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Research Article

Evaluation of biomarkers for clinical activity of systemic lupus erhythematodes

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Abstract

The scope of the present clinical study is to conduct research on biomarkers of clinical activity of SLE (Human IFN-gamma, Human IFN-alpha, Human sICAM-1, Human sVCAM-1, Human sIL-2R, and TNF-R) and to determine the connection between laboratory biomarkers and clinical and immunological activity of the disease. In the present study, we included 48 patients with SLE (43 women and 5 men) age 53 years, with an average value of 4.32 points (min 0 max 12 points) of activity of SLE by SLEDAI-2K, and 34 healthy controls (31 women and 3 men). Basic clinical symptoms include arthritis and skin damage. The analysis of biomarkers in serum is statistically significantly higher than healthy controls for human IFN-alpha and human sVCAM-1. The analysis of biomarkers in the serum of human IFN-gamma, human sICAM-1, TNF-R, and human IL-12p40/p70 is not statistically significantly higher than healthy controls. The correlation analysis between disease activity assessed with SLEDAI-2K and biomarkers did not confirm a statistically significant relationship. Conclusion. Human IFN-alpha and human sVCAM-1 are sensitivity markers and play an important role in the activity of the disease in the Bulgarian population with SLE.

Keywords

biomarkers, SLE

Introduction

Systemic lupus erythematosus (SLE) is a chronic, autoimmune, multisystemic connective tissue disease. SLE affects more often the female sex (female/male ratio is approximately 9:1), with peak incidence between 20 and 40 years of age (Aringer et al. 2019). 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 (Botto 2016; Califf 2018). 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 (Califf 2018). 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

Traditionally, the definition of autoantibodies as antinuclear antibodies (ANA), anti-extractable nuclear antigen antibodies (e.g., anti-Ro/SSA, anti-La/SSB, anti-snRNP, and anti-Sm), and anti-double-stranded DNA (anti-dsDNA) is used in the diagnosis and monitoring of SLE (Catalina et al. 2020).

Disease activity in SLE was assessed using composite disease activity indices: SLE Disease Activity Index (SLEDAI), System Lupus Activity Measure (SLAM), European Consensus Lupus Activity Measure (ECLAM), and British Isles Lupus Assessment Group Index (BILAG). These include various clinical and laboratory parameters. Assessment of these scales requires appropriate training for accurate interpretation and completion, making daily application more difficult to implement. The value of conventional tests measuring serum complement and autoantibodies as markers of SLE disease activity is being reviewed.

In Bulgaria, there have been no studies of biomarkers to establish the activity, development, and prognosis of the SLE disease. Our idea is to establish the relationship between the presence of biomarkers for clinical activity of SLE (human IFN-gamma, human IFN-alpha, human sICAM-1, human sVCAM-1, human sIL-2R, and human IL-12p40/p70) and the clinical development and course of the disease, comparing the values of the biomarkers with a healthy control group in Bulgaria. This information will be extremely useful in preventing severe organ damage and reducing disability and mortality among SLE patients in Bulgaria.

Assessing a patient with SLE includes five steps:

1. Making a diagnosis.
2. Evaluation of disease activity by activity index: SLEDAI-2H (Systemic Lupus Erythematosus Disease Index).
3. Assessment of the probable prognosis of the disease and its severity.
4. Choice of therapy.

Globally, in recent years, it has been proven that some patients with SLE are characterized by increased expression of type I IFN-regulated genes. A large number of human genes (up to 10%) are under the control of type I IFN. Genes regulated by IFN of type I and type II overlap widely and are believed to contribute to the regulation of gene expression in SLE patients (Batliwalla et al. 2009; Botto 2016). In 2017, a test method was proposed that allows for a 5000-fold increase in sensitivity and thus detects significantly high levels of circulating IFN-a in the serum of patients with SLE.

IL-1 is a major inducer of type II IFN is IL-18 (a cytokine of the IL-1 family) and has been proposed as a biomarker of disease activity assessment. 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 in the assessment of SLE activity. ST2/IL-1R4 mediates IL-33 signaling; the soluble form of the receptor prevents the interaction of IL-33 with the membrane. Soluble IL (sIL)-1R4 levels are increased in active SLE and strongly correlate with disease activity index and with levels of anti-dsDNA and anti-C1q antibodies (Leffler et al. 2014). When the diagnostic value of sIL-1R4 was assessed directly 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 (Botto 2016). Examination of gene expression profiles in peripheral blood mononuclear cells of patients with SLE revealed a striking pattern of increased expression of IFN-inducible genes (hereafter referred to as the "IFN signature"). The IFN signature predicted more severe disease, such as cerebritis and nephritis, and hematologic involvement in these patients. This information can give us answers to the questions for Bulgarian patients with SLE:

- whether changes in IFN signature expression correlate with
- attacks of the disease
- new involvement of organs and systems
- response to treatment in patients with SLE? (Batliwalla et al. 2009).

Other soluble biomarkers of disease activity that will provide useful information are:

- soluble cytokine receptors (sIL-2 receptor),
- soluble adhesion molecules (sICAM and sVCAM).

Other important players in inflammation in affected organs are cell adhesion molecules, which are expressed on the vascular endothelium and interact with leukocyte integrins, allowing their extravasation into inflamed tissues.

In lupus nephritis, expression of vascular cell adhesion molecule 1 (VCAM-1) is upregulated in glomerular epithelial cells, mesangium, and proximal tubular cells; activated leukocyte CAM (ALCAM) is also overexpressed on macrophages and glomerular endothelium. Urinary VCAM-1 and ALCAM are elevated in active lupus nephritis and can distinguish active renal involvement from active non-renal disease. Other adhesion molecules emerging as biomarkers in lupus nephritis are E-selectin and ICAM, which are also detected in urine by a sensitive and specific rapid assay (Aringer et al. 2019; Leffler et al. 2014).

Objectives of the study

To conduct a study of biomarkers of disease activity in the SLE hospital and to establish the relationship between laboratory biomarkers and the clinical and immunological activity of the SLE hospital.

Materials and methods

1. Taking an anamnesis about the course of SLE, obtaining information about age, gender, duration of the disease, and therapy carried out.
2. Evaluation of disease activity by activity index: SLEDAI-2H (Systemic Lupus Erythematosus Disease Index).
3. Blood collection for marker research and serum analysis: human IFN-gamma, human IFN-alpha, human sICAM-1, human sVCAM-1, human sIL-2R, and human IL-12p40/p70.
4. Final: statistical processing of the data, shaping of the final scientific product, realization of publications, and preparation of the scientific report.
5. The statistical processing was done with SPSS 19.0. Descriptive analysis was performed with an estimation of the arithmetic mean and standard deviation. An analysis of the difference in means was performed, and the statistical significance of the differences was tested using models for testing the mean difference (ANOVA, t-test). The results of these models are detected with applied non-parametric analogues (Kolmogorov-Smirnov Z test), the aim being to eliminate possible influence from non-compliance with the requirements of the parametric models and at the same time to use a maximally strong parametric statistical method. For evaluation of tested correlation dependences, correlation analysis was applied using Pearson's coefficients, which were validated with analogues—Spearman's coefficients.

To estimate the level of significance of certain empirical characteristics, levels based on assumptions about the distribution of the tested characteristics are used. 0.05 is taken as the limit value for the level of significance. The respective significance estimates of a particular empirical feature of the tests described above are compared with this cut-off 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

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 hours. The main therapy for the disease in 28 of the patients with SLE was with hydroxychloroquine, 200 to 400 mg daily. Nine patients with SLE were treated with azathyoprine 100 mg daily; four patients were treated with methotrexate 15 mg weekly; and four patients were treated with methylprednisolone and endoxan 1 gram monthly. All patients accept methylprednisolone at a daily dose of 4 to 16 mg. Patients with increased values of anti-dsDNA are 27.1%.

The results of the quantification of human IFN-gamma showed an average concentration of 0.4 pg/ml for SLE patients and 0.32 pg/ml for healthy individuals, $p > 0.05$.

The concentration of human IFN-alpha in patients with SLE is statistically insignificantly higher compared to healthy individuals: 14.91 pg/ml (min 0 max 628.05 pg/ml) versus 7.94 pg/ml (min 0 max 218.85 pg/ml) – $p < 0.05$ (Fig. 1).

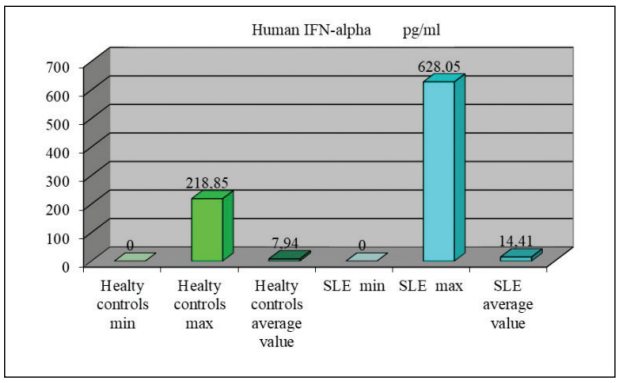

Figure 1. Results of the quantification of human IFN-alpha.

The results for the concentration of human sICAM-1 for the group of patients with SLE were 438.36 ng/ml (min 205.3 max 958.5 ng/ml), and for the healthy subjects it was an average of 387.05 ng/ml (min 208.8 max 597.3 ng/ml), $p > 0.05$.

The results for the concentration of human sVCAM-1 for the group of patients with SLE were 1060.66 ng/ml (min 151 max 3072 ng/ml), and for the healthy subjects it was an average of 384.38 ng/ml (min 373 max 1740.5 ng/ml) – $p < 0.05$ (Fig. 2).

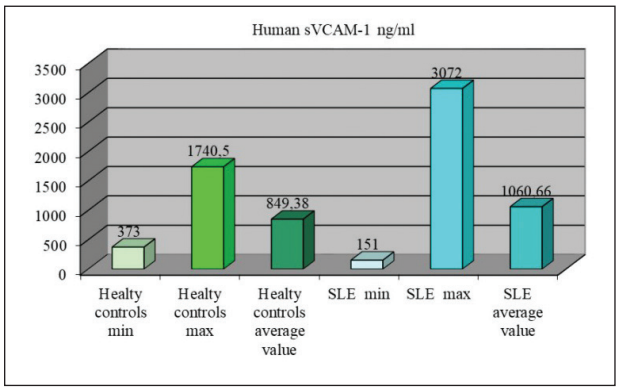

Figure 2. Results for the concentration of human sVCAM-1.

The results for the concentration of human sIL-2R Biovendor in the group of SLE patients averaged 4.81 ng/ml (min 1.7 max 11.34 ng/ml) and 3.76 ng/ml (min 1.18 max 8.32 ng/ml) for the control group, $p < 0.05$ (Fig. 3).

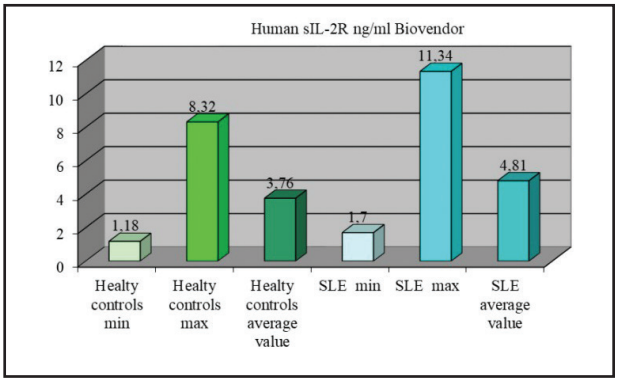

Figure 3. Results for the concentration of human sIL-2R.

The results for the concentration of human IL-12p40/p70 had an average value of 157.61 pg/ml (min 0 pg/ml max 1231.94 pg/ml) for the group of patients with SLE and 108.78 pg/ml (min 0 pg/ml max 401.46 pg/ml) for the control group; the difference between the two groups is significant $p > 0.05$ (Fig. 4).

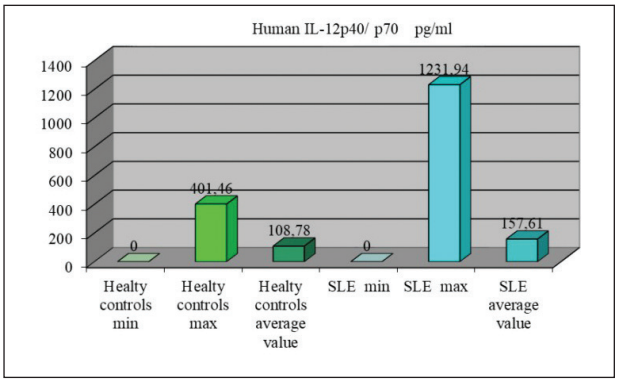

Figure 4. Results for the concentration of human IL-12p40/p70.

The correlation analysis between disease activity assessed with SLEDAI-2K and biomarkers did not confirm a statistically significant relationship (Table 1).

Table 1. Correlation analysis between SLEDAI-2K and biomarkers.

		SLEDAI-2K*
Human IFN-gamma pg/ml	Pearson Correlation	-0.206
	Sig. (2-tailed)	0.161
Human IFN-alpha pg/ml	Pearson Correlation	-0.109
	Sig. (2-tailed)	0.463
Human sICAM-1 ng/ml	Pearson Correlation	-0.189
	Sig. (2-tailed)	0.198
Human sVCAM-1 ng/ml	Pearson Correlation	0.114
	Sig. (2-tailed)	0.441
Human sIL-2R ng/ml Biovendor	Pearson Correlation	0.009
	Sig. (2-tailed)	0.954
Human IL-12p40/ p70 pg/ml	Pearson Correlation	0.065
	Sig. (2-tailed)	0.659

Discussion

Lupus is associated with multisystem inflammation resulting from an abnormal immunological response. Recent years have seen significant advances in understanding of inflammation and, in particular, the role of the interaction between the vascular endothelium, mediators, and immune effector cells. Anti-endothelial cell antibodies, cellular adhesion molecules, soluble forms of adhesion molecules, chemokines, and cytokines directly or indirectly affect endothelial cells, causing inflammatory damage to the vessel wall, as their roles have been discussed. Analysis of our results showed a statistically significant difference in results for human sVCAM-1 and sIL-2R.

P. Cieslik et al. (2008) found that in SLE patients with vasulitis, plasma sVCAM-1, soluble E-selectin, and sICAM-1 levels are increased (Cieslik et al. 2008). Pizzaro et al. (2007) found that the concentration of sVCAM-1 is increased in SLE and is associated with the activity of the indicated and especially renal damage. In patients with nosocomial, hematological, and vascular activity, the concentration of sVCAM-1 was elevated and statistically significant. In our study, a quantitatively higher concentration of human sICAM-1 was found in patients with SLE compared to healthy individuals (Pizzaro et al. 2007).

Sponk et al. (1994) confirms that the concentrations of sICAM-1 and sE-selectin do not increase and remain within normal limits in all examined patients and exacerbations. sVCAM-1 concentrations increase in parallel with disease activity during SLE exacerbations. Hajialino et al. (2018) found higher serum concentrations of sICAM-1 and endothelin-1 in 60 SLE patients compared to healthy controls (Hajialino et al. 2018). There were no significant correlations between serum concentrations and organ involvement or disease activity (Sponk et al. 1994).

Interferon-α (IFNα) is a primary pathogenic factor in systemic lupus erythematosus (SLE), and high IFNα levels may be associated with particular clinical manifestations. The prevalence of individual clinical and serologic features differs significantly by race. This study indicates that serum IFNα activity is strongly and consistently associated with autoantibodies and not independently associated with clinical features in SLE. IFNα may be more relevant to humoral tolerance and initial pathogenesis than later clinical disease manifestations. IFN-α signaling can be suppressed by different strategies: direct neutralization by an anti-IFN-α antibody or suppression of IFN-α synthesis using an anti-IFN-α receptor antibody. Our study confirms that the presence of an increased concentration of human IFN-alpha in the serum of patients with SLE is an indicator of increased disease activity (Weckerle et al. 2011; Wiedeman 2012).

In our study, there was no statistically significant difference in the concentration of human IFN-alpha, human IFN-gamma, human sICAM-1, and human IL-12p40/p70 between SLE patients and healthy individuals. They can be increased in some autoimmune conditions, but in this case, they have no sensitivity in the examined patients from the Bulgarian population. The increased concentration of human IL-12 p40/p70 in the serum of patients proves its key role in the induction of disease activity in

autoimmune processes. Lauwervs and Van Snick (2011) found that biologically active IL-12 is a 70 kDa heterodimeric cytokine (IL-12 p70) produced mainly by antigen-presenting cells and composed of disulfide-linked a (p35) and b (p40) chains. Production of the p40 subunit is regulated independently of that of IL-12 p70. Serum p40 titers were significantly higher in patients with SLE compared with patients with rheumatoid arthritis ($P < 0.0001$) or controls. IL-12 p70 was not detected in any healthy serum. Serum p40 monomers (but not IL-12 p70 titers) are elevated in SLE patients, commensurate with disease activity (Lauwervs and Van Snick 2011).

The study of biomarkers in patients with SLE in Bulgaria shows that human sVCAM-1 and human sIL-2R are important biomarkers for identifying and predicting the beginning or end of the exacerbation of the disease. They serve for clinical assessment of disease activity and the correct therapeutic approach. The biggest challenge in identifying and developing specific biomarkers for SLE is its complex etiopathogenesis and clinical heterogeneity.

Long et al. (2022) confirms that the concentration of serum sIL-2Rα in patients with SLE is elevated and closely related to disease activity. Elevated sIL-2Rα in the peripheral blood of patients with SLE is associated with an imbalance of immune regulation. Soluble IL-2Rα is a promising target for the treatment of SLE in the future (Long et al. 2011).

Biomarkers human IFN-alpha, human IFN-gamma, human sVCAM-1, and human IL-12 p40/p70 may be informative at different points in the disease process, such as at diagnosis, during exacerbation, at damage assessment of end organs, or when assessing response to treatment. A „Lupus Biomarker Panel“ needs to be validated, which will include assays for individual molecules as well as „molecular biomarkers“ (Bethesda 2017). A very good theoretical preparation and clinical experience are necessary for the full treatment of patients with SLE.

Conclusion

Human sVCAM-1 and sIL2r are sensitive biomarkers of disease activity in SLE. They are important and sensitive biomarkers to identify and predict the onset or end of disease exacerbations. The studied biomarkers for SLE activity can be used to assess the clinical status of SLE in the Bulgarian population. Each patient with SLE must be considered individually due to individual heterogeneity in clinical manifestations, degree of disease progression, and perhaps pathogenetic mechanisms.

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