CCL-19 LEVELS IS ASSOCIATED WITH ORGAN DAMAGE AND LUPUS NEPHRITIS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract: Background: Systemic lupus erythematosus (SLE) is a remarkably heterogeneous autoimmune disease. Despite tremendous efforts, our knowledge of serum leves of CCL-19 in severe forms of SLE is still limited. We investigated the serum levels of CCL19 of SLE, with special emphasis on irreversible organ damage and active lupus nephritis (LN).
Methods:We used immunoassay to assess the serum levels of CCl19 in Bulgarian patients with SLE (n = 35) and age-matched healthy control subjects (n = 13). Patients were divided into two main groups - patients with hematological form of the disease (patients with anemic syndrome, leukopenia, thrombocytopenia) and patients with lupus nephritis. Demographic and physical data were studied, as well as some scales for disease activity,All statistical analyses were performed on linearized data (linear ddCq) for each analyte. Statistical tests (Mann–Whitney–Wilcoxon test, Benjamini–Hochberg correction, Spearman correlations,) were performed using the SPSS program. P corr value < 0.05 was considered significant.
Results: Serum levels of CCL19 were significantly elevated in patients with systemic lupus compared to healthy controls (P corr < 0.05). Patients with a hematological form of the disease have a higher level of the studied chemokine than patients with lupus nephritis (P corr < 0.0006). Very high levels of CCL19 were found in active lupus nephritis, the same patients were referred for a kidney biopsy that found lupus nephritis III-IV class. Serum levels of CCL19 are strongly correlated with the severity of anemic syndrome, leukopenia and thrombocytopenia. Serum CCL19 levels have been shown to be useful in predicting active renal disease (P corr < 0.05).
Conclusions: This highly sensitive assay for CCL19 identifies patients with active lupus nephritis and haematological abnormalities in patients with SLE. Their exact role and suitability as biomarkers in the diagnosis of SLE deserve further study.CCL-19 levels is associated with organ damage and lupus nephritis in patients with systemic lupus erythematosus
Keywords: CCL-19, lupus nephritis, systemic lupus erythematosus

1. BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic disease that causes inflammation in connective tissues, such as cartilage and the lining of blood vessels(1). The signs and symptoms of SLE vary among affected individuals, and can involve many organs and systems, including the skin, joints, kidneys, lungs, central nervous system, and blood-forming system (1, 2, 3). About a third of people with SLE develop kidney disease (nephritis) (1, 4). Heart problems may also occur in SLE, including inflammation of the heart valves. The inflammation characteristic of SLE can also damage the nervous system, and may result in abnormal sensation and weakness in the limbs; seizures; stroke; and difficulty processing, learning, and cognitive impairment. Anxiety and depression are also common in SLE (5, 6).
SLE is characterized by production of pathogenic autoantibodies directed against nucleic acids and their binding proteins, reflecting a global loss of self-tolerance (reviewed in [1]). The loss of tolerance with subsequent immune dysregulation is a consequence of genetic factors, in the setting of environmental triggers and stochastic events, with recent studies implicating over 30 genetic loci in disease pathogenesis (2, 5).Aberrant innate immune responses play a significant role in the pathogenesis of SLE, contributing both to tissue injury via release of inflammatory cytokines as well as to aberrant activation of autoreactive T and B cells, with the latter leading to pathogenic autoantibody production and resultant end-organ injury (6). Autoantigenic nucleic acids and their binding proteins are required for self-antigen specific activation of autoreactive lymphocytes. Autoantigens complexed with their cognate autoantibodies also directly contribute to activation of innate immune cells via Fc receptor (FcR)-mediated uptake of complexes (or in the case of autoreactive B cells, initial engagement of the B cell antigen receptor by autoantigens per se), with the nucleic acid component of these complexes upon endosomal trafficking engaging intracellular Toll-like receptors (TLRs) with subsequent innate and B cell activation (7, 8, 9, 10, 11).
The kidney is a primary site of tissue injury in lupus (1, 4, 6). Nephritis results from glomerular deposition of immune complexes of autoantibodies and autoantigens, with engagement of FcRs on immune cells along with

complement fixation (12). These effector mechanisms initiate infiltration and activation of tissue-infiltrating macrophages that promote the inflammatory response with resultant tissue injury (12, 13). The contributions of autoantibody isotypes to tissue injury in the kidney has not been well understood, although those associated with Th1 responses are thought to predominate in the human and murine diseases (14). As autoantibodies are critical for the pathogenesis of SLE and resultant tissue injury, B cell depletion is an attractive therapeutic option in disease. Lupus nephritis is the diagnosis applied to people with renal inflammation occurring in the context of SLE (8). It occurs in 39% of people. The World Health Organisation (WHO) graded the disease in 1982, based on histological features, as follows: grade I = normal kidney or minor abnormalities; grade II = mesangial proliferation; grade III = focal proliferative glomerulonephritis; grade IV = diffuse proliferative glomerulonephritis; grade V = membranous disease; and grade VI = sclerosing glomerulonephritis. This overview covers treatments of WHO grades III to V (4,6).

The search for lupus biomarkers to diagnose, monitor, stratify, and predict individual response to therapy is currently more intense than ever before (15). This effort is essential for several reasons. First, epidemic overdiagnosis and underdiagnosis of lupus, even by certified rheumatologists, leads to errors in therapy with concomitant side effects which may be more serious than the disease itself. Second, identification of lupus flares remains as much an art as it is a science. Third, the capacity to stratify patients so as to predict those who will develop specific patterns of organ involvement is not currently possible but would potentially lead to preventive therapeutic strategies. Fourth, only one new drug for the treatment of lupus has been approved by the US Food and Drug Administration in over 50 years.

Chemokine (C-C motif) ligand 19 (CCL19) is a protein that in humans is encoded by the CCL19 gene (16). This gene is one of several CC cytokine genes clustered on the p-arm of chromosome 9. Cytokines are a family of secreted proteins involved in immunoregulatory and inflammatory processes. The cytokine encoded by this gene may play a role in normal lymphocyte recirculation and homing. It also plays an important role in trafficking of T cells in thymus, and in T cell and B cell migration to secondary lymphoid organs. CCL19) is belonging to the CC chemokine family that is also known as EBI1 ligand chemokine (ELC) and macrophage inflammatory protein-3-beta (MIP-3-beta). (16). This chemokine elicits its effects on its target cells by binding to the chemokine receptor chemokine receptor CCR7, it attracts certain cells of the immune system, including dendritic cells and antigen-engaged B cells, CCR7+ central-memory T-Cells (17, 18, 19).

The aim of this study is to investigate the serum levels of CCL19 of patients with SLE, with special emphasis on irreversible organ damage and active lupus nephritis .

2. METHODS

We used immunoassay to assess the serum levels of CCl19 in Bulgarian patients with SLE (n = 35) and age-matched healthy control subjects (n = 13). Patients were divided into two main groups - patients with hematological form of the disease (patients with anemic syndrome, leukopenia, thrombocytopenia) and patients with lupus nephritis. Demographic and physical data were studied, as well as some scales for disease activity,All statistical analyses were performed on linearized data (linear ddCq) for each analyte. Statistical tests (Mann–Whitney–Wilcoxon test, Benjamini–Hochberg correction, Spearman correlations,) were performed using the SPSS program. P-correlations value < 0.05 was considered significant.

3. RESULTS

Demographic indicators of the population of patients suffering from systemic lupus compared with healthy controls are presented in Table 1

Table 1. Baseline characteristics of patients with systemic lupus compared with healthy controls

		Total n= 48	**SlE n=35**	**Controls n=13**	**p**
Age		48.7±17.5	48.5±15.7	48.7±18	0.8
Sex	Female	74.9%	82.8%	72.9%	0.1
Haemoglobin	< 10 g/l		89.1%	0%	**0.001**
Platelets	$< 100.10^9$		61.9%	0%	**0.001**

leukocytes <3,5.10^9	31.1%	0%	**0.001**
Lymphocytes <1,5.10^9	30.8%	0%	**0.001**
Pain (VAS 0-100)	57.8±27.3	14±27.7	0.02
Patient Global Assessment (0-100)	86.2±26	11.8±7.9	0.001
Phisician Global Assessment (0-100)	64.2±12.3	7.1±10.4	**0.001**
HAQ	8.2±5.5	1.2±0.9	0.001
ESR (mm/h)	51.8±21.2	5.2±21.9	**0.010**
CRP (mg/L)	1.9±2.8	3.08±9	0.2
ANA (IU/mL)	133.5±243.1	17.5±94.1	**0.001**
Anti –ds-DNA(IU/mL)	379.5±162.9	5.41±6.2	**0.001**
SLEDAI	18.2±5.5	0	

Serum levels of CCL19 were significantly elevated in patients with systemic lupus compared to healthy controls ($P_{corr} < 0.05$). CCL19 was detected in a total of 5.35±1.02 in patients with systemic lupus, the level of CCL19 in control subjects was 0.021±0.39, the difference between the two groups was significant ($P_{corr} < 0.05$). The level of CCL19 in patients with active WHO stage 3 and 4 lupus nephritis, proven by renal biopsy, is 12.61±2.67, which is significantly higher than in healthy controls and than the mean in patients with lupus($P_{corr} < 0.05$).
Patients with haematological abnormalities but no overt lupus nephritis have CCL19 levels of 6,34±2.66, which is a significantly higher level than that of healthy controls and that of patients with lupus nephritis($P_{corr} < 0.05$).
The independent predictors for the development of lupus nephritis are presented in Table 2, based on a logistic regression model.

Table 2. Independent predictors of lupus nephritis development based on logistic regression model

	Odds ratio	**95% C.I.**	
		Lower	**Upper**
Phisician Global Asessment (0-100)	1,016	,983	1,051
ESR(mm/h)	1,018	,992	1,044
ESR (mm/h)	,633	,021	19,250
ANA (IU/mL)	,851	,223	3,245
Anti –ds-DNA(IU/mL)	,568	,145	2,225
Haematological abnormalities (anemia, thrombocytopenia, leukopenia, lymphopenia)	***2,899***	***1,795***	***2,382***
CCL19 (pg/ml)	***3.441***	***6.009***	***4.541***
SLEDAI	***3,238***	***2,353***	***5,410***

4. DISCUSSION

Until recently, the development of SLE biomarkers has primarily been focused on biomarkers that may assist in making a precise diagnosis or monitoring disease activity (16). Attempts have begun toward discovering biomarkers that might aid in predicting the onset of SLE in susceptible individuals and/or development of flares (systemic or organ-specific) in patients with established SLE, predicting disease outcome, and assessing the effectiveness of therapeutic interventions (16, 17). These 'next-generation' SLE biomarkers will be particularly important because more sensitive and specific markers for the onset or flare of SLE disease activity may allow proactive institution of therapeutic and even preventive strategies so that the therapeutic efficacy can be enhanced while treatment-related side effects can be minimized.

In the burgeoning era of biologic therapeutics, some of which have resulted from recent biomarker studies, a new class of pharmacodynamic biomarkers is needed to aid identification of patients who might respond favorably to a particular biologic, selection of the type and dose of biologics used, and evaluation of therapeutic efficacy.

Owing to the extreme complexity of the disease in its diagnosis, course and organ-specific manifestations as described above, we will most likely depend upon a panel of SLE biomarkers that will be used by physicians, scientists and industry for patient care, research, and drug discovery. This potentially daunting task will require collaborative efforts and novel approaches moving forward (19).

5. CONCLUSIONS

This highly sensitive assay for CCL19 identifies patients with active lupus nephritis and haematological abnormalities in patients with SLE. Their exact role and suitability as biomarkers in the diagnosis of SLE deserve further study.

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