast enhancing agent. Additionally fast spin echo T_2 weighted, transversal and sagittal slices were obtained.

The transverse diameter of the periaortic cuff, its longitudinal extension, and contrast enhancement (compared to the adjacent psoas muscle), the existence of urinary tract obstruction, and vascular involvement (renal or iliac vessels or the inferior vena cava) were evaluated in a blinded manner.

Enzyme-linked immunosorbent assay

At the time of imaging blood was drawn and the serum immediately separated, deep frozen at -20°C, and stored for later examination. Serum OPN concentrations were quantified by a commercially available enzyme-linked immunosorbent assay kit as described by the manufacturer (Abcam, Cambridge, UK). Sera of healthy volunteers served as controls.

Statistical analysis

Data are presented as median (range) unless stated otherwise. The Mann-Whitney U-test was used to analyse the differences between two subgroups. The distribution of clinical characteristics (sex, cardiovascular risk factors, and cardiovascular diseases) among groups was tested with Fisher's exact test. To analyse the relationship between serum OPN concentrations and the transverse diameter of the periaortic cuff Spearman's rank correlation coefficient was calculated. Data analysis was performed using the SPSS software (ver. 17, International Business Machines, New York, United States). The graphs were created using GraphPad Prism (ver. 5, GraphPad Software, California, USA). Bottom and top of the bars in boxplot graphs represent the 25th and 75th percentile, respectively. The whiskers represent minimum and maximum, the line within each bar denotes the median. All reported p-values are two-sided, p<0.05 was considered statistically significant.

Results

The sera of 22 patients with IRF, treated at the Department of Rheumatology and Clinical Immunology of the University Medical Center Freiburg between 2000 and 2010, were included. At the time of examination the patients had a median disease duration of 12 months (range 0–104) and 18 of them (82%) were receiving immunosuppressive treatment (glucocorticoids, and/or cyclophosphamide, azathioprine, mycophenolic acid, cyclosporine). Serum OPN concentrations were higher in IRF patients than in controls (Table I). There was no correlation between disease duration and serum OPN concentrations (n=22; ϱ=-0.149; p=0.507).

The 6 patients with a transverse diameter of the periaortic cuff of more than 10mm had higher serum OPN concentrations (n=6; median 548ng/ml, range 284–967) than the 16 patients who had a diameter of less than 10mm (n=16; median 264ng/ml, range 67-624; p=0.004; Fig. 1). A positive correlation between the serum OPN concentrations and the diameter was observed (n=22; ϱ=0.549; p=0.008), while serum CRP concentrations (p=0.200) and ESR (p=0.880) did not correlate.

Serum OPN concentrations were increased in patients with contrast enhancement of the periaoritc cuff in imaging studies (n=12; median 424ng/ml,

range 246–967) compared to those patients without (n=9; median 224ng/ml, range 67–323; $p<0.001$; Fig. 1).

Twelve patients had no typical clinical signs and symptoms of IRF (flank/back/abdominal pain, fever, night sweats, or weight loss) while 10 had one or more of these symptoms at the time of examination. Comparing the serum OPN concentrations of asymptomatic (n=12; median 237ng/ml, range 67–629) to those of symptomatic patients (n=10; median 414ng/ml, range 246–967), the concentrations were higher in the latter ($p=0.031$; Fig. 1). Table II shows the results of these subgroup analyses in comparison to those of serum CRP concentrations and ESR.

Both the distribution of patients receiving immunosuppressive/glucocorticoid treatment and the treatment duration were similar between the subgroups. The patients with a diameter of less than 10mm received significantly lower doses of glucocorticoids than the patients with a greater diameter, while the glucocorticoid doses in the other subgroups were similar (Table III).

The exact number of smoked pack years (py) was known in 21 of 22 patients: The 13 patients who smoked more than 20py (n=13; median 40py, range 20–70; median 356ng/ml, range 67–967) did not have significantly different serum OPN concentrations than the 8 patients who smoked less (n=8; median 6py, range 0–15; median 303ng/ml, range 113–468; $p=0.607$). Likewise, there was no significant difference in serum OPN concentrations between patients with and without cardiovascular risk factors (data not shown, all p-values >0.1). The distribution of these risk factors and the prevalence of cardiovascular diseases were not significantly different between the aforementioned subgroups (data not shown, $p>0.05$ for all comparisons).

Table II. Laboratory findings in the subgroups.

Variable	Diameter <10mm	Diameter >10mm	p-value
OPN (ng/ml)	264 (67-624); n=16	548 (284-967); n=6	0.004
CRP (mg/l)	5 (0-23); n=14	7 (0-155); n=6	0.449
ESR (mm/h)	15 (1-45); n=9	18 (10-22); n=3	1.000
	No contrast enhancement	Contrast enhancement	
OPN (ng/ml)	224 (67-323); n=9	424 (246-967); n=12	<0.001
CRP (mg/l)	3 (0-23); n=7	8 (0-155); n=12	0.299
ESR (mm/h)	18 (3-26); n=6	11 (1-45); n=5	0.792
	Asymptomatic	Symptomatic	
OPN (ng/ml)	237 (67-629); n=12	414 (246-967); n=10	0.031
CRP (mg/l)	4 (0-23); n=10	5 (0-155); n=10	0.665
ESR (mm/h)	21 (9-26); n=5	11 (1-45); n=7	0.432

OPN: osteopontin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; data are presented as median (range).

Table III. Distribution of immunosuppressive drugs and treatment duration in the subgroups.

Patients	Diameter <10mm	Diameter >10mm	p-value
Any treatment (yes/no)	14 / 2	4 / 2	0.292
Treatment duration (months)	9.5 (1-104); n=14	29 (10-45); n=4	0.181
Glucocorticoids (yes/no)	11 / 5	4 / 2	1.000
Treatment duration (months)	9 (1-104); n=11	29 (10-45); n=4	0.237
Dosage (mg/day per os)	5 (2.5-40); n=11	12.5 (10-50); n=4	0.004
	No contrast enhancement	Contrast enhancement	
Any treatment (yes/no)	8 / 1	9 / 3	0.603
Treatment duration (months)	14 (1-104); n=8	26 (1-53); n=9	0.980
Glucocorticoids (yes/no)	7 / 2	7 / 5	0.642
Treatment duration (months)	10 (1-104); n=7	26 (1-53); n=7	1.000
Dosage (mg/day per os)	5 (5-7.5); n=7	10 (2.5-50); n=7	0.278
	Asymptomatic	Symptomatic	
Any treatment (yes/no)	10 / 2	8 / 2	1.000
Treatment duration (months)	13.5 (1-104); n=10	18 (1-53); n=8	0.745
Glucocorticoids (yes/no)	8 / 4	7 / 3	1.000
Treatment duration (months)	13.5 (1-104); n=8	10 (1-53); n=7	0.976
Dosage (mg/day per os)	5 (5-50); n=8	10 (2.5-40); n=7	0.574

Glucocorticoid dosage given as prednisone equivalent; data are presented as median (range).

Discussion

In addition to imaging studies, serum biomarkers as surrogate markers for disease extent and activity would be desirable in IRF patients. In several reports, ESR and CRP had only limited success in reflecting the course of disease and its severity or were only useful in a particular subset of patients (2-5). Therefore, additional markers are needed. Since OPN plays an important role in other fibrotic diseases (8-10), we examined its serum concentrations in this cohort of IRF patients.

This is the first study showing that serum OPN concentrations are elevated in IRF patients compared to healthy controls. The serum OPN concentrations correlated with the extent of the fibrotic mass and were particularly high in patients with extensive periaortic fibrosis (>10mm in diameter). Furthermore, the presence of contrast enhancement in imaging studies, indicating active inflammation in the fibrotic tissue (12), and typical symptoms, were associated with high serum OPN concentrations.

Since there are ambivalent data on glucocorticoid treatment and OPN synthesis (13-14), we analysed the differences between treatment regimens in the subgroups in detail. Only patients with a perioartic cuff of more than 10mm received higher doses of glucocorticoids.

In the 4 patients in that subgroup the increased glucocorticoid doses could be explained by higher disease activity (2 patients with new onset ureteral involvement and 2 patients with worsening imaging findings and progressive symptoms), although we cannot clearly rule out effects of glucocorticoid treatment *per se*.

OPN is known to play a critical role in both inflammatory processes (15) and fibrogenesis (10). Since we observed a correlation not only with the extent of the periaortic fibrotic mass, but also with contrast enhancement in imaging studies, both mechanisms might be reflected by elevated OPN serum concentrations in IRF.

Our data support previous observations that ESR and CRP were of minor value in the disease assessment of IRF (2-5), since they were less likely to reflect disease characteristics of IRF.

Cardiovascular risk factors and diseases might confound results since they are both common among IRF patients and can be associated with elevated serum OPN concentrations (16). However, the serum OPN concentrations of patients with a high prevalence of cardiovascular risk factors and diseases (16) were considerably lower than the concentrations we observed. Additionally, there were no differences in serum OPN concentrations between IRF patients with and those without cardiovascular risk factors and there was a similar distribution of these risk factors among the subgroups (diameter <10mm *vs*. >10mm; contrast enhancement *vs*. no contrast enhancement; asymptomatic *vs*. symptomatic). These data suggest that the elevated serum OPN concentrations are likely to be related to the underlying IRF.

OPN plays a pivotal role in inflammation and fibrogenesis and it was implicated in the development of smoking-associated interstitial lung diseases (10). Albeit smoking more than 20py is known to be a risk factor for the development of IRF (17) and the majority of IRF patients has a smoking history (18), we could not observe any associations between serum OPN concentrations and smoking behavior in this study (smokers *vs*. non-smokers, <20py *vs*. >20py).

Our analysis is limited by the small number of patients and therefore needs to be interpreted with caution. Nevertheless, the data suggest a direct relationship between serum OPN concentrations, MRI findings, and clinical IRF manifestations. However, more powerful trials will be needed to confirm OPN as a biomarker in IRF patients.

In conclusion, serum OPN concentrations were elevated in IRF patients and reflected both clinical and imaging findings. Future investigations are necessary to prove the suitability of OPN for disease monitoring in IRF patients.

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