**Is Neuroserpin a New Biomarker in Patient with Rheumatoid Arthritis?**

Sevil ARABACI TAMER, Gönül GUROL, Ibrahim TEKEOGLU, Halil HARMAN, and Ihsan Hakkı CIFTCI

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**Abstract**— Rheumatoid arthritis (RA) which is an autoimmune inflammatory disease characterized by inflammation of the synovial tissue in joints. Inflammatory markers are used clinical evaluation of patients with RA. Neuroserpin (NS) is an inhibitor of tissue-plasminogen-activator and it shows neuroprotective and anti-inflammatory activity. So, the aim of this study investigate the association between NS and disease activity in RA. Selected patients were divided into four groups based on their DAS28 scores: remission group (RG), (DAS28 < 2.6); low disease activity group (LDAG), (DAS28 > 2.6–3.2); moderate disease activity (MDAG), (DAS28 > 3.2–5.1); high disease activity group (HDAG), (DAS28 > 5.1). Healthy subjects (HS) served as controls. Our results demonstrate, for the first time, that NS is significantly different in RA patients relative to healthy subjects (P = 0.014) and it shows correlation with ESR and CRP (p=0.005). So, we recommend that NS may be associated with inflammation in RA.

**Keywords**— Inflammation, Neuroserpin, Tissue plasminogen activator, Rheumatoid arthritis

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**I. INTRODUCTION**

RHEUMATOID arthritis (RA) which is an autoimmune inflammatory disease characterized by inflammation of the synovial tissue in joints can cause joint destruction [1],[2]. The prevalence of rheumatoid arthritis ranges from 0.5-1.0% in the entire world. The annual incidence of RA in most European countries ranges from approximately 0.4 to >2.5 per 1,000 adults, increasing with age [1]. Elevated concentrations of inflammatory mediators are characteristic of autoimmune disease accompanied by chronic or recurrent inflammation [3].

In a patient with inflammatory arthritis, the presence of a rheumatoid factor (RF), anti-citrullinated protein (Anti-CCP) antibody, elevated C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) suggests a diagnosis of rheumatoid arthritis [4]. Rheumatoid factor (RF), which is the immunologic hallmark of RA, is not specific for RA, as it is found in 5% of healthy individuals and in 10-20% of those over the age of 65 years [5]. Both RF and anti-CCP are regarded as serological markers of RA [6],[7]. Anti-CCP antibodies has demonstrated a similar sensibility (55-80%), but a higher specificity (96-98%) for RA [8]. Hence, anti-CCP antibodies have been included in the RA classification criteria [9]. In addition, CRP is a highly conserved protein belonging to the pentraxin family, and a key component of the acute-phase response to infection and inflammation. CRP with the widespread clinical use is a sensitive but nonspecific marker of inflammation [10],[11]. However, CRP concentration is commonly used in RA as a biomarker of systemic inflammation, and included as a surrogate marker of disease activity [12]. Patients with RA suffer from the increased CRP levels leading to platelet activation and thrombosis [13]. The serine protease tissue plasminogen activator (tPA) activates plasminogen to its active form of plasmin, which subsequently degrades fibrin [14]. Patients affected by RA present an increased risk of thromboembolism which is an important cause of morbidity and mortality [15]. During thrombus formation, tPA is inhibited by neuroserpin and PAI-1 which released from platelets [16],[17].

Neuroserpin (NS) is an inhibitor of tPA, with a role in physiological processes such as synaptic plasticity and memory [18],[19]. Also NS has shown neuroprotective effects in animal models of cerebral ischemia. The neuroprotective properties of neuroserpin may be related to the inhibition of excitotoxicity, inflammation, as well as blood brain barrier disruption that occur after acute ischemic stroke [20]. Moreover, NS possesses anti-inflammatory activity in systemic arteries [21].

To the best of our knowledge, the association of neuroserpin with indices of disease severity has not been studied in patients with RA. So, the present study investigated the relationship between NS clinical activity of disease in patients with RA.

**II. MATERIALS AND METHODS**

All patients (40 women, 10 men) participating in this study were admitted to the rheumatology outpatient facility at the Medical Faculty Hospital, Sakarya University, in October 2014. RA diagnosis was based on the diagnostic criteria revised in 2010 by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [9]. Ten healthy individuals admitted to our outpatient hospital for a checkup were selected as healthy subjects. All patients are seropositive. Patients who did not meet the criteria for RA; were treated for another rheumatic disease in the past; had a history of diabetes mellitus, renal...
failure, or liver failure; and were on TNF blockers, were excluded from the study. Disease activity was determined by Disease Activity Score 28 (DAS28), which was calculated using results from clinical and laboratory data. DAS28 remission criteria, including levels of CRP, swollen and tender joint counts, and global health assessment, were used to determine whether the disease was in remission. A DAS28 score between 2.6–3.2 indicates low disease activity, 3.2–5.1 indicates moderate activity, and > 5.1 indicates high disease activity [22].

Selected patients were then divided into four groups based on their DAS28 scores: remission group (RG), 8 patients (DAS28 < 2.6); low disease activity group (LDAG), 7 patients (DAS28 > 2.6–3.2); moderate disease activity group (MDAG), 14 patients (DAS28 > 3.2–5.1); high disease activity group (HDAG), 11 patients (DAS28 > 5.1). Ten healthy subjects (HS) served as controls. Patient demographic data, including age, gender, smoking habit, and clinical data, such as duration of symptoms, duration of disease, and delay in diagnosis, were recorded.

Rheumatoid factor (RF) was measured by nephelometry (Beckman Coulter IMMAGE® 800, USA); levels greater than 20 U/mL were considered positive. Anti-Cyclic Citrullinated Peptide (anti-CCP) levels were measured using an enzyme linked immunosorbent assay (ELISA; Abbott Diagnostics, USA); those greater than 0.5 U/mL were considered positive. NS (Eastbiopharm, China) was assessed by ELISA from serum samples.

A. Ethics approval

Ethics approval was obtained from the local Ethics Committee of Sakarya University.

B. Statistical analysis

Statistical analysis was performed using SPSS software, version 20.0 (IBM SPSS statistics). Values are presented as the mean ± standard deviation (SD). Differences between groups and healthy subjects were determined by one-way analysis of variance (ANOVA). Correlation coefficients between ESR, Anti-CCP and CRP with RFIgG and NS using Pearson’s test at 95 % confidence were determined. P values less than 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curve was used for determining the sensitivity and specificity of the laboratory markers.

III. RESULTS

Age, sex, and smoking habits were similar between RA patients and HS. Average times of disease duration (DD), duration of symptoms (DS), and delay in diagnosis (DED) were 41.26 ± 36.95, 53.04 ± 38.18, and 6.92 ± 5.01 months, respectively. These results and clinical characteristics of RA patients are demonstrated in Table I. DD and DS were determined to be significantly different among study groups (P < 0.001); however, delay in diagnosis did not significantly differ among groups (Table. II).

A significant difference between HS and patients in erythrocyte sedimentation rate (ESR) values was observed in Fig. 1a (P < 0.001). Within groups, CRP values were significantly different (Fig. 1b). Moreover, a significant difference was revealed among MDAG individuals and HS (P = 0.001). Patients with RA had significantly higher RF values (P < 0.001) than HS. Anti-CCP levels of RG compared to MDAG were different, but not statistically significant (P = 0.071). In addition, anti-CCP levels were significantly different (P = 0.008) in HS compared to patient groups (Fig. 1c). When HS were compared to MDAG and HDAG patients, serum levels of NS were significantly different (P = 0.008 and 0.031, respectively). All RA groups were significantly different from HS (P = 0.014). Fig. 1d shows the relationship between neuroserpin levels and stage of disease activity.

![Fig. 1 Relationship between ESR(a), CRP(b), Anti-CCP(c) and neuroserpin(d) levels and disease activity](image)

Also, NS showed correlation with ESR and CRP (p=0.005) (Table. III). The ROC curves for parameters were drawn and area under the curve for these tests was measured. The area under the curve for NS was 0.834±0.05, RFIgG was
Anti-CCP was 0.988±0.012 (Table IV, Fig. 2).

### TABLE III
CORRELATION COEFFICIENTS BETWEEN ESR AND CRP WITH RF IgG AND NEUROSERPIN IN RA PATIENTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RF IgG</th>
<th>Anti-CCP</th>
<th>Neuroserpin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>0.292*</td>
<td>-0.170</td>
<td>0.296*</td>
</tr>
<tr>
<td>CRP</td>
<td>0.099</td>
<td>-0.209</td>
<td>0.287*</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficient (r) at 95 % confidence was significant at 0.05 levels (two-tailed).

### TABLE IV
ACCURACY ANALYSIS OF THE LABORATORY PARAMETERS IN RA PATIENTS

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Std. Error</th>
<th>Asymptotic Sig.</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroserpin</td>
<td>0.834</td>
<td>0.050</td>
<td>0.000</td>
<td>0.737</td>
<td>0.932</td>
</tr>
<tr>
<td>RF IgG</td>
<td>0.969</td>
<td>0.018</td>
<td>0.000</td>
<td>0.922</td>
<td>1.000</td>
</tr>
<tr>
<td>ESR</td>
<td>0.987</td>
<td>0.012</td>
<td>0.000</td>
<td>0.900</td>
<td>1.000</td>
</tr>
<tr>
<td>CRP</td>
<td>0.711</td>
<td>0.068</td>
<td>0.025</td>
<td>0.579</td>
<td>0.844</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>0.988</td>
<td>0.012</td>
<td>0.000</td>
<td>0.900</td>
<td>1.000</td>
</tr>
</tbody>
</table>

The test result variable(s): Neuroserpin, ESR, CRP has at least one tie between the positive actual state group and the negative actual state group.

a. Under the nonparametric assumption,
b. Null hypothesis: true area = 0.5

Fig. 2 ROC curve of laboratory parameters in RA patients

### IV. DISCUSSION

Our results demonstrate, for the first time, that NS levels are significantly different in RA patients relative to HS (P = 0.014). There are many studies lately related to the activity of plasminogen in patients with rheumatoid arthritis effect in the literature. Tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) are the two main proteases responsible for cleaving the inactive plasminogen into active plasmin. tPA activity are influenced by endogenous serine protease inhibitors, including plasminogen activator inhibitor-1 (PAI-1), protease nexin-1 (PN-1) and neuroserpin [23]. tPA play an important role in the modulation of inflammation [24]. Kopeikina et al.[25] reported that significant elevation of t-PA and PAI-1 were found in RA patients compared to healthy subjects. Also, The up-regulated expression of PAI-1/PAI-2 in synovial fluid and plasma were significantly higher in RA than healthy subjects, suggesting PAI-1 may play an important role in the pathogenesis of RA [26]. The involvement of inflammatory mediators in PAI-1/t-PA imbalance was proposed from the relation of fibrinolytic abnormalities with the activity of systemic inflammatory process [25].

Neuroserpin is a member of the serine protease inhibitor or serpin superfamily of proteins. It is widely expressed in the central nervous system, has been recently detected in different organs such as pancreas, heart, kidney and testis and plays an important role in the regulation of tissue plasminogen activator [27],[28]. NS is associated with many neurological diseases. In Alzheimer’s disease, NS has been shown to be up-regulated and it is linked to elevated thyroid hormone receptor-β1 and plays an important role both in the accumulation of brain amyloid plaques and loss of cognitive abilities [29],[30]. Wan et al.[31] showed that NS was upregulated in the injury area of spinal cord, accompanied with the activation of microglia and tPA, which suggested that NS also plays a neuroprotective role in spinal cord compression. Neuroprotective properties of neuroserpin may be related to the inhibition of excitotoxicity, inflammation, as well as blood brain barrier disruption that occur after acute ischemic stroke [20]. NS also as a regulator of neuronal development through a non-inhibitory mechanism and suggest a basis for neuroserpin's effects on complex emotional behaviors and recent link to schizophrenia [32]. NS possesses anti-inflammatory activity in systemic arteries, modifies T helper cell responses, and significantly reduces plaque formation in mouse aortic allografts [21]. As far as we know, NS has not been studied in patient with RA. So, our results demonstrate for the first time in the literature that NS may serve as an inflammatory marker in RA patients.

In addition to these findings, ESR values were significantly different when compared to patients and the healthy subjects (p<0.001). CRP values of HDAG in respect to RG were different (p=0.002). Patients with RA had significantly higher RF and anti-CCP values compared to the healthy subjects’ levels (p = 0.008, p <0.001 respectively).

After exposure to inflammatory stimuli, endothelial cells in virtually every tissue express PAI-1 [33]. Wällberg-Jonsson et al. [34] reported hemostatics factors of endothelial origin, i.e., PAI-1 mass, D-dimer, vWF, circulating immune complexes, and cardiolipin (IgM, IgA) correlated with ESR, and, with the exception of vWF, to accumulated disease activity in rheumatoid arthritis. It was shown that circulating immune complexes correlated significantly with IgM antibodies against oxidized low density lipoprotein and cardiolipin. It has been reported that while healthy endothelium defence mechanisms cease under risk factors and inflammation, they inducing by proinflammatory cytokines such as interleukin-(IL) 1beta and...
tumor necrosis factor-(TNF) alpha, by CRP, and CD40/CD40 ligand interactions express adhesion molecules. Recent studies have shown higher levels of mass concentrations of tPA and PAI-1 in addition to higher levels of VWF, sVCAM-1, sICAM-1 and sE-selectin in RA patients compared with healthy controls [35]-[37].

V. CONCLUSION

NS may be associated with RA disease activity. So our results demonstrated that this association may be due to inflammatory pathway. We may suggest that NS is a new biomarker in determination of RA disease activity.

ACKNOWLEDGMENT

All authors are involved in the manuscript preparation. The authors thank the Sakarya University Research Project Committee for financial support of the project.

REFERENCES


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**Table II**

**DEMOGRAPHIC AND LABORATORY PARAMETERS AMONG STUDY GROUPS**

|   | HS     | RG     | LDAgger | MDagger | HDagger | p
---|--------|--------|---------|---------|---------|---
| n (%) | 10 (8.5%) | 16 (21.5%) | 16 (21.5%) | 28 (37.4%) | 15 (20%) | < 0.001
| DS*  | NA     | 32.08 ± 14.66 | 31.78 ± 21.42 | 48.71 ± 26.87 | 93.66 ± 54.15 | < 0.001
| DD*  | NA     | 28 ± 16.94 | 26.81 ± 21 | 39.89 ± 23.09 | 84.86 ± 54.38 | < 0.001
| DED* | NA     | 6.43 ± 4.83 | 7.06 ± 3.64 | 7.78 ± 5.25 | 9.93 ± 5.73 | 0.23
| ESR¹ | 7.8 ± 3.94 | 23.41 ± 8.42 | 27.32 ± 7.81 | 33.12 ± 11.37 | 46.76 ± 14.89 | < 0.001
| CRP³ | 4.58 ± 2.53 | 6.09 ± 3.72 | 8.79 ± 7.82 | 9.49 ± 6.89 | 15.70 ± 5.46 | 0.001
| RF IgG¹  | 16.47 ± 2.46 | 91.93 ± 100.06 | 154.97 ± 232.21 | 207.97 ± 235.98 | 396.43 ± 265.48 | < 0.001
| anti-CCP¹   | 3.92 ± 1.22 | 116.24 ± 122.32 | 203.51 ± 228.18 | 331.80 ± 386.64 | 162 ± 174.60 | 0.008
| NSP¹  | 12.16 ± 1.5 | 14.28 ± 2.40 | 15.48 ± 4.14 | 16.11 ± 2.42 | 18.08 ± 3.47 | 0.014

*Presented data were sorted according to mean ± SD (months). *Range in women 10 mm/h; range in men 20 mm/h. *Range: 1–960 mg/L. *U/mL, *ng/mL. *One-way ANOVA (within groups). NA: not applicable.