B-Cell-Attracting Chemokine-1 (BCA-1/CXCL13) in Systemic Lupus Erythematosus, Its Correlation to Disease Activity and Renal Involvement

Sawsan Said Hafez, Wessam El Sayed Saad, Noha Hussien Shedid

Departments of Clinical & Chemical Pathology and Internal Medicine and Rheumatology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune disease in which cytokines such as B lymphocyte chemoattractant (BLC or CXCL13) may play important roles in pathogenesis. The aim of our study was to investigate the implications of CXCL13 in SLE diagnosis and its correlation with disease activity and renal involvement. The study included 50 adult female patients with SLE and 30 age-matched female healthy individuals serving as a control group. Patients' Group was further subdivided according to disease activity calculated by SLE Disease Activity Index (SLEDAI). All studied individuals were subjected to assessment of serum CXCL13 by ELISA. A highly significant stepwise progressive increase in CXCL13 level was recorded through controls, inactive SLE and active disease (P < 0.01). Moreover, it correlated positively with SLEDAI and proteinuria (P < 0.01). At a cut-off level 80 pg/mL, CXCL13 could discriminate active SLE from inactive (AUC=0.989, sensitivity 100% & specificity 96%). In conclusion, an increased level of CXCL13 is a distinctive feature in SLE. CXCL13 correlates with disease activity and renal involvement.

Systemic lupus erythematosus (SLE, lupus) is a prototype of systemic autoimmune disease. Immune system activation in SLE is characterized by exaggerated B-cell and T-cell responses and loss of immune tolerance against self-antigens. Production and defective elimination of antibodies, circulation and tissue deposition of immune complexes, and complement and cytokine activation contribute to clinical manifestations that range from fatigue and joint pain to severe, life-threatening organ damage (Kong et al., 2009; Yee et al., 2011).

Renal involvement is a common manifestation in course of SLE and may occur at any time. In SLE nephritis, the pattern of glomerular injury is primarily related to the formation of the immune deposits in situ, due major to anti-double-stranded DNA (anti-dsDNA) antibodies and anti-C1q. Immune complexes deposits can induce the inflammatory response by activation of adhesion molecules on endothelium, resulting in the recruitment of proinflammatory leukocytes, activated and damaged glomerular cells, infiltrating macrophages, B and T cells produced cytokines that play a pivotal role as inflammatory mediators to extend renal injury (Kiriakidou et al., 2013).

Chemokines (CXC) are the important factors that regulate leukocyte recruitment into the inflamed tissue; they participate in the chemotraction of numerous immunoreactive cells and play an important role in the development of inflammatory conditions and progression of autoimmune disease such as SLE (Alzawawy et al., 2009).

The chemokine CXC ligand 13 protein (CXCL13), also known as B-cell-attracting chemokine-1(BCA-1) or B-lymphocyte chemoattractant (BLC), is a small cytokine belonging to the CXC chemokine family. It is expressed in secondary lymphoid tissues by stromal cells such as follicular dendritic cells (FDCs), where it contributes to lymphorganogenesis as well as to regulating antibody responses (Steinmetz et al., 2008). As its name suggests, this chemokine is
selectively chemotactic for B cells belonging to both the B-1 and B-2 subsets, and elicits its effects by interacting with chemokine receptor CXCR5 (Lee et al., 2010). CXCR5 is expressed by B cells and a subset of CD4+ T cells termed follicular helper T (T\textsubscript{FH}) cells that are critical for driving T cell-dependent antibody responses. CXCL13 and its receptor CXCR5 control the organization of B cells within follicles of lymphoid tissues (Lalor et al., 2010). CXCL13 can induce the trafficking of CXCR5+ T lymphocyte subset designated as follicular helper T lymphocytes (TFH) which are specifically involved in high affinity IgG production in germinal centers developed within B cell follicles of secondary lymphoid tissues including lymph nodes, spleen, and tonsils (Wong et al., 2010).

In humans, aberrant CXCL13 expression has been observed in inflamed tissues of patients with various autoimmune diseases such as rheumatoid arthritis (RA) (Sherif et al., 2013), SLE (Steinmetz et al., 2008), Sjögren's syndrome, multiple sclerosis (MS), and neuroinflammation (Lalor et al., 2010; Hytönen et al., 2014). The expression of CXCL13 is often associated with leukocyte infiltration and the development of tertiary lymphoid structures in the tissues (Hytönen et al., 2014).

The role of CXCL13 is particularly interesting in the course of lupus nephritis (LN), since aberrant CXCL13 expression is sufficient to induce the formation of ectopic lymphoid tissues in non-lymphoid organs and thus could be responsible for the accumulation of inflammatory cells in the kidneys in SLE (Adalid-Peralta et al., 2008). Furthermore, it is believed that the presence of ectopic lymphoid tissues promotes the local activation of T and B cells leading to exacerbation of disease (Segerer & Schlondorff, 2008). This emerging new concept was an inspiration to investigate the diagnostic value of serum CXCL13 levels in SLE patients as well as its correlation with disease activity and renal involvement in lupus nephritis patients.

**Subjects and Methods**

I. Subjects

This study was conducted on 50 adult female SLE patients fulfilling the updated American College of Rheumatology (ACR) revised criteria for the classification of SLE (Hochberg et al., 1997). Patients were recruited from rheumatology outpatient clinic and internal medicine department, rheumatology division, Ain Shams University Hospital, Cairo. In addition to 30 apparently healthy female age matched subjects serving as a control group, all of whom willingly participated in the study. Subjects with other inflammatory diseases or malignancy were excluded from the study.

A) Patients’ Group: This group included 50 females, with a mean age of 32.1±11.8 years. Full history taking and clinical examination were performed for all patients. Assessment of disease activity was done using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier et al., 1992). SLE Patients were classified according to disease activity into two subgroups:

1. Inactive SLE subgroup (n= 28): This subgroup included 28 female patients (SLEDAI < 6), with a mean age of 31.3±11.6 years.

2. Active SLE subgroup (n= 22): This subgroup included 22 female patients (SLEDAI ≥ 6), with a mean age of 32.4±11.4 years.

This subgroup was further subdivided according to presence or absence of nephritis into: active SLE with nephritis (lupus nephritis, LN; n=12), active SLE without nephritis (n=10). LN was defined as having histopathological evidence of immune complex-mediated glomerulonephritis, plus the current renal portion of SLEDAI. This consists of the four kidney-related items of the SLEDAI which are hematuria (> 5 RBCs/HPF after exclusion of stone, infection or other causes), pyuria (> 5 WBCs / HPF after exclusion of infection), proteinuria (> 0.5 g/24 hour, either of new onset or recent increase of more than 0.5 g /24 hour) and urinary casts (granular or red blood cell) (Bombardier et al., 1992).

B) Control Group: This group included 30 age matched female healthy subjects, with a mean age of 33.8±13.6 years, with no evidence of any rheumatologic disorder or chronic medical illness serving as a control group.
II. Samples

A) Blood Samples:

Six milliliters of venous blood were collected under complete aseptic precautions from each subject. The collected blood was divided among an EDTA tube for complete blood picture, a citrated tube for ESR, and a plain test tube for serum separation. After clotting, samples were centrifuged at 1000 Xg for 15 minutes, and sera were separated into 2 aliquots. 1st aliquot for the assay of serum creatinine, serum anti-double stranded-DNA (Anti-dsDNA) antibodies and serum complement 3 and 4 (C3 and C4). The second aliquot was stored at – 20 °C for the subsequent assay of serum CXCL13. Hemolysed samples were discarded, repeated freezing and thawing was avoided.

B) Urine Samples:

The first voided morning urine sample was collected from each subject in the study. The first portion of the sample was collected in a clean container to be used for routine urine analysis, second one for measurement of protein creatinine ratio (Pr/Cr).

III. Methods

Analytical Methods:

1. Complete Blood Count (CBC): CBC was done on Coulter LH 750 hematology analyzer (Beckman Coulter International SA, Rue Juste-Olivier 22, PO Box 1059, CH – 1260 Nyon, Switzerland).
2. Erythrocyte Sedimentation Rate (ESR): was determined in the first hour (mm/hour) by Westergren method.
3. Serum creatinine and 24 h urine protein: were measured on Synchron CX-5 Delta autoanalyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634, 3100, USA).
4. Serum anti-double stranded-DNA (Anti-dsDNA) antibodies: Quantitative measurement was determined by EIA (anti-dsDNA Kit, ORGENTEC Diagnostika GmbH, Carl-Zeiss Strarabe49, Germany), according to the manufacturer instructions.
5. Serum levels of complement 3 and 4 (C3 and C4): were measured by nephelometric method using Minineph™ human C3 and C4 kit (Binding site Group Ltd, Birmingham, UK), according to the manufacturer instructions.
7. Assessment of serum CXCL13 levels: Assay was carried out using a commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by Quantikine (R&D Systems, Inc., McKinley Place NE, Minneapolis, United States of America), according to the manufacturer instructions. Briefly, a monoclonal antibody specific for CXCL 13 has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any CXCL 13 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for CXCL 13 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of CXCL 13 bound in the initial step. The color development was stopped and the intensity of the color was measured at 450 nm using ELISA reader. To deduce the concentration of CXCL 13 in serum samples and control material, a standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. The best fit curve was drawn through the points on the graph. Zero standard absorbance was subtracted from all recorded absorbance. Results were expressed as pg/mL.

Statistical Methods

Statistical analysis was carried out on personal computer using IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) for data analysis. Data were expressed using the mean and standard deviation (SD) for quantitative parametric data, and median and interquartile range (IQR) for quantitative non parametric data. Nominal data were expressed as frequency and percentage. Comparative statistics was done using t tests for parametric data and Wilcoxon’s rank sum test in case of non-parametric data. Correlation analysis was performed by Spearman’s rank correlation ($r_s$). P values <0.05 were considered significant. Receiver operating characteristic curve (ROC) analysis was applied to assess the diagnostic performance of CXCL13 to differentiate between SLE patients and controls, patients with and without activity and patients with and without LN.

Results

Results are presented in tables (1, 2) and figures (1-6).

Fifty female patients with SLE were analyzed. The main laboratory descriptive data and clinical characteristics are depicted in table 1.
As regards serum CXCL 13 levels, the median (IQR) serum concentration of CXCL-13 was significantly higher in SLE patients [77.5 (45–200) pg/mL] compared to healthy controls [21 (25-15) pg/mL] (P<0.01). The CXCL13 levels in active SLE patients (SLEDAI ≥6) were 4-folds higher compared to those in inactive SLE patients (SLEDAI<6) [200 (147.5–220) pg/mL] versus [45 (38.5–65) pg/mL, P<0.01]. Of note, the patients with inactive SLE still had higher CXCL13 levels than healthy controls (P<0.01) (Figure 1).

A positive correlation was observed between circulating CXCL13 levels and SLEDAI in all patients with SLE (r_s=0.822, P <0.01) (Table 2), and in patient with active disease (r_s=0.946, P <0.01) (Figure 2). Likewise, CXCL13 concentrations showed a positive correlation with anti-dsDNA titres (r_s=0.600, P<0.01) as well as ESR (r_s= 0.726, P< 0.01). However, a negative correlation was seen with C3 (r_s= -0.535, P<0.01) and C4 (r_s= -0.428, P<0.01) (Table 2).

Although median (IQR) CXCL13 concentrations were higher in patients with renal involvement (210 (157.5–280) pg/mL, n=12) compared with those without renal involvement (180 (130–212.5) pg/mL, n=10), it didn't reach a statistical significance (P>0.05) (Figure 3). Interestingly, a positive correlation was observed between circulating CXCL13 levels and urinary proteinuria in patients with SLE and in active disease (r_s=0.678, r_s=0.855; P <0.01; respectively) (Figure 4). Furthermore, serum CXCL13 had a positive correlation with serum creatinine in all SLE patients (r_s=0.343, P<0.05).
Figure 1. Serum levels of CXCL13 (pg/mL) in SLE patients (active & inactive disease) compared to control subjects.

Figure 2. Correlation between serum CXCL13 (pg/mL) levels and disease activity (SLEDAI) in patients with active SLE.

Table 2. Correlation analysis between serum CXCL13 and all studied parameters in SLE patient.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$r_s$</th>
<th>$P$ value</th>
<th>Parameter</th>
<th>$r_s$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.035</td>
<td>NS</td>
<td>C3 (mg/dL)</td>
<td>-0.535</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>0.726</td>
<td>&lt;0.01</td>
<td>C4 (mg/dL)</td>
<td>-0.428</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>-0.079</td>
<td>NS</td>
<td>Creatinine (mg/dL)</td>
<td>0.343</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WBCs (cell/HPF)</td>
<td>0.011</td>
<td>NS</td>
<td>Proteinurea (g/24h)</td>
<td>0.678</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lymphocyte (cell/HPF)</td>
<td>-0.101</td>
<td>NS</td>
<td>Anti-dsDNA antibodies (IU)</td>
<td>0.600</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plt ($10^3$/mm$^3$)</td>
<td>0.009</td>
<td>NS</td>
<td>SLEDAI (Points)</td>
<td>0.822</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

$r_s$: Spearman's correlation coefficient; $P > 0.05$: Non-significant correlation (NS).
Conversely, serum CXCL13 expression could not be correlated to age, hemoglobin, platelets or white blood cells in both active and inactive SLE subgroups ($P > 0.05$ in all). Therefore, the increase in CXCL13 did not simply reflect a raised leukocyte population.

ROC curve analysis was constructed to evaluate the diagnostic performance of serum CXCL13. The best cutoff value of CXCL 13 for discriminating SLE patients from control group was 32 pg/mL. This had a diagnostic sensitivity 90%, specificity 100%, PPV 100%, NPP 86% and efficiency 94% with area under the curve (AUC) 0.927 (Figure 5). Moreover, the best cutoff value of CXCL 13 for discriminating active SLE (with and without lupus nephritis) subgroup from inactive SLE subgroup was 80 pg/mL. This had a diagnostic sensitivity 100%, specificity 96%, PPV 96%, NPP 100% and efficiency 98% with AUC 0.989 (Figure 6). However, the best cutoff value of CXCL 13 for discriminating active SLE with lupus nephritis from active SLE without lupus nephritis was 200 pg/mL. This had a diagnostic sensitivity 58%, specificity 70%, PPV 70%, NPP 58% and efficiency 67% with AUC 0.719.
Figure 5. ROC curve analysis showing the diagnostic performance of CXCL 13 in discriminating SLE patients from control subjects. The best cut-off = 32 pg/mL, AUC= 0.927

Figure 6. ROC curve analysis showing the diagnostic performance of CXCL 13 in discriminating active SLE patients (with & without lupus nephritis) from those inactive. The best cut-off = 80 pg/mL, AUC= 0.989

Discussion

The present study was conducted in an attempt to determine whether serum levels of CXCL13 would correlate with SLE disease variables, disease activity indices as well as renal involvement.

In the present study, we have confirmed the significant increase in plasma concentration of CXCL13 in SLE patients and the elevated plasma concentration of CXCL13 correlated significantly with SLE disease activity (ds-DNA antibodies & SELDAI score). Our results were in concordance with the previous studies (Schiffer et al., 2009; Wong et al., 2010; Lee et al., 2010; Ezzat et al., 2011). Moreover, in the present study, serum CXCL13 level showed a positive
correlation with ESR ($r_s=0.726$ & $P<0.01$). These results were similar to those found by Ezzat and his colleagues (2011), where both ESR and CXCL13 levels are increased in case of inflammation. On the other hand, negative correlations were observed between serum CXCL13 and both C3 ($r_s=-0.535$ & $P<0.01$) and C4 ($r_s=-0.428$ & $P<0.01$). This confirms the previous finding by Ezzat and his colleagues (2011). However, Schiffer and his colleagues (2009) did not find a significant correlation between serum CXCL13 and the complement factor C3 that may be not consumed in all their active patients.

We noted that CXCL13 serum levels remained significantly elevated in SLE patients with inactive disease compared to healthy controls, probably indicating aberrant B-cell trafficking even in remission. Previous studies indicated that elevated CXCL13 may relate with the immunopathogenesis mediated by the function of follicular helper T cells (T<sub>FH</sub>) cells in autoimmune disease (Wong et al., 2010). CXCL13 can induce the trafficking of distinct CXCR5+ T cells designated as T<sub>FH</sub> which are specifically involved in high-affinity IgG production in germinal centers developed within B cell follicles of secondary lymphoid tissues including lymph nodes, spleen, and tonsils (Dörner et al., 2011).

As regard renal involvement, our study demonstrated that median CXCL13 serum levels were higher in patients with lupus nephritis compared to patients without renal involvement but this elevation did not reach a statistical significance. Further longitudinal studies may be required to establish role of CXCL13 in SLE with and without renal involvement. The finding of a positive correlation between serum CXCL13 and proteinuria ($P<0.01$) and serum creatinine ($P<0.05$) is of special interest.

The correlation of CXCL13 and renal involvement in SLE patients could be attributed to that CXCL13 has a central role in B-cell trafficking via interaction with its corresponding receptor CXCR5 on the B-cell surface (Steinmetz et al., 2009); both were highly expressed in the renal cortex from patients with lupus nephritis (Schiffer et al., 2008). Thus, CXCL13 is sufficient to induce the formation of ectopic lymphoid tissues in non-lymphoid organs, leading to exacerbation of SLE disease via accumulation of inflammatory cells in renal tissues (Adalid-Peralta et al., 2008 & Worthmann et al., 2014). Intrarenal B-cell aggregates are described in different forms of inflammatory kidney disease, including LN, and correlate with a worse clinical outcome (Steinmetz et al., 2008 & Jordan et al., 2013). Therefore, high local expression in the kidneys in patients with lupus nephritis may lead to high CXCL13 serum levels that found in our study and previous studies (Schiffer et al., 2009; Lee et al., 2010; Wong et al., 2010; Ezzat et al., 2011).

We estimated the potential use of CXCL13 as a marker for renal involvement in SLE patients by ROC curve analysis, the best cutoff value of CXCL13 for discriminating active SLE with lupus nephritis from active SLE without lupus nephritis was 200 pg/mL. This had a diagnostic sensitivity 58%, specificity 70%, and efficiency 67% with AUC 0.719. These results were comparable to those of Schiffer and his colleagues (2009) who reported that, the best cutoff value of serum CXCL13 to differentiate renal from non-renal involvement in SLE was 162.2 pg/mL. This value had a diagnostic sensitivity 61% and specificity 72% with AUC= 0.693.

In conclusion, increased level of CXCL13 has a distinctive feature in SLE, even in patients with inactive disease. CXCL13 correlates with disease activity and renal involvement. So, it could be added to other laboratory parameters to monitor the disease course and progression.
References


