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SHORT COMMUNICATION

CXCL4 in undifferentiated connective tissue disease at risk for systemic sclerosis (SSc) (previously referred to as very early SSc)

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Abstract The aim of the study was to evaluate CXCL4 levels in undifferentiated connective tissue disease at risk for SSc (UCTD-SSc-risk) and confirm its increase and investigate its prognostic value. Serum CXCL4 levels were measured in 45 patients and 24 controls. CXCL4 was significantly higher in UCTD-SSc-risk patients than in controls. It resulted higher in patients with a shorter disease duration and in those lacking capillaroscopic alterations. We confirm that CXCL4 levels are increased in UCTD-risk-SSc patients. Further studies are needed to investigate the role of CXCL4 assessment in UCTD-risk-SSc.

Keywords Systemic sclerosis · Undifferentiated connective tissue disease

Introduction

Very early (or early) SSc is a condition characterized by Raynaud's phenomenon (RP) plus SSc marker autoantibodies and/or distinct capillaroscopic alterations [1, 2]. It carries a significant, but not absolute risk of evolution into definite SSc [3, 4]. Because of this prognostic evidence as well as to avoid any nosographic confusion with early definite SSc or early diffuse SSc [5–7], it should be properly referred to as undifferentiated connective tissue disease (UCTD) at risk for SSc (UCTD-SSc-risk) [8].

Van Bon et al. [9] recently reported increased platelet factor 4 (CXCL4) levels in both the plasma and skin of patients with systemic sclerosis (SSc) in whom they resulted to be associated with distinct clinical features and to predict a poorer prognosis. They also pointed out increased values in patients with very early SSc, but did not investigate any association between them.

The aim of this study is to confirm increased CXCL4 levels in patients with strictly defined UCTD-SSc-risk and to evaluate whether this finding is associated with any disease feature and/or is predictive of evolution into definite disease [3, 4].

Materials and methods

Forty-five patients with UCTD-SSc-risk consecutively admitted from November 1st 2000 to December 31st 2013 were enrolled in the study after giving a written informed consent.

Classification

UCTD-SSc-risk was diagnosed in patients with RP and either SSc marker autoantibodies or distinct capillaroscopic alterations or both, who at admission neither satisfied the 2013 ACR/EULAR criteria for SSc [10] nor presented any manifestation indicative of SSc sine scleroderma [11].

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Assessment at admission

Patients underwent a detailed history, an accurate physical examination, routine laboratory investigations, nailfold capillaroscopy, autoantibodies detection and investigation of preclinical organ involvement as already described [4].

Subsetting

Patients were subdivided into 3 subsets [12]: subset I (patients with both SSc marker autoantibodies and capillaroscopic abnormalities), subset II (patients with only marker autoantibodies, i.e., anti-centromere or anti-DNA topoisomerase I or anti-RNA polymerase III or anti-Fibrillarin or anti-Th/To or anti-Pm-Scl), and subset III (patients with only capillaroscopic abnormalities, i.e., megacapillaries and/or avascular areas) (Table 1).

Serum marker quantification

To avoid any influence of platelet-derived chemokines [13], we measured CXCL4 levels in the serum of 45 patients at admission and in 24 osteoarthritis/fibromyalgia control subjects matched for sex and age. Peripheral blood was obtained at baseline by venipuncture. Serum was separated by centrifugation at 1500*g* for 10 min, aliquoted and stored at −20 °C. CXCL4 levels were measured by a suspension fluorescence-based immunoassay (Merk Millipore, Billerica, MA, USA) using a Luminex 200 instrument (Luminex Corporation, Austin, TX, USA). Serum samples were diluted 1:40,000, and final concentrations of CXCL4 were expressed as ng/ml. No patient was under glucocorticoid and/or immunosuppressive treatment at the time of blood drawing.

Follow-up

After enrollment, patients were re-evaluated at yearly intervals, up to December 31, 2015, by history taking, clinical examination, EKG, B-mode-echocardiography, esophageal barium X-ray, lung function testing and lung high-resolution tomography to assess whether and when each of them satisfied the ACR/EULAR classification criteria disease score $\geq$9) [10] and/or criteria for SSc sine scleroderma [11].

Statistical analysis

We used the SPSS for Windows software (version 16.0) for statistical analysis. Categorical data were analyzed by Chi-square test. Continuous data were compared by the Mann–Whitney *U* test. A p value <0.05 was considered significant. Risk was assessed by assessing hazard ratios in univariate and step-wise regression analysis.

Informed consent

Informed consent was obtained from all individual participants included in the study.

The study was approved by the Ethics Committee.

Results

Serum CXCL4 was significantly higher in the 45 UCTD-SSc-risk patients [median 4.84 ng/ml; interquartile range (1.83–10.82)] than in controls [median 1.43 ng/ml; interquartile range (0.21–2.70), $p = 0.0001$]. In detail, serum CXCL4 resulted to be significantly higher in 28 subset I [median 4.74 ng/ml; interquartile range (1.67–10.18), $p = 0.0012$] and in 13 subset II patients [median 7.0 ng/ml; interquartile range (2.9–16.8), $p = 0.0002$] than in controls, while no difference was detected between 4 subset III patients [median 2.32 ng/ml; interquartile range (1.7–3.3)] and the 24 controls. Moreover, serum CXCL4 levels were almost significantly higher in 13 subset II patients (i.e., those lacking microcirculatory abnormalities as detected by nailfold videocapillaroscopy) than in 32 subset I + III patients ($p = 0.07$).

No other association was detected between CXCL4 levels and either disease duration or autoantibody specificity or presence of any (esophageal and/or cardiac and/or lung) preclinical internal organ involvement.

At December 31, 2015, 4 patients had been lost to follow-up, 1 patient died (pulmonary embolism). The remaining 40 had been monitored for 6–101.4 months (median 25.4 months; interquartile range 12.4–47.4; 19.04 patient/years follow-up). At the study closure, 26/40 (65 %) patients had evolved into definite SSc (at 6–101.4 months from enrollment into the study; median 24.35 months interquartile range 8–41.5). Of these, 9 only had CXCL4 values at baseline >95th percentile of the values recorded in controls (8.30 ng/ml). Nevertheless, 2 out of the 3 patients with the highest values (Fig. 1) had evolved into definite SSc.

Figure 2 shows the receiver operator curve devoted to identify the performance of baseline serum CXCL4 in predicting the evolution into definite SSc. A baseline CXCL4 value >8.3 ng/ml was found to identify patients evolved into definite SSc during follow-up with a 30 % sensitivity and 86 % specificity (AUC 0.69).

Discussion

CXCL4 is a 7.8-kDa protein that comprises from 2 to 3 % of the protein content of activated platelets and is considered one of the most anti-angiogenic chemokines [13].

Table 1 Demographic, serological, capillaroscopic features and functional abnormalities indicative of preclinical internal organ involvement in 45 patients divided into 3 subsets

Features	Whole cohort	Subset I	Subset II	Subset III
Sex (F/M)	43/2	27/1	12/1	4/0
Age (years: median; range)	42 (37–48.5)	41 (34–45)	42 (39.2–50.2)	56 (47–62)
Disease duration from RP (years: median; range)	2 (1–5.25)	3 (2–6)	2 (1–4.2)	2.5 (1.5–17)
Marker autoantibody positivity	41	28	13	0
Anti-centromere	30 (66.6 %)	21/28 (75 %)	9/13 (69.23 %)	
Anti-DNA topoisomerase I	10/45 (22.2 %)	6/28 (21.4 %)	4/13 (30.7 %)	
Anti-PM-Scl	1/45 (2.2 %)	1/28 (3.5 %)		
Capillaroscopic alterations	32	28	0	4
Megacapillaries	28/32 (87.5 %)	24/28 (85.7 %)		4/4 (100 %)
Avascular areas	4/32 (12.5 %)	4/28 (14.2 %)		0
DLCO <80 % of the predicted value	17/45 (37.8 %)	8/28 (28.5 %)	6/13 (46.1 %)	3/4 (75 %)
E/A < 1[a]	0	0	0	0
Low esophageal sphincter pressure <15 mm Hg	8/29 (27.6 %)	5/18 (27.7 %)	2/9 (22.2 %)	1/2 (50 %)

RP Raynaud's phenomenon, *DLCO* diffusing lung capacity for carbon monoxide, *E/A* early/atrial ratio

[a] In patients aged <50 years, with an arterial blood pressure <130/85 mmHg

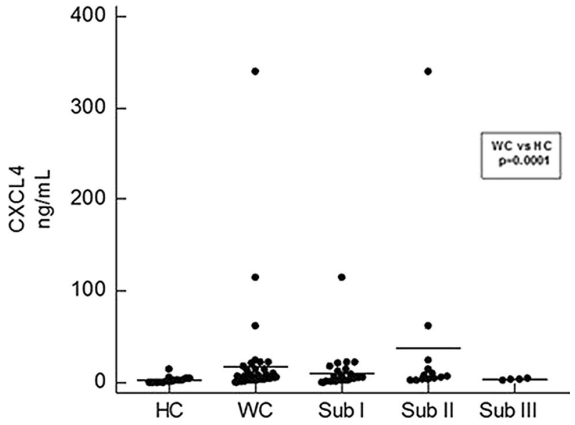

Fig. 1 CXCL4 levels in healthy controls (HC), in the whole cohort (WC) of UCTD-SSc-risk patients

In their pivotal paper, van Bon et al. [9] reported a correlation between CXCL4 levels and the extent of skin fibrosis in patients affected by SSc. Moreover, they found higher CXCL4 levels were associated with lung fibrosis and pulmonary arterial hypertension and resulted to be predictive of faster decline in DLCO, faster progression of skin fibrosis and higher incidence of lung fibrosis in 79 SSc patients followed up for 18 months. These authors also mentioned increased CXCL4 values in "very early" SSc, but did not investigate either the presence of associations or the prognostic value of the finding.

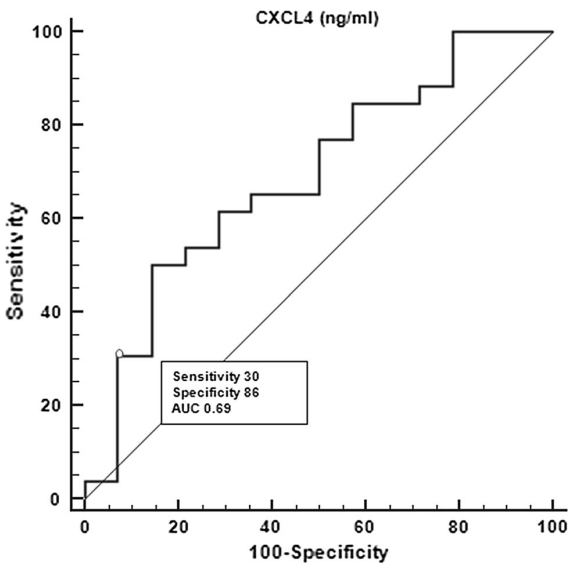

Fig. 2 Receiver operator curve showing the relationship between baseline serum CXCL4 and evolution into definite SSc

We undertook the present study in order to address these aspects. Unlike van Bon et al. [1], who assessed CXCL4 levels in plasma, we investigated the chemokine levels in the serum, because increased levels of chemokine in plasma can depend on its release from platelets [14]. Since platelet activation occurs in SSc, we opted to assess CXCL4 levels in the serum in order to better evaluate the

amount of the circulating chemokine probably secreted by plasmocytoid dendritic cells, which constitute its main source in SSc [9].

Despite the different origin of the samples used, we confirm the original report by van Bon et al. [9] that CXCL4 levels are higher in UCTD-risk-SSc patients, who had been referred by them as "very early SSc."

We investigated the presence of associations between CXCL4 levels and disease duration from the onset of RP, disease subsetting, autoantibody specificity, preclinical internal organ involvement as well as the value as a prognostic marker of evolution into definite SSc.

We found that CXCL4 levels are almost significantly higher in patients lacking microcirculatory abnormalities as detected by nailfold videocapillaroscopy. These associations should be evaluated further in a larger series of patients also assessed for other factors influencing angiogenesis in SSc, for example vascular endothelial growth factor (VEGF). Notably, megacapillaries, which were the only microvascular abnormality in most our patients (28/45), may represent a compensatory mechanism, promoted by VEGF that might be inhibited by CXCL4 [15].

As far as CXCL4 prognostic value is concerned, ROC analysis pointed out a significant specificity, suggesting a potential role of its evaluation in the assessment of the UCTD-risk-SSc patient at baseline. Such a role is, however, challenged by its low sensitivity.

The prognostic value of CXCL4 in definite SSc pointed out by van Bon et al. [9] has been recently challenged by Volkmann et al. [16] who investigated patients enrolled into Scleroderma Lung Study 2. This aspect, therefore, also awaits to be clarified in definite SSc.

In conclusion, we confirmed that increased CXCL4 levels are detected in UCTD-SSc-risk patients. Further studies on a larger, possibly multicentric series, are needed to assess the value of CXCL4 and other biomarkers in predicting the evolution in UCTD-SSc-risk. At present, predicting prognosis in SSc relies on the definition of the subset [3, 4] and the presence of preclinical organ involvement at admission [4].

Compliance with ethical standards

Conflict of interest None.

References

1. Avouac J, Fransen J, Walker UA, et al. EUSTAR Group. Preliminary criteria for the very early diagnosis of systemic sclerosis: results of a Delphi Consensus Study from EULAR Scleroderma Trials and Research Group. Ann Rheum Dis. 2011;70:476–81.
2. Matucci-Cerinic M, Bellando-Randone S, Lepri G, Bruni C, Guiducci S. Very early versus early disease: the evolving definition of the 'many faces' of systemic sclerosis. Ann Rheum Dis. 2013;72:319–21.
3. Koenig M, Joyal F, Fritzler MJ, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients with validation of proposed criteria for early systemic sclerosis. Arthritis Rheum. 2008;58:3902–12.
4. Valentini G, Marcoccia A, Cuomo G, et al. Early systemic sclerosis: analysis of the disease course in patients with marker autoantibodies or capillaroscopic positivity or both. Arthritis Care Res. 2014;66:1520–7.
5. Wuttge DM, Lood C, Tufvesson E, et al. Increased serum type I interferon activity in early systemic sclerosis is associated with antibodies against Sjögren's syndrome antigens and nuclear ribonucleoprotein antigens. Scand J Rheumatol. 2013;42:235–40.
6. Camargo CZ, Sekiyama JY, Arismendi MI, Kayser C. Microvascular abnormalities in patients with early systemic sclerosis: less severe morphological changes than in patients with definite disease. Scand J Rheumatol. 2015;44:48–55.
7. Frech TM, Murtaugh M, Gordon JK et al. Longitudinal assessment of gastrointestinal symptoms in the prospective registry of early systemic sclerosis cohort [abstract]. Arthritis Rheumatol. 2015; 67 suppl 10.
8. Valentini G. Undifferentiated connective tissue disease at risk for systemic sclerosis (SSc) (so far referred to as very early/early SSc or pre-SSc). Autoimmun Rev. 2015;14:210–3.
9. van Bon L, Affandi AJ, Broen J, et al. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N Engl J Med. 2014;370:433–43.
10. Van den Hoogen F, Khanna D, Fransen J, et al. Classification criteria for systemic sclerosis: an ACR-EULAR collaborative initiative. Arthritis Rheum. 2013;65:2737–47.
11. Poormoghim H, Lucas M, Fertig N, Medsger TA Jr. Systemic sclerosis sine scleroderma: demographic, clinical, and serologic features and survival in forty-eight patients. Arthritis Rheum. 2000;43:444–51.
12. Valentini G, Marcoccia A, Cuomo G, et al. Early systemic sclerosis: marker autoantibodies and videocapillaroscopy patterns are each associated with distinct clinical, functional and cellular activation markers. Arthritis Res Ther. 2013;15:R63.
13. Aidoudi S, Bujakowska K, Kieffer N. Bikfalvi A The CXC-chemokine CXCL4 interacts with integrins implicated in angiogenesis. PLoS ONE. 2008;3:e2657.
14. Wiesner T, Bugl S, Mayer F, Hartmann JT, Kopp H-G. Differential changes in platelet VEGF, Tsp, CXCL12, and CXCL4 in patients with metastatic cancer. Clin Exp Metastasis. 2010;27:141–9.
15. Distler JHW, Gay S, Distler O. Angiogenesis and vasculogenesis in systemic sclerosis. Rheumatology. 2006;45:iii26–7.
16. Volkmann ER, Tashkin DP, Roth M and Scleroderma Lung Study II Group. CXCL4 does not predict extent or progression of interstitial lung disease in systemic sclerosis [abstract]. Arthritis Rheumatol. 2015; 67 suppl 10.