

ASSESSMENT OF THE PRODUCTS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) GENETIC PREDISPOSITION BIOMARKERS IN BULGARIA

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Abstract

The scope of the current clinical trial is to investigate the products of systemic lupus erythematosus (SLE) genetic predisposition biomarkers and to determine those biomarkers that correlate with the clinical course of the disease. The present study includes 48 patients with SLE (43 women and 5 men) age 53 years with 4.32 points (min. 0 max. 12 points) of activity of SLE by SLEDAI-2K and 35 healthy controls (32 women and 3 men). Clinical symptoms include arthritis and skin damage. The result of quantitative determination of Human IL-6 in patients with SLE is statistically significantly higher 2.71 pg/ml than in healthy controls. The result of quantitative determination of Human sIL-2R in patients with SLE is statistically significantly higher 11.7 ng/ml than in healthy controls 8.9 ng/ml. The result of quantitative determination of Human Interleukin-1 – Receptor Antagonist, Human IL-10, Human IL-15 in serum of patients with SLE is not statistically significantly higher than in healthy controls. Analysis of the results shows a statistically significant difference in the results for Human IL-6, Human sIL-2R, Human Thrombomodulin (CD141), Anti-C1-q. They are sensitivity markers and products for genetic predisposition in Bulgarian population with SLE.

Key words: biomarkers, SLE

Introduction. Systemic lupus erythematosus (SLE) is a chronic, autoimmune, multisystemic connective tissue disease. SLE affects more often the female

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sex (female/male ratio is approximately 9:1), with peak incidence between 20 and 40 years of age [1]. Autoimmune diseases differ in their clinical manifestations, organ and tissue damage, laboratory findings, and response to treatment; all of these differences may be related to genetic variability. The strategy of searching for pathogenic genetic mutations and their associations with the production of autoantibodies and cytokine secretion is crucial for the development of successful personalised programmes for patients with severe autoimmune diseases such as SLE. More than 100 genetic loci associated with the development of SLE have recently been identified. The genetic loci associated with a predisposition to SLE may also contribute to the development of other autoimmune diseases. The same trend can be observed in the opposite direction – a family history of an autoimmune disease increases the risk of SLE. A biomarker can be defined as a genetic, biological, biochemical or molecular event whose changes correlate with genetic predisposition, pathogenesis and/or disease manifestations and can be assessed qualitatively and/or quantitatively in laboratories [2, 3]. Biomarkers are biomolecules that are detected in blood, urine or tissues. Finding them in certain concentrations in combination with other symptoms can indicate the onset, development and prognosis of SLE. Biomarkers are successfully used for diagnosis, prognosis and therapy monitoring. They are crucial in research and clinical practice. The clinical manifestations of SLE are the result of a multifaceted autoimmune process unique to each patient, which develops during the course of the disease and is characterized by the production of autoantibodies, damage to the body's immune-complex and complement-mediated response, and vasculopathy [3]. Based on these etiopathogenic characteristics of SLE, a categorization of biomarkers for SLE into different classes is carried out:

I. Biomarkers for susceptibility to SLE / Genetic factors

II. Biomarkers for diagnosis

III. Biomarkers for SLE activity

Genetic factors, whether protective or provocative, play a major role in determining whether an individual will develop SLE. Genetic susceptibility is influenced by environmental factors, although these interactions remain poorly identified. The identification of “lupus genes”, primarily through candidate gene association studies and single nucleotide polymorphism (SNP) association scans, has shown that SLE is a disease with complex genetic inheritance and no single genetic predisposition. It is likely that the thresholds for autoimmunity and the onset of the disease are regulated by multiple genes. Early genetic studies focused on genes that were previously considered key components of immune responses, such as the major histocompatibility complex (MHC) genes. Specific alleles of the MHC (called human leukocyte antigen (HLA) in humans) have been found to be associated with SLE across ethnic groups. Recent studies have identified several susceptibility loci that contain many candidate genes with known immune functions [4].

1) Complement deficiency

The genes that are most commonly called “lupus genes” are those encoding components of the classical complement pathway. Complete deficiency of one of the early components, C1q, C1r, C1s, C4, or C2, is a strong, though rare, risk factor for the development of SLE and/or lupus-like diseases.

Much more common is partial deficiency of complement genes, which is reflected in variably low levels of circulating complement proteins (e.g., C4) [3, 5]. Partial deficiency of C4, especially C4A deficiency, occurs in 30–40% of patients with SLE and is associated with susceptibility to lupus [2, 6].

2) Other products of systemic lupus erythematosus (SLE) genetic predisposition biomarkers that will provide useful information are:

- cytokines (e.g. IL-6, IL-10, IL-15),
- soluble cytokine receptors (sIL-2 receptor)
- Interleukin 10 (IL-10)

IL-10 is a major inducer of type II IFN and has been proposed as a biomarker for assessing disease activity. Among the IL-1 family of cytokines and receptors, the soluble form of ST2/IL-1 receptor 4 (IL-1R4) has recently been proposed as a novel biomarker for assessing SLE activity. ST2/IL-1R4 mediates IL-33 signalling. The soluble form of the receptor prevents the interaction of IL-33 with the membrane receptor. Soluble IL (sIL)-1R4 levels are elevated in active SLE and strongly correlate with the disease activity index and with the levels of anti-dsDNA and anti-C1q antibodies. When the diagnostic value of sIL-1R4 was directly assessed by multivariate analysis, sIL-1R4 was similar to anti-dsDNA and IL-18BP in identifying patients with active nephritis and was the most relevant variable in distinguishing active from inactive patients [5].

The aim of this clinical study is to investigate biomarkers of genetic predisposition to systemic lupus erythematosus (SLE) and to identify those biomarkers that correlate with the clinical course of the disease.

Patients and methods

1. Conducting a history of the course of SLE, accompanying diseases with a detailed assessment of risk factors, obtaining information on age, gender, duration of the disease, therapy performed, genetic predisposition.

2. Assessment of disease activity using an activity index – SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index).

3. Taking blood for marker testing and serum analysis – Interleukin-1 – Receptor Antagonist Human, Human IL-6, Human IL-10, Human IL-15, Human sIL-2R, Human Thrombomodulin (CD141), Anti-C1-q.

4. Statistical processing of data, formation of the final scientific product, implementation of publications, and preparation of the scientific report.

Statistical processing was done with SPSS 19.0. Descriptive analysis was performed with an estimate of the arithmetic mean and standard deviation. An analysis of the difference in mean values was performed, and the statistical signifi-

cance of the differences was tested using mean difference testing models (ANOVA, *t*-test). The results of these models were detected with applied non-parametric analogues (Kolmogorov–Smirnov Z test), the aim being to eliminate any influence from non-compliance with the requirements of the parametric models and at the same time to use the most powerful, parametric statistical method.

To assess the tested correlation dependencies, correlation analysis was applied using Pearson's coefficients, which were validated with analogues – Spearman's coefficients.

To assess the level of significance of certain empirical characteristics, levels based on assumptions about the distribution of the tested features are used. The threshold value for the level of significance is 0.05. The corresponding estimates of the significance of a certain empirical characteristic of the above-described tests are compared with this threshold value of 0.05. If it is less than 0.05, the tested effect is considered statistically significant, if it is greater than 0.05, the tested effect is considered statistically insignificant.

Results. The study included forty-eight SLE patients (43 women and 5 men with an average age of 53 years) with an average SLEDAI-2K disease activity score of 4.32 points (min. 0 max. 12 points) and thirty-five healthy subjects/controls (32 women and 3 men with an average age of 53 years). Primary disease involvement includes arthritis and mucocutaneous manifestations. Twelve of the patients had renal involvement with proteinuria greater than 0.5 g/24 h. The primary treatment for the disease in 28 of the patients with SLE was hydroxychloroquine at a dose of 200 to 400 mg daily. Nine patients were treated with azathioprine 100 mg daily, four patients with methotrexate 15 mg weekly, and four patients with methylprednisolone and endoxan, 1 gram each monthly. All patients received methylprednisolone at a daily dose of 4 to 16 mg. The proportion of patients with elevated anti-dsDNA levels was 27.1%.

The result of the quantitative determination of Human IL-6 in patients with SLE was statistically significantly higher – 2.71 pg/ml (min. 0, max. 16.32 pg/ml) – compared to healthy controls – 0.9 pg/ml (min. 0, max. 9.71 pg/ml) – $p < 0.05$ (Fig. 1).

The result of the quantitative determination of human sIL-2R (Invitrogen) in patients with SLE was statistically significantly higher – 11.7 ng/ml – compared to that in healthy controls – 8.9 ng/ml. The normal range for human sIL-2R in serum is up to 20 ng/ml ($p < 0.05$).

The result of the quantitative determination of the human interleukin-1 receptor antagonist in the serum of patients with SLE is 738.2 pg/ml. The result in healthy controls was 604.32 pg/ml. The result was higher in patients with SLE, $p > 0.05$.

The result of the quantitative determination of human IL-15 was 0 pg/ml in both groups, $p > 0.05$.

The result of the quantitative determination of Human Thrombomodulin

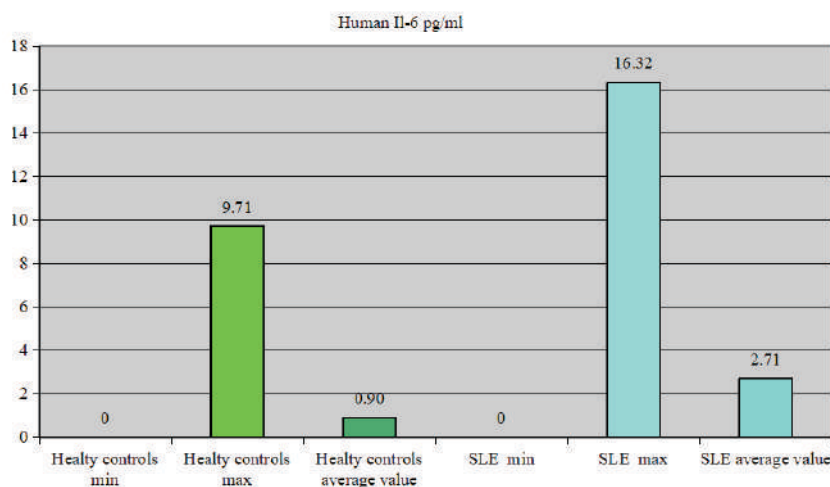


Fig. 1. Results of the quantification of Human IL-6 (< 0.05)

(CD141) in the serum of patients with SLE was 872.66 pg/mL, while in healthy controls it was 1216.78 pg/mL, $p < 0.05$ (Fig. 2).

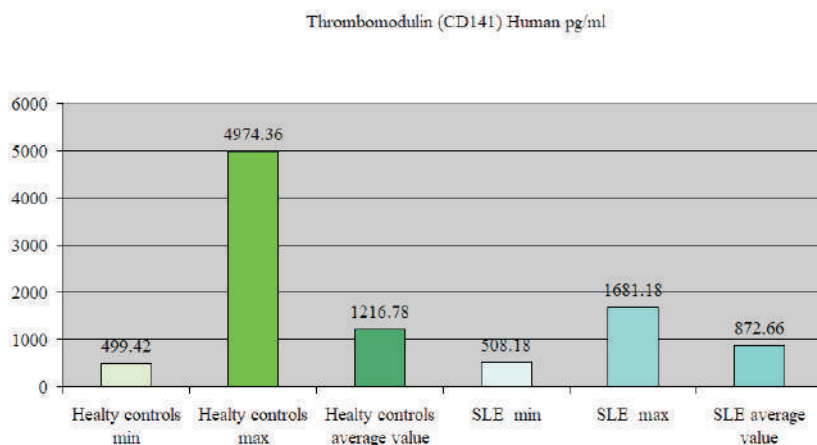


Fig. 2. Results of the quantification of Human Thrombomodulin (CD141) (< 0.05)

The result of the quantitative determination of anti-C1-q was 3.27 U/ml (min. 0, max. 37.5 U/ml), $p < 0.05$. A result is considered positive at a value above 10 U/ml. Positive levels of anti-C1-q antibodies were detected in five patients with SLE. This demonstrates that anti-C1-q antibodies are a marker of SLE activity (Fig. 3).

Analysis of the results shows a statistically significant difference in the results for Human IL-6, Human sIL-2R, Human Thrombomodulin (CD141), Anti-C1-q.

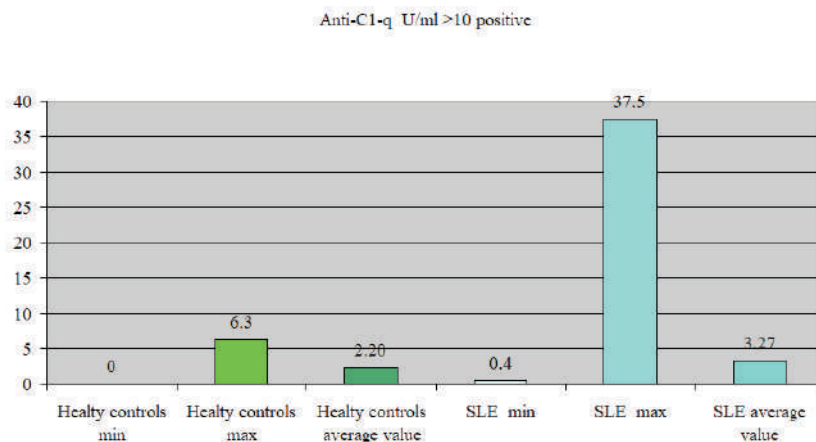


Fig. 3. Results of the quantification of Anti-C1-q ($p < 0.05$)

Discussion. The analysis of the results shows a statistically significant difference in the results for Human IL-6. The concentration of Human IL-6 in patients with SLE is statistically significantly higher compared to healthy individuals – 2.71 pg/ml (min. 0 max. 16.32 pg/ml) versus 0.9 pg/ml (min. 0 max. 9.71 pg/ml) – $p < 0.05$. The presence of a statistically significant difference of this product of systemic lupus erythematosus (SLE) genetic predisposition assumes increased inflammatory activity in patients with SLE compared to healthy individuals. Interleukin-6 (IL-6) is a proinflammatory cytokine that mediates pleiotropic functions in immune responses and inflammatory diseases. The literature lacks studies, with a clinical perspective, on the relationship between IL-6 serum levels and the characteristics of the disease in patients with systemic lupus erythematosus (SLE). In the present work, we aimed to analyze the association between circulating IL-6 and disease manifestations in a well-characterized series of patients with SLE [1, 7]. Elevated IL-6 in active disease may be explained by its role in promoting B-cell differentiation, antibody production, and acute-phase response, mechanisms that become more pronounced during disease flares.

Complement component 1q (or simply C1q) is a protein complex included in the complement system, which is part of the innate immune system. As a chronic inflammatory autoimmune disease, systemic lupus erythematosus (SLE) is characterized by tissue damage in multiple organs caused by autoantibodies and the deposition of immune complexes. A possible role of the complement system in the development of autoimmune conditions such as SLE could be its contribution to the mechanisms of clearance of apoptotic debris, as part of homeostasis in the human body. The capacity of C1q to bind early apoptotic cells can be significantly reduced in the presence of anti-C1q antibodies. In our study the result of quantitative determination of Anti-C1-q is a statistically significant difference between the two groups [2].

The endothelial glycoprotein thrombomodulin regulates coagulation, vascular inflammation and apoptosis. The concentration of thrombomodulin from our clinical study shows a statistically significant difference between the two groups. Plasma levels of thrombomodulin are increased in a certain number of pathologies associated with endothelium lesion: atheromatous arterial disease, disseminated intravascular coagulation syndrome, and also in systemic lupus erythematosus where the levels of plasma thrombomodulin are related to the severity of the pathology. Serum levels of thrombomodulin were significantly higher in SLE. It may prove to be a potential indicator for early and more aggressive treatment.

A quantitatively higher concentration of Interleukin-1 – Receptor Antagonist Human was found in patients with SLE compared to healthy individuals. It neutralizes the action of Interleukin-1. This fact shows that the concentration of the powerful inflammatory mediator Interleukin-1 is higher in patients than in healthy individuals and is considered a product of the inflammatory-immune response in SLE [5].

Deficient or excessive expression of the IL-10 receptor on circulating leukocytes does not seem to be a major factor in the pathogenesis of SLE. However this does not exclude a functional defect, and molecular and signalling studies are required to substantiate this observation. The increased circulating levels of IL-10 in some SLE patients is confirmed in other studies. In summary, although IL-10 may play a central role in SLE pathogenesis, abnormal expression of IL-10R on circulating leukocyte cell surface membranes does not appear to be present in this population of SLE patients [3].

IL-15 is critical for autoimmune and inflammatory diseases and previous studies show that the increased expression of IL-15 is associated with rheumatoid arthritis, sarcoidosis, inflammatory bowel disease, and SLE. An IL-15 mRNA isoform lacking exon 6 in activated macrophages and B cells, which acts as a natural antagonist for IL-15 and may prove to be a promising new therapeutic agent for the treatment of IL-15-mediated inflammatory diseases [3].

In our study no difference in the concentration of Human IL-1ra, IL-10, Human IL-15 was found between patients with SLE and healthy individuals. They can be elevated in some autoimmune conditions, but in this case they have no sensitivity in the studied patients from the Bulgarian population.

Conclusion. Given our growing understanding of the key role of intrinsic characteristics in the predisposition to SLE and the increasing need for precision medicine, focusing on personalized treatment of SLE using genetic or immunological markers may represent a promising therapeutic approach. Analysis of the results shows a statistically significant difference in the levels of Human IL-6, Human sIL-2R, Human Thrombomodulin (CD141), and anti-C1q. These are sensitive markers and the end products of the genes that encode them. They can be used to determine genetic predisposition to the development of SLE in the Bulgarian population.

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