Is Neuroserpin a New Biomarker in Patient with Rheumatoid Arthritis?

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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

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-1which 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

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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 (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 demostrated 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).

TABLE I
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY GROUPS

Characteristics	RA patients	HS
Age, mean ± SD (years)	47.04 ± 10.51	45.9 ± 10.64
Sex (% women)	80.0	20.0
Cigarette smoking (%)	34.7	40.0
DS, mean ± SD (months)	53.04 ± 38.18	NA
DD, mean ± SD (months)	41.26 ± 36.95	NA
DED, mean ± SD (months)	6.92 ± 5.01	NA

NA, not applicable; HS, healthy subjects; DD, disease duration; DS, duration of symptoms; DED, delay in diagnosis

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.

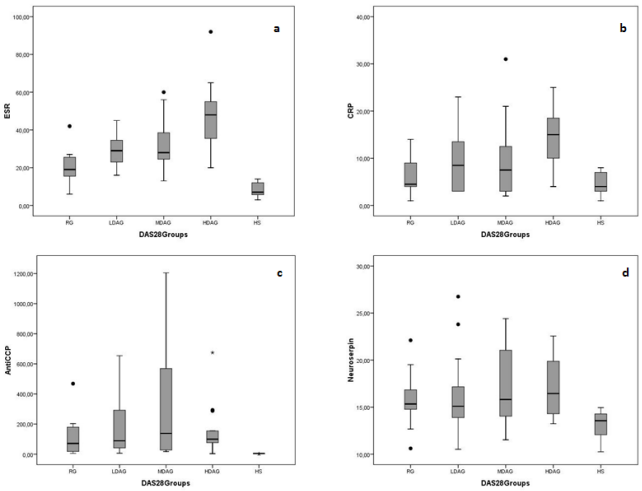

Fig. 1 Relationship between ESR(a),CRP(b),Anti-CCP(c) and neuroserpin (d) levels and disease activity

Also, NS showed correlation with ESR and CRP (p=0,005) (Table. III). The ROC curves for paramethers 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

0,969±0.18, ESR was 0,987±0.012, CRP was 0,711±0.068, 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

Parameter	RFIgG	Anti-CCP	Neuroserpin
ESR	0,292*	-0,170	0,296*
CRP	0,099	-0,209	0,287*

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

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Neuroserpin	,834	,050	,000	,737	,932
RFIgG	,969	,018	,000	,922	1,000
ESR	,987	,012	,000	,000	1,000
CRP	,711	,068	,025	,579	,844
AntiCCP	,988	,012	,000	,000	1,000

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

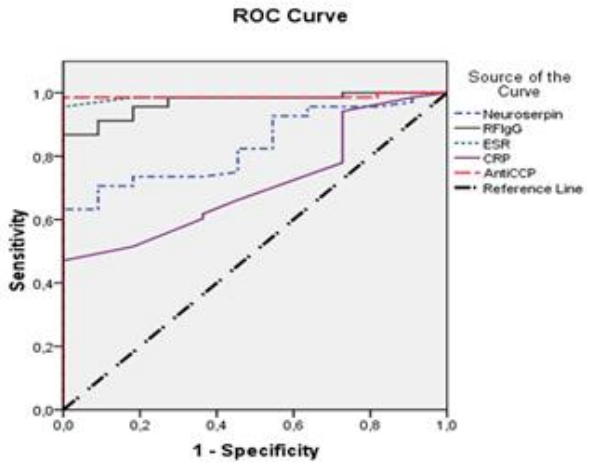

Fig.2 ROC curve of laboratory parameters in RA patients

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 micorglia 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]. Wållberg-Jonsson et al. [34] reported hemostatic 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.

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References

[1] M. S. Drutskaya, G. A. Efimov, R.V. Zvartsev, A. A. Chashchina, D.M. Chudakov, S.V. Tillib, et al., "Experimental Models of Arthritis in Which Pathogenesis Is Dependent on TNF Expression, " Biochemistry (Mosc), vol. 79(12), pp. 1349-57, Dec 2014. http://dx.doi.org/10.1134/S0006297914120086

[2] G.M. Guidelli, T. Barskova, M.G. Brizi, G. Lepri, A. Parma, R. Talarico, et al., "One year in review: novelties in the treatment of rheumatoid arthritis", Clin Exp Rheumatol, Feb 2015. (Epub ahead of print)

[3] J.F. Solus , C.P. Chung, A. Oeser, C. Li, Y.H. Rho, K.M. Bradley, et al., "Genetics of serum concentration of IL-6 and TNFα in systemic lupus erythematosus and rheumatoid arthritis: a candidate gene analysis", Clin Rheumatol, Feb 2015. (Epub ahead of print) http://dx.doi.org/10.1007/s10067-015-2881-6

[4] A.M. Wasserman, "Diagnosis and management of rheumatoid arthritis", Am Fam Physician, vol. 84(11), pp. 1245-52, Dec 2011.

[5] S.Sh. Farid, G. Azizi, and A. Mirshafiey, "Anti-citrullinated protein antibodies and their clinical utility in rheumatoid arthritis", Int J Rheum Dis, vol. 16(4), pp. 379-86, Aug 2013. http://dx.doi.org/10.1111/1756-185X.12129

[6] J. Avouac, L. Gossec, and M. Dougados, "Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review ", Ann Rheum Dis, vol. 65(7), pp. 845-51, Jul 2006. http://dx.doi.org/10.1136/ard.2006.051391

[7] G.J. Pruijn, A. Wiik, and W.J. van Venrooij "The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis ", Arthritis Research & Therapy, vol. 12, pp. 203, Feb 2010. http://dx.doi.org/10.1186/ar2903

[8] S. Dubucquoi, and E. Solau-Gervais, "Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases ", Ann Rheum Dis, vol. 63, pp. 415–419, Apr 2004. http://dx.doi.org/10.1136/ard.2003.008623

[9] D. Aletaha, and T. Neogi, "Rheumatoid arthritis classification criteria: an American College of Rheumatology /European League Against Rheumatism collaborative initiative ", Arthritis Rheum, vol. 62, pp. 2569–2581, Sep 2010 http://dx.doi.org/10.1002/art.27584

[10] M.B. Pepys, and G.M. Hirschfield, " C-reactive protein: a critical update ", J Clin Invest, vol. 111(12), pp. 1805-12, Jun 2003. http://dx.doi.org/10.1172/JCI200318921

[11] C.G. Ammitzbøll, R. Steffensen, M. Bøgsted, K. Hørslev-Petersen, M.L. Hetland, P. Junker, et al., " CRP genotype and haplotype associations with serum C-reactive protein level and DAS28 in untreated early rheumatoid arthritis patients ", Arthritis Res Ther, vol. 16(5), pp. 475, Oct 2014. http://dx.doi.org/10.1186/s13075-014-0475-3

[12] S. Tarp, E.M. Bartels, H. Bliddal, D.E. Furst, M. Boers, B. Danneskiold-Samsøe, et al., "Effect of nonsteroidal antiinflammatory drugs on the C-reactive protein level in rheumatoid arthritis: a meta-analysis of randomized controlled trials ", Arthritis Rheum, vol. 64(11), pp. 3511-21, Nov 2012. http://dx.doi.org/10.1002/art.34644

[13] V.F. Panoulas, G.S. Metsios, A.V. Pace, H. John, G.J. Treharne, M.J. Banks, et al., "Hypertension in rheumatoid arthritis ", Rheumatology, vol. 47, pp. 1286–1298, Sep 2008. http://dx.doi.org/10.1093/rheumatology/ken159

[14] G. Cesarman-Maus, and K.A.Hajjar, "Molecular mechanisms of fibrinolysis ", Br J Haematol, vol. 129, pp. 307–321, May 2005. http://dx.doi.org/10.1111/j.1365-2141.2005.05444.x

[15] A. Mameli, D. Barcellona, and F. Marongiu, "Rheumatoid arthritis and thrombosis ", Clin Exp Rheumatol, vol. 27(5), pp. 846-55, Sep-Oct 2009.

[16] H. Brogren, L. Karlsson, M. Andersson, L. Wang, D. Erlinge, and S. Jern, "Platelets synthesize large amounts of active plasminogen activator inhibitor 1 ", Blood, vol. 104, pp. 3943–3948, Dec 2004. http://dx.doi.org/10.1182/blood-2004-04-1439

[17] M.W. Gramling, and F.C. Church, "Plasminogen activator inhibitor-1 is an aggregate response factor with pleiotropic effects on cell signaling in vascular disease and the tumor microenvironment ", Thromb Res, vol. 125(5), pp. 377-81, May 2010. http://dx.doi.org/10.1016/j.thromres.2009.11.034

[18] G.A. Silverman, P.I. Bird, R.W. Carrell, F.C. Church, P.B. Coughlin, P.G. Gettins, et al., "The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature", J. Biol. Chem, vol. 276, pp. 33293–33296, Sep 2001. http://dx.doi.org/10.1074/jbc.R100016200

[19] R. Noto, M.G. Santangelo, M. Levantino, A. Cupane, M.R. Mangione, D. Parisi et al., "Functional and dysfunctional conformers of human neuroserpin characterized by optical spectroscopies and Molecular Dynamics ", Biochim Biophys Acta, vol. 1854(2), pp. 110-7, Feb 2015. http://dx.doi.org/10.1016/j.bbapap.2014.10.002

[20] R. Rodríguez-González, T. Sobrino, M. Rodríguez-Yáñez, M. Millán, D. Brea, E. Miranda, et al., "Association between neuroserpin and molecular markers of brain damage in patients with acute ischemic stroke ", J Transl Med, vol. 11, pp. 9:58, May 2011.

[21] G. Munuswamy-Ramanujam, E. Dai, L. Liu, M. Shnabel, Y.M. Sun, M. Bartee, et al., "Neuroserpin, a thrombolytic serine protease inhibitor (serpin), blocks transplant vasculopathy with associated modification of T-helper cell subsets ", Thromb Haemost, vol. 103(3), pp. 545-55, Mar 2010. http://dx.doi.org/10.1160/TH09-07-0441

[22] G. Wells, J.C. Becker, J. Teng, M. Dougados, M. Schiff, J. Smolen, et al., "Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate ", Ann Rheum Dis, vol. 68(6), pp. 954-60, June 2009. http://dx.doi.org/10.1136/ard.2007.084459

[23] M. Sashindranath, A.L. Samson, C.E. Downes, P.J. Crack, A.J. Lawrence, Q.X. Li, et al., "Compartment- and context-specific changes in tissue-type plasminogen activator (tPA) activity following brain injury and pharmacological stimulation ", Lab Invest, vol. 91(7), pp. 1079-91, Jul 2011. http://dx.doi.org/10.1038/labinvest.2011.67

[24] L. Lin, and K. Hu, "Tissue plasminogen activator and inflammation: from phenotype to signaling mechanisms", Am J Clin Exp Immunol, vol. 3(1), pp. 30-6, Feb 2014.

[25] L.T. Kopeikina, E.F. Kamper, V. Koutsoukos, Y. Bassiakos, and I. Stavridis, "Imbalance of tissue-type plasminogen activator (t-PA) and its specific inhibitor (PAI-1) in patients withrheumatoid arthritis associated with disease activity ", Clin Rheumatol, vol. 16(3), pp. 254-60, May 1997. http://dx.doi.org/10.1007/BF02238960

[26] C. Yang, and F. Huang, "Detection and significance of plasminogen activator inhibitor in synovial tissue, synovial fluid and plasma from

patients with rheumatoid arthritis ", Zhonghua Nei Ke Za Zhi, vol. 39(10), pp. 690-3, Oct 2000.

[27] D. Belorgey, J.A. Irving, U.I. Ekeowa, J. Freeke, B.D. Roussel, E. Miranda, et al., "Characterisation of serpin polymers in vitro and in vivo ", Methods, vol. 53(3), pp. 255-66, Mar 2011. http://dx.doi.org/10.1016/j.ymeth.2010.11.008

[28] J. Chéret, N. Lebonvallet, L. Misery, and C. Le Gall-Ianotto, "Expression of neuroserpin, a selective inhibitor of tissue-type plasminogen activator in the human skin ", Exp Dermatol, vol. 21(9), pp. 710-1, Sep 2012. http://dx.doi.org/10.1111/j.1600-0625.2012.01541.x

[29] S. Fabbro, K. Schaller, and N.W. Seeds, "Amyloid-beta levels are significantly reduced and spatial memory defects are rescued in a novel neuroserpin-deficient Alzheimer's disease transgenic mouse model ", J Neurochem, vol. 118(5), pp. 928-38, Sep 2011. http://dx.doi.org/10.1111/j.1471-4159.2011.07359.x

[30] B. Subhadra, K. Schaller, and N.W .Seeds, "Neuroserpin up-regulation in the Alzheimer's disease brain is associated with elevated thyroid hormone receptor-β1 and HuD expression ", Neurochem Int, vol. 63(5), pp. 476-81, Nov 2013. http://dx.doi.org/10.1016/j.neuint.2013.08.010

[31] S. Wan, Z. Feng, Z. Chen, X. Wang, Y. Cao, Y. Shao, et al., "Neuroserpin upregulates in the early period of sustained spinal cord compression ", Clin Lab, vol. 58(9-10), pp. 891-6. 2012.

[32] T.W. Lee, J.M. Montgomery, and N.P. Birch, "The serine protease inhibitor neuroserpin regulates the growth and maturation of hippocampal neurons through a non-inhibitory mechanism ", J Neurochem, vol. 121(4), pp. 561-74, May 2012. http://dx.doi.org/10.1111/j.1471-4159.2011.07639.x

[33] M.S. Sawdey, and D.J. Loskutoff, "Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta ", J Clin Invest, vol. 88(4), pp. 1346-53, Oct 1991. http://dx.doi.org/10.1172/JCI115440

[34] S. Wållberg-Jonsson, J.T. Cvetkovic, K.G. Sundqvist, A.K. Lefvert, S. Rantapää-Dahlqvist, "Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis ", J Rheumatol, vol. 29(5), pp. 875-82, May 2002.

[35] S.W. Jonsson, C. Backman, O. Johnson, K. Karp, E. Lundström, K.G. Sundqvist, et al., "Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis ", J Rheumatol, vol. 28(12), pp. 2597-602, Dec 2001.

[36] A. McEntegart, H.A. Capell, D. Creran, A. Rumley, M. Woodward, and G.D. Lowe, "Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis ", Rheumatology (Oxford), vol. 40(6), pp. 640-4, Jun 2001. http://dx.doi.org/10.1093/rheumatology/40.6.640

[37] P.H. Dessein, B.I. Joffe, and S. Singh, "Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis ", Arthritis Res Ther, vol. 7(3), pp. R634-43. Mar 2005. http://dx.doi.org/10.1186/ar1717

TABLE II
DEMOGRAPHIC AND LABORATORY PARAMETERS AMONG STUDY GROUPS

	HS	RG	LDAG	MDAG	HDAG	*P*[f]
n (%)	10 (8.5%)	16 (21.5%)	16 (21.5%)	28 (37.4%)	15 (20%)	
DS[a]	NA	32.08 ± 14.66	31.78 ± 21.42	48.71 ± 26.87	93.66 ± 54.15	< 0.001
DD[a]	NA	28 ± 16.94	26.81 ± 21	39.89 ± 23.09	84.86 ± 54.38	< 0.001
DED[a]	NA	6.43 ± 4.83	7.06 ± 3.64	7.78 ± 5.25	9.93 ± 5.73	0.23
ESR[b]	7.8 ± 3.94	23.41 ± 8.42	27.32 ± 7.81	33.12 ± 11.37	46.76 ± 14.89	< 0.001
CRP[c]	4.58 ± 2.53	6.09 ± 3.72	8.79 ± 7.82	9.49 ± 6.89	15.70 ± 5.46	0.001
RFIgG[d]	16,47 ± 2,46	91,93 ± 100,06	154,97 ± 232,21	207,97 ± 235,98	396,43 ± 265,48	< 0.001
anti-CCP[d]	3.92 ± 1.22	116.24 ± 122.32	203.51 ± 228.18	331.80 ± 386.64	162 ± 174.60	0.008
NSP[e]	12.16 ± 1.5	14.28 ± 2.40	15.48 ± 4.14	16.11 ± 2.42	18.08 ± 3.47	0.014

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