Serum CXCL13 Chemokine is Not a Marker for Active Lyme Borreliosis

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The chemokine CXCL13 has been identified as a diagnostic marker for early Lyme neuroborreliosis in cerebrospinal fluid (CSF) but not in serum (1–4). However, serum CXCL13 levels have been shown to be elevated in autoimmune diseases, like lupus erythematosus (5) and dermatomyositis (6).

Patients presenting to the dermatology department with various chronic or recurrent clinical complaints may have high levels of anti-*Borrelia burgdorferi (Bb)* antibodies. As persistent infection of tissues with *Bb* is characterized by infiltration with T- and B-lymphocytes and plasma cells, leading to continuous immune activation, we investigated whether the B-cell chemokine CXCL13 could be a marker for active borrelial infection requiring treatment (4, 7).

The aim of this study was to assess whether CXCL13 serum levels are elevated in patients with classical Lyme borreliosis (LB) and in those with persisting positive Lyme serology, compared with healthy controls and patients with dermatological diseases other than LB.

MATERIALS AND METHODS

We prospectively analysed 80 patients with positive anti-*Bb* immunoglobulin M (IgM)- and/or IgG antibodies and various subjective symptoms of mean duration 13.5 months (median 4.5 months) and defined them as "seropositives". The symptoms were documented by a questionnaire (wandering arthralgias, myalgias or joint swelling, history of tick bite and erythema migrans (EM), flu-like symptoms, non-specific and distressing symptoms, such as tinnitus, hearing loss, difficulties concentrating, burning sensations and fatigue). In 29 of these 80 patients, all of whom had confirmed positive IgG immunoblots (IB) and clinical history and complaints compatible with LB (8), treatment with doxycycline 200 mg for 30 days or intravenous ceftriaxone 2 g for 20 days was performed. Nine of the 29 patients responded well to antibiotic therapy, with considerable regression of the symptoms and were classified retrospectively as confirmed LB requiring treatment.

In addition, we studied 53 patients with classical LB: 13 with physician-diagnosed EM, 10 with borrelia lymphocytoma (BL), and 30 with acrodermatitis chronica atrophicans (ACA) before antibiotic therapy. Ninety-seven patients with various dermatological diagnoses were also analysed, in addition to 300 blood donors. The study was approved by the ethics committee of the Medical University of Graz.

All patients were examined for Borrelia antibodies by enzyme-linked immunoassay (ELISA) (IDEIA *Borrelia burgdorferi* IgM/IgG OXOID, Cambridgeshire, UK) confirmed by IB (Mikrogen *recom*Blot BorreliaNB IgG and IgM Mikrogen, Neuried, Germany). CXCL13 was measured by ELISA (Quantikine Human CXCL13/BLC/BCA-1 Immunoassay, R&D Systems, GmbH. Wiesbaden, Germany) (1, 2). Statistical analysis was performed with the statistical software package SPSS, Version 16.0.

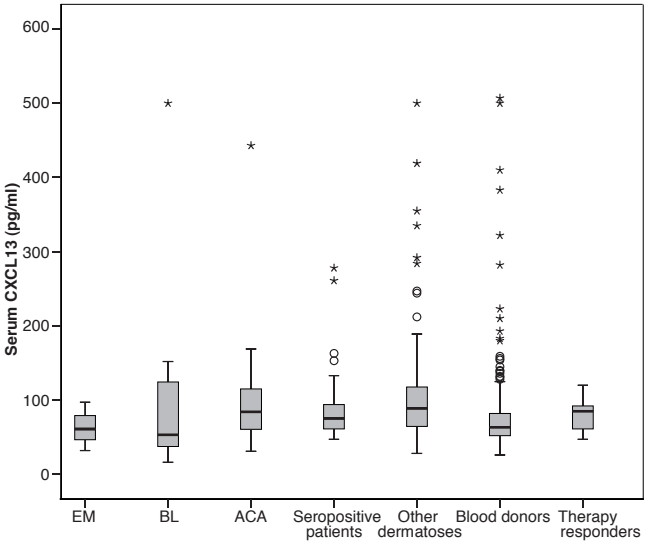

Fig. 1. CXCL13 serum levels (pg/ml) in all patient groups. EM: erythema migrans; BL: borrelia lymphocytoma; ACA: acrodermatitis chronica atrophicans.

RESULTS

The 80 "seropositives", 96% of which were confirmed by IB, with non-specific clinical symptoms had significantly higher CXCL13 levels than blood donors (median 75.5 pg/ml compared with median 63.5 pg/ml, $p = 0.001$), but CXCL13 levels were not significantly higher in the 9 seropositive patients with subsiding symptoms after therapy ($p = 0.135$) (Fig. 1). Within the patient group, as well as in blood donors, however, CXCL13 levels were highly variable (35–261 pg/ml in patients and range 26–500 pg/ml in blood donors). After therapy, CXCL13 concentrations of the 29 antibiotic-treated patients were reduced to a median of 65 pg/ml ($p = 0.148$). High antibody titres did not correlate with elevated CXCL13 levels. There was no difference in CXCL13 levels in the 4% not-confirmed "seropositives".

The highest CXCL13 concentrations were found in the serum of patients with newly diagnosed BL before therapy (500 and 125 pg/ml), but altogether in the 10 BL patients, investigated after therapy and retrospectively, this was not statistically significant.

In contrast, ACA patients before therapy showed significantly elevated CXCL13 levels compared with blood donors (median 84 pg/ml, $p = 0.006$). CXCL13 levels were lower in the EM-group (mean 56.8; median

61 pg/ml), even lower than in blood donors (mean 76.3; median 63.5 pg/ml).

The control group of 97 patients with various dermatoses had the second highest levels of CXCL13 after BL patients (mean 184 pg/ml; median 110 pg/ml) (Table SI; available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1144) and levels above 105 pg/ml in some patients with squamous cell carcinoma (1/4), basal cell carcinoma (3/7), malignant melanoma (4/16), mycosis fungoides (1/1), Merkel cell carcinoma (1/1), systemic mastocytosis (1/1), psoriasis vulgaris (3/15, especially with psoriatic arthritis), erysipelas (4/7), pityriasis rubra pilaris (1/1), pemphigus vulgaris (1/2), urticaria (1/5), and atopic dermatitis (2/3).

DISCUSSION

Serum CXCL13 concentration varies considerably within all investigated patient groups and blood donors.

"Seropositives" with various symptoms had statistically higher CXCL13 serum levels than blood donors, but lower levels than patients with ACA and seronegative non-LB patients. After antibiotic therapy, serum CXCL13 levels decreased in all treated patients, both with and without symptom improvement, while antibody titres showed no changes. Similarly, in a previous study, Shin et al. (9) found no difference in CXCL13 levels in synovial fluid and tissue between patients with antibiotic-responsive and antibiotic-refractory Lyme arthritis. Most of the clinical symptoms described have a high prevalence in the general population and cannot be used to differentiate between LB and non-LB patients, as has been shown previously by Donta et al. (10) and Seltzer et al. (11). On the other hand, in an Austrian study, the seropositivity rate in the local population was up to 70%, depending on age and the risk of exposure to ticks (12). The seropositive individuals investigated did not have complaints compatible with chronic LB.

Only in ACA, defined by high anti-*Bb* antibody titres with numerous bands in the IgG immunoblot (13), as well as in the sera of patients with newly diagnosed BL were CXCL13 serum levels markedly higher than in blood donors, thus explaining the stronger B-cell presence. Similarly, CXCL13 has been detected in the skin of BL by quantitative reverse transcription-PCR (14). Patients with EM had low serum CXCL13 levels, as the early phase of infection is not characterized by B-cell activation, in contrast to chronic infection (15).

Since CXCL13 levels correlate with B-cell activation and were found to be elevated in patients with psoriasis vulgaris and lupus erythematosus, further studies are needed to clarify whether different CXCL13 concentrations within these control groups have clinical relevance, or whether this chemokine could be used as a new biomarker. In LB, serum CXCL13, similarly to anti-*Bb* antibody titres, cannot be used as a marker for disease activity or to indicate the need for antibiotic therapy.

REFERENCES

1. Rupprecht TA, Pfister HW, Angele B, Kastenbauer S, Wilske B, Koedel U. The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis. Neurology 2005; 65: 448–450.
2. Wutte N, Berghold A, Zenz W, Daghofer E, Löffler S, Kleinert G, et al. Serum CXCL13 chemokine in adult and pediatric Lyme neuroborreliosis. Acta Neurol Scand 2011. doi: 10.1111/j.1600-0404.2010.01477.x [Epub ahead of print].
3. Senel M, Rupprecht TA, Tumani H, Pfister HW, Ludolph AC, Brettschneider J. The chemokine CXCL13 in acute neuroborreliosis. J Neurol Neurosurg Psychiatry 2010; 1: 929–933.
4. Narayan K, Dail D, Li L, Cadavid D, Amrute S, Fitzgerald-Bocarsly P, et al. The nervous system as ectopic germinal center: CXCL13 and IgG in Lyme neuroborreliosis. Ann Neurol 2005; 57: 813–823.
5. Lee HAT, Shiao YM, Wu TH, Chen WS, Hsu YH, Tsai SF, et al. Serum BLC/CXCL13 concentrations and renal expression of CXCL13/CXCR5 in patients with systemic lupus erythematosus and lupus nephritis. J Rheumatol 2010; 37: 45–52.
6. De Paepe B, Creus K, De Bleecker J. Role of cytokines and chemokines in idiopathic inflammatory myopathies. Curr Opin Rheumatol 2009; 21: 610–616.
7. Pachner AR, Gelderblom H, Cadavid D. The rhesus model of Lyme neuroborreliosis. Immunol Rev 2001; 183: 186–204.
8. Hofmann H.Leitlinien für Kutane Manifestationen der Lyme Borreliose. In: www.awmf.de; Korting HC, ed. Berlin, Germany: Dermatologische Qualitätssicherung. ABW Wissenschaftsverlag, GmbH; 2009: Nr. 013/044
9. Shin JJ, Glickstein LJ, Steere AC. High levels of inflammatory chemokines and cytokines in joint fluid and synovial tissue throughout the course of antibiotic-refractory Lyme arthritis. Arthritis Rheum 2007; 56: 1325–1335.
10. Donta ST. Late and chronic Lyme disease: symptom overlap with chronic fatigue syndrome & fibromyalgia. Med Clin North Am 2002; 86: 341–349.
11. Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED. Long-term outcomes of persons with Lyme disease. JAMA 2000; 283: 609–616.
12. Cetin EH, Sotoudeh M, Auer H, Stanek G. Paradigm Burgenland: risk of Borrelia burgdorferi sensu lato infection indicated by variable seroprevalence rates in hunters. Wien Klin Wochenschr 2006; 118: 677–681.
13. Wilske B, Fingerle V, Herzer P, Hofmann A, Lehnert G, Peters H, et al. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med Microbiol Immunol 1993; 182: 255–270.
14. Müllegger RR, Means TK, Shin JJ, Lee M, Jones KL, Glickstein LJ, et al. Chemokine signatures in the skin disorders of Lyme borreliosis in Europe: predominance of CXCL9 and CXCL10 in erythema migrans and acrodermatitis and CXCL13 in lymphocytoma. Infect Immun 2007; 75: 4621–4628.
15. Boer A, Bresch M, Dayrit J, Falk TM. Erythema migrans: a reassessment of diagnostic criteria for early cutaneous manifestations of borreliosis with particular emphasis on clonality investigations. Br J Dermatol 2007; 156: 1263–1271.