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Hemostasis Biomarkers in Multiple Sclerosis

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ABSTRACT

Objective: To investigate the plasma levels of hemostasis components in multiple sclerosis (MS) and their association with clinical and MRI outcomes.

Methods: We studied 138 MS patients (85 with relapsing-remitting, RR-MS and 53 with progressive, P-MS) with mean age of 54 years; 72.5% female; median EDSS 3.5; mean disease duration 21 years) and 42 age- and sex-matched healthy individuals (HI). All subjects were examined with 3T MRI and clinical examinations. Plasma levels of hemostasis factors (procoagulant, factor XII, FXII) and inhibitors (tissue factor pathway inhibitor (TFPI), thrombomodulin (TM), heparin cofactor II (HCII), disintegrin-like and metalloprotease with thrombospondin type 1 motif 13 (ADAMTS13), and plasminogen activator inhibitor 1 (PAI-1) were evaluated by magnetic Luminex assays and ELISA. Associations between hemostasis plasma levels and clinical and MRI outcomes were assessed.

Results: Lower ADAMTS13 levels were found in MS patients compared to HI (p=0.008), and in MS patients presenting with cerebral microbleeds compared to those without (p=0.034). Higher PAI-1 levels were found in MS patients compared to HI (p=0.02). TFPI levels were higher in the P-MS subgroup compared to RR-MS patients (p=0.011) and

compared to HI (p=0.002). No significant associations between hemostasis plasma levels and clinical or MRI outcomes were found.

Conclusions: Decreased ADAMTS13, particularly in MS patients with cerebral microbleeds, increased PAI-1 and TFPI levels were observed in MS patients, which deserves further investigation. No relationship between hemostasis plasma levels and measures of disease severity was detected.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) that causes irreversible and progressive accumulation of physical and cognitive disability.[1]

The pathogenesis of MS involves blood brain barrier (BBB) breakdown, extravasation of immune cells, inflammation and neurodegeneration in the CNS, which results in formation of lesions and development of brain atrophy.[2] MRI provides sensitive and selective biomarkers capable of quantitating the extent of BBB breakdown, lesion and atrophy accumulation in MS patients.

There is an increasing interest to explore in more detail the coagulation pathway in MS, which is an important physiological effector mechanism involved in the crosstalk between inflammation, immunity and neurodegeneration.[3, 4] Activation of the coagulation pathway triggers rapid proteolysis of a series of coagulation factors in the intrinsic and/or extrinsic pathways, that converge at the prothrombinase complex and eventually cause the fibrin clot (Figure 1). In particular, the inhibitors of hemostasis proteases such as plasminogen activator inhibitor 1 (PAI-1), tissue factor pathway inhibitor (TFPI), the thrombin inhibitor heparin cofactor II (HCII) and the main receptor for activation of the

anticoagulant protein C, thrombomodulin (TM), are key regulators of fibrinolysis and coagulation. Moreover, the disintegrin-like and metalloprotease with thrombospondin type 1 motif 13 (ADAMTS13), is a main regulator of von Willebrand factor-dependent primary hemostasis.[5-7]

Histopathological studies have reported extensive deposition of coagulation pathway proteins in MS lesions.[8-11] Factor XII (FXII), the initiator of the intrinsic pathway, is involved in adaptive immune responses in MS and FXII deposits are found near CD87+ dendritic cells in MS brain tissue.[10] Moreover, increased plasma FXII activity has also been reported in MS patients.[10] In MS, the extrinsic coagulation pathway is potentially activated upon BBB breakdown, which exposes perivascular and astrocyte tissue factor (TF) and leads eventually to thrombin generation and fibrin deposition.[12] TF and extensive fibrin deposition have been found in chronic active MS plaques.[8] Additionally, there is evidence for impaired clearance of fibrin in MS neurodegeneration, particularly in the late progressive stages of the disease.[9, 11]

PAI-1 levels have been reported to be higher in MS patient during exacerbations [13, 14] and genetic polymorphisms of PAI-1, linked to lower PAI-1 plasma levels, are associated with increased risk of developing MS.[15]

TM, a thrombin receptor that mediates protein C activation, and TFPI, which acts on the TF-activated factor VII complex, are coagulation inhibitors expressed on the vascular endothelium. Evidence for increased cerebrospinal fluid (CSF) TM synthesis in progressive MS patients was detected.[16] However, several other studies of plasma or serum TM in MS patients have yielded discordant results.[17-19] TFPI also suppresses production of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6, and increases production of the anti-inflammatory cytokine, interleukin-10.[20]

TFPI, HCII and ADAMTS13 have not been investigated systematically in MS. Moreover, previous studies have examined only single or a limited number of hemostasis components. The present study measured a panel of six hemostasis components, investigating their associations with clinical and MRI measures of disease severity in a large cohort of MS patients. We hypothesized that altered proteins levels would be associated with focal extravascular leakage of blood components that can be measured on MRI by evaluating cerebral microbleed (CMB) frequency and quantitative susceptibility mapping (QSM) values (an indirect measure of iron deposition) of deep gray matter (DGM) structures.

MATERIALS AND METHODS

Study Population

The study data were obtained from subjects who participated in a case-control study of cardiovascular, environmental and genetic risk factors for disease progression in patients with MS (CEG-MS study; IRB ID: MODCR00000352).[21] Inclusion and exclusion criteria are reported in the Supplement data.

All subjects underwent to neurological and MRI examinations and provided blood samples. The Expanded Disability Status Scale (EDSS) was assessed in MS patients. The data collected included demographic and clinical information. The study protocol was approved by the local Institutional Review Board and all participants gave their written informed consent.

Assays for Hemostasis Components

Hemostasis components were measured in EDTA plasma samples by analysts who were blinded to sample status.

FXII and HCII protein levels were measured using ELISA kits (LS-F10418, LifeSpan Biosciences, Seattle, WA, USA; CSB-E09492h, Cusabio, Wuhan, Hubei, China) following

the manufacturer's instructions. Total PAI-1 levels were assayed using Milliplex™ magnetic bead kits (human neurodegenerative disease panel 3, HNDG3MAG-36K, Merck Millipore, Germany) whereas ADAMTS13, TM and TFPI protein levels were similarly measured using custom-designed Luminex Screening Assays magnetic bead kits (Luminex R&D Systems Inc., Minneapolis, MN, USA). Data were acquired using the Luminex® 100 system and analyzed using Bioplex Manager Software version 6.0 (both from Biorad Laboratories, Hercules, CA). The calculated inter-assay coefficient of variations for ADAMTS13, FXII, HCII, TM, TFPI and PAI-1 were 2.1%, 4.3%, 9.6%, 3.0%, 4.5% and 5.7%, respectively.

MRI Acquisition and Image analysis

Subjects were examined on a General Electric 3T Signa Excite HD 12.0 scanner (Milwaukee, WI) using an eight-channel head and neck coil. Details of the acquisition protocol and MRI analyses are provided in the Supplement data.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences software (version 24, IBM Corp. Armonk, NY, USA).

The Kolmogorov–Smirnov test was used to test for normality of continuous variables. The Fisher's exact test was used to compare differences in categorical variables and Student's *t*-test was used to compare age and MRI measurements between total MS and HI groups.

The associations among the protein levels, and with demographic characteristics, EDSS and disease duration, were assessed with Spearman's rank correlation.

Comparisons of protein levels for MS vs. HI and relapsing-remitting-MS (RR-MS) vs. progressive-MS (P-MS) were conducted with the Mann–Whitney test. The associations of protein levels with clinical and MRI outcomes were assessed with the partial correlation using age and gender as covariates. The Kruskal-Wallis test, followed by Mann-Whitney test

was used to investigate whether various disease-modifying treatments (DMTs) are associated with protein levels.

Logistic regression analysis was used to determinate associations of hemostasis components with the presence of CMBs.

The Benjamini-Hochberg method was used to adjust for the multiple comparisons with a target false discovery rate of $q \leq 0.05$. The tables and results present the unadjusted p-values and adjusted p-values (q-values) for those associations when unadjusted p-values were ≤ 0.05 using two-tailed tests.

RESULTS

Demographic and Clinical Characteristics

The study included 138 total MS patients (85 RR-MS, 53 P-MS) and 42 HI. The demographic and clinical characteristics of the study sample are summarized in Table 1. For the purpose of the analyses, 46 secondary-progressive (SP) and 7 primary-progressive MS were categorized in P-MS group. The demographic characteristics of the MS and HI groups were similar. As expected, brain MRI measures (Table 2) were significantly different between the MS and HI groups.

Hemostasis Components Levels

The hemostasis components levels in the MS and HI groups are summarized in Figure 2. ADAMTS13 were lower in MS compared to HI (1548±481 ng/mL vs. 1733±562 ng/mL; p=0.008). PAI-1 levels were higher in the MS group compared to HI (121.1±64.9 ng/mL vs. 103.6±71.4 ng/mL; p=0.02). No significant differences in FXII, HCII, TFPI and TM levels were observed between MS and HI groups. However, TFPI levels were higher in the P-MS compared to RR-MS patients (45.1±16.3 ng/mL vs. 37.9±15.9 ng/mL, p=0.011) and compared to HI (36.0±11.8 ng/mL, p=0.002).

Association among Hemostasis Components

In MS, TFPI was positively associated with TM (r=0.24, p=0.004). PAI-1 was positively associated with FXII (r=0.28, p=0.001) and inversely correlated with HCII (r=-0.21, p=0.014).

These associations were not detected in HI (TFPI vs. TM r=0.134, p=0.398; PAI-1 vs. FXII r=-0.057, p=0.718; PAI-1 vs. HCII r=-0.081, p=0.608). In HI, trends for positive associations were found between TFPI and PAI-1 (r=0.33, p=0.032) and between TM and ADAMTS13 (r=0.34, p=0.027).

Clinical Associations of Hemostasis Components

The hemostasis components levels were not associated with EDSS nor disease duration.

No significant differences were detected according to the type of DMTs (Supplement Figure) in proteins levels (ADAMTS13, p=0.83; FXII, p=0.20; HCII, p=0.77; TFPI, p=0.10; TM, p=0.69; PAI-1, p=0.17). In addition, comparison between treated (n=111) vs. not treated (n=27) MS patients did not yield significant differences.

Associations of Hemostasis Components with Presence of Cerebral Microbleeds

CMBs were present in 8.7% of MS and 7.1% of HI (p=1.0). Association between hemostasis components and the presence of CMBs are reported in Table 3. ADAMTS13 showed significantly lower levels in MS patients with CMBs compared to those without (1257±459 ng/mL vs. 1566±479 ng/mL; p=0.034). ADAMTS13 levels in HI with CMBs were not significantly different than those without (1630±623 ng/mL vs. 1758±562 ng/mL; p=0.7). No significant associations were observed between coagulation inhibitors and number of CMBs.

Association of Hemostasis Components and MRI Measures

The associations between the hemostasis biomarker levels and MRI outcomes were evaluated (Supplement Tables 1 and 2). None of these associations were significant when adjusted for multiple comparisons.

In MS, higher FXII levels showed a trend for correlation with lower ventricular (r=-0.19, p=0.027, q=0.42) and higher DGM (r=0.18, p=0.047, q=0.42) volumes, while higher HCII levels showed a trend for correlation with lower brain (r=-0.21, p=0.017, q=0.42) and cortical (r=-0.18, p=0.046, q=0.49) volumes and higher DGM volume (r=0.19, p=0.034, q=0.42). Higher TFPI levels showed a trend for correlation with lower DGM volume (r=-0.18, p=0.041, q=0.42).

In HI, a trend for association between higher HCII levels and lower cortical volume (r=-0.36, p=0.024, q=0.43) was detected. Higher PAI-1 levels showed a trend for correlation with higher QSM of DGM (r=0.41, p=0.013, q=0.43).

DISCUSSION

In this paper, we measured the plasma levels of six key hemostasis components, FXII, PAI-1, TM, ADAMTS13, HCII and TFPI in a large cohort of MS patients and assessed their associations with clinical and MRI outcomes.

We found some limited evidence for dysregulation of associations among hemostasis protein levels in plasma. In MS patients, levels of TFPI and TM, both anticoagulant and anti-inflammatory proteins, were positively associated. Differently, PAI-1 was associated with higher levels of FXII and lower levels of HCII, which altogether are expected to enhance both deposition and stability of fibrin. These associations were not detected in HI.

Our study is the first to investigate HCII in MS patients. HCII exclusively inactivates thrombin by a complex with glycosaminoglycans, such as heparin, thus regulating both hemostasis and cellular effects of thrombin.[22] The cellular effects which are mediated by protease-activated receptors, include tube formation, migration and proliferation of endothelial cells. Hence, HCII is required for maintenance of angiogenesis.[23] We did not detect significant differences between MS and HI or in relation to DMTs and MRI outcomes.

FXII is a pro-coagulant component in the intrinsic coagulation pathway at the interface with innate inflammation.[10] FXII activity has been reported higher in RR-MS and SP-MS compared to HI, and it was associated with relapses and shorter relapse-free period, independently from immunomodulatory therapy.[10] However, we did not find differences, nor any association with DMTs, in FXII protein concentration, which is a potentially improved measure of the autoimmune function as compared to coagulation activity assays, which depends on several plasma factors.

We detected higher PAI-1 levels in MS patients compared to controls, as previously reported.[13, 14] Further, PAI-1 levels were not associated with clinical or MRI outcomes.

In addition we did not find any associations of clinical or MRI outcomes with TM, which has been suggested to confer protection from demyelination in a mouse model of MS.[24] The literature data on TM levels in MS are discordant.[17-19] As previously reported, we did not find differences in TM levels between MS vs. HI, but we failed to confirm an association between TM levels and more severe disability.[19] Despite the greater sample size in our study, we failed to confirm an association between GA and TM levels.[18]

We observed significantly higher TFPI levels in P-MS subgroup when compared to RR-MS and to HI, and an association between TM and TFPI in MS. These are novel data because TFPI has not been systematically investigated in MS. It is known that age influences the levels of hemostasis components.[25] Indeed, correlations of hemostasis components

levels with MRI outcomes were assessed using age as a covariate in our statistical analysis. Taking into account this aspect, our data should be interpreted with caution, as increased TFPI levels in P-MS may be in part due to the older age of this group.

In MS, we found lower levels of ADAMTS13, whose deficiency is associated with microangiopathic hemolytic anemia.[26] Acquired ADAMTS13 deficiency and thrombotic microangiopathy were described in two MS patients treated with interferon-beta.[27, 28] We did not find lower ADAMTS13 levels in MS patients treated with interferon-beta, or other DMTs. On the other hand, decreased ADAMTS13 levels were detected in MS patients with CMBs, which have been reported to be more frequent in MS patients of older age.[21] In animal models, ADAMTS13 was found to attenuate brain injury after intracerebral hemorrhage, and it was suggested as a new therapeutic strategy for intracerebral hemorrhage, able to regulate pathological inflammation and BBB function.[29] In light of these observations, the finding of decreased ADAMTS13 levels, particularly in MS patients with CMBs, is intriguing.

Our results should be confirmed by future investigations. Because inhibition of FXII has been proposed as an important mechanism in the pathogenesis of MS, the potential influence on its levels, as well as the impact on the disease activity, deserve additional studies. The main limitation of the present study is that patients were not evaluated at the time of relapse or occurrence of contrast enhancing lesions. Thus, our cohort is not representative of the underlying acute inflammatory activity. Future investigations of hemostasis components levels should address this issue by including MS patients in an active phase of the disease along with the use of inflammatory/non-inflammatory CNS conditions as comparator groups.

In conclusion, one of the first extensive surveys of hemostasis inhibitor levels in plasma of MS patients detected decreased ADAMTS13 levels, and particularly in those who presented CMBs, increased PAI-1 levels, and increased TFPI in P-MS group. We did not find a relationship between hemostasis components and clinical and MRI outcomes. Clinical usefulness of plasma levels of these hemostasis proteins as biomarkers of disease progression and treatment effects in MS is likely limited.

Table 1. Demographic and clinical characteristics of the cohort.

	All MS	**RR-MS**	**P-MS**	**HI**
Sample size *n*	138	85	53	42
Female, *n* (%)	100 (72.5)	60 (70.6)	40 (75.5)	31 (73.8)
Age, years	54.3 (10.8)	50.1 (10.7)	60.9 (7.2)	51.0 (14.3)
Age onset in years	32.9 (9.5)	32.6 (9.1)	33.3 (10.1)	-
Disease duration, years	21.1 (10.6)	17.0 (8.8)	27.6 (10.0)	-
EDSS, median (IQR)	3.5 (4)	2 (1.5)	6 (2.5)	-
Annual relapse rate	0.2 (0.4)	0.2 (0.4)	0.1 (0.3)	-
DMT status, *n* (%)				
Interferon-beta	45 (32.6)	30 (35.3)	15 (28.3)	
Glatiramer acetate	42 (30.4)	23 (27.1)	19 (35.9)	
Natalizumab	5 (3.6)	4 (4.7)	1 (1.9)	-
Other DMT*	19 (13.8)	13 (15.3)	6 (11.3)	
No DMT	27 (19.6)	15 (17.6)	12 (22.6)	

Legend: MS: Multiple Sclerosis; RR-MS: Relapsing Remitting Multiple Sclerosis; P-MS: Progressive Multiple Sclerosis; HI: Healthy Individuals; EDSS: Expanded Disability Status Scale; IQR: interquartile range; SD: standard deviation; n: number; DMT: disease-modifying treatment.

*Other DMTs included intravenous immunoglobulin, mitoxantrone and methotrexate.

Descriptive analysis between MS and HI were performed using Fisher's exact test and Student t-test.

Table 2. MRI characteristics of the cohort.

	All MS	**RR-MS**	**P-MS**	**HI**	**MS vs. HI *p*-value**
T2-LV, ml	15.8 (19.0)	11.8 (15.9)	22.2 (21.9)	0.2 (0.6)	< 0.001
T1-LV, ml	2.9 (6.2)	2.0 (4.6)	4.4 (8.1)	0.0 (0.0)	< 0.001
NBV, ml	1438 (92.1)	1469 (82.4)	1387 (85.2)	1528 (97.9)	< 0.001
NCV, ml	591 (48.6)	606 (44.8)	567 (44.8)	630 (53.3)	< 0.001
LVV, ml	55.1 (27.0)	50.7 (25.2)	62.3 (28.5)	32.2 (14.5)	< 0.001
DGM volume, ml	53.6 (7.1)	55.5 (6.5)	50.4 (6.9)	60.5 (46.4)	< 0.001
QSM DGM	26.1 (5.9)	25.5 (5.6)	27.0 (6.1)	25.3 (6.4)	0.470

Legend: MS: Multiple Sclerosis; RR-MS: Relapsing Remitting Multiple Sclerosis; P-MS: Progressive Multiple Sclerosis; HI: Healthy Individuals; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter; QSM: quantitative susceptibility mapping.

Lesion and brain volumes are expressed in milliliters and QSM values in part per billion.

P-values were derived using Student t-test.

Table 3. Associations of hemostasis components with cerebral microbleeds in multiple sclerosis patients.

	Cerebral Microbleeds		*p*-value
	Present	**Not Present**	
Sample size *n*	12	113	
ADAMTS13 (ng/mL)	1257±459	1566±479	0.034
FXII (µg/mL)	43.5±10.5	40.6±13.4	0.31
HCII (ng/mL)	19.7±16.8	18.5±12.0	0.97
TFPI (ng/mL)	43.0±21.7	39.7±15.2	0.76
TM (ng/mL)	7.8±3.0	7.3±2.1	0.40
PAI-1 (ng/mL)	135±104	121±61.9	0.93

Legend: ADAMTS13: A Disintegrin-like And Metalloprotease with ThromboSpondin type 1 motif 13; FXII: Factor XII; HCII: Heparin Cofactor II; TFPI: Tissue Factor Pathway Inhibitor; TM: Thrombomodulin; PAI-1; Plasminogen activator inhibitor-1.

Levels mean values±SD and the *p*-values from logistic regression analysis are shown.

FIGURE LEGENDS

Figure 1. Schematic representation of the coagulation pathways. The components investigated in the study are highlighted.

Legend: ADAMTS13: A Disintegrin-likeAnd Metalloprotease with ThromboSpondin type 1 motif 13; FXII: Factor XII; HCII: Heparin Cofactor II; TFPI: Tissue Factor Pathway Inhibitor; TM: Thrombomodulin; PAI-1: Plasminogen activator inhibitor-1; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter; QSM: quantitative susceptibility mapping.

Figure 2. Hemostasis components levels in healthy individuals, relapsing-remitting and progressive multiple sclerosis. The p-values from a Mann–Whitney test are provided. The error bars indicate the standard error of the mean.

Legend: ADAMTS13: A Disintegrin-likeAnd Metalloprotease with ThromboSpondin type 1 motif 13; FXII: Factor XII; HCII: Heparin Cofactor II; TFPI: Tissue Factor Pathway Inhibitor; TM: Thrombomodulin; PAI-1: Plasminogen activator inhibitor-1; LV: lesion volume; NBV: normalized brain volume; NCV: normalized cortical volume; LVV: lateral ventricular volume; DGM: deep grey matter; QSM: quantitative susceptibility mapping.

REFERENCES

[1]. Benedict RH, Zivadinov R. Risk factors for and management of cognitive dysfunction in multiple sclerosis. *Nat Rev Neurol*. 2011 **7:**332-342.

[2]. Cramer SP, Simonsen H, Frederiksen JL, Rostrup E, Larsson HB. Abnormal blood-brain barrier permeability in normal appearing white matter in multiple sclerosis investigated by MRI. *Neuroimage Clin*. 2014 **4:**182-189.

[3]. Chu AJ. Tissue factor, blood coagulation, and beyond: an overview. *Int J Inflam*. 2011 **2011:**367284.

[4]. Ryu JK, Petersen MA, Murray SG, *et al.* Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. *Nat Commun*. 2015 **6:**8164.

[5]. Esmon CT. Inflammation and the activated protein C anticoagulant pathway. *Semin Thromb Hemost*. 2006 **32 Suppl 1:**49-60.

[6]. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. *Blood Rev*. 2015 **29:**17-24.

[7]. Zheng XL. Structure-function and regulation of ADAMTS-13 protease. *J Thromb Haemost*. 2013 **11 Suppl 1:**11-23.

[8]. Han MH, Hwang SI, Roy DB, *et al.* Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature*. 2008 **451:**1076-1081.

[9]. Yates RL, Esiri MM, Palace J, Jacobs B, Perera R, DeLuca GC. Fibrin(ogen) and neurodegeneration in the progressive multiple sclerosis cortex. *Ann Neurol*. 2017 **82:**259-270.

[10]. Gobel K, Pankratz S, Asaridou CM, *et al.* Blood coagulation factor XII drives adaptive immunity during neuroinflammation via CD87-mediated modulation of dendritic cells. *Nat Commun*. 2016 **7:**11626.

[11]. Gveric D, Herrera B, Petzold A, Lawrence DA, Cuzner ML. Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. *Brain*. 2003 **126:**1590-1598.

[12]. Wang S, Reeves B, Pawlinski R. Astrocyte tissue factor controls CNS hemostasis and autoimmune inflammation. *Thromb Res*. 2016 **141 Suppl 2:**S65-67.

[13]. Akenami FO, Koskiniemi M, Farkkila M, Vaheri A. Cerebrospinal fluid plasminogen activator inhibitor-1 in patients with neurological disease. *J Clin Pathol*. 1997 **50:**157-160.

[14]. Onodera H, Nakashima I, Fujihara K, Nagata T, Itoyama Y. Elevated plasma level of plasminogen activator inhibitor-1 (PAI-1) in patients with relapsing-remitting multiple sclerosis. *Tohoku J Exp Med*. 1999 **189:**259-265.

[15]. Zivkovic M, Starcevic Cizmarevic N, Lovrecic L, *et al.* The role of TPA I/D and PAI-1 4G/5G polymorphisms in multiple sclerosis. *Dis Markers*. 2014 **2014:**362708.

[16]. Frigerio S, Ariano C, Bernardi G, *et al.* Cerebrospinal fluid thrombomodulin and sVCAM-1 in different clinical stages of multiple sclerosis patients. *J Neuroimmunol*. 1998 **87:**88-93.

[17]. Tsukada N, Matsuda M, Miyagi K, Yanagisawa N. Thrombomodulin in the sera of patients with multiple sclerosis and human lymphotropic virus type-1-associated myelopathy. *J Neuroimmunol*. 1995 **56:**113-116.

[18]. Festoff BW, Li C, Woodhams B, Lynch S. Soluble thrombomodulin levels in plasma of multiple sclerosis patients and their implication. *J Neurol Sci*. 2012 **323:**61-65.

[19]. Balkuv E, Varoglu AO, Isik N, *et al.* The effects of thrombomodulin and activated protein C on the pathogenesis of multiple sclerosis. *Mult Scler Relat Disord*. 2016 **8:**131-135.

[20]. Espada S, Stavik B, Holm S, *et al.* Tissue factor pathway inhibitor attenuates ER stress-induced inflammation in human M2-polarized macrophages. *Biochem Biophys Res Commun*. 2017 **491:**442-448.

[21]. Zivadinov R, Ramasamy DP, Benedict RR, *et al.* Cerebral Microbleeds in Multiple Sclerosis Evaluated on Susceptibility-weighted Images and Quantitative Susceptibility Maps: A Case-Control Study. *Radiology*. 2016 **281:**884-895.

[22]. Coughlin SR. How the protease thrombin talks to cells. *Proc Natl Acad Sci U S A*. 1999 **96:**11023-11027.

[23]. Ikeda Y, Aihara K, Yoshida S, *et al.* Heparin cofactor II, a serine protease inhibitor, promotes angiogenesis via activation of the AMP-activated protein kinase-endothelial nitric-oxide synthase signaling pathway. *J Biol Chem*. 2012 **287:**34256-34263.

[24]. Wolter J, Schild L, Bock F, *et al.* Thrombomodulin-dependent protein C activation is required for mitochondrial function and myelination in the central nervous system. *J Thromb Haemost*. 2016 **14:**2212-2226.

[25]. Favaloro EJ, Franchini M, Lippi G. Aging hemostasis: changes to laboratory markers of hemostasis as we age - a narrative review. *Semin Thromb Hemost*. 2014 **40:**621-633.

[26]. Dimopoulou D, Dimosiari A, Mandala E, Dimitroulas T, Garyfallos A. Autoimmune Thrombotic Thrombocytopenic Purpura: Two Rare Cases Associated with Juvenile Idiopathic Arthritis and Multiple Sclerosis. *Front Med (Lausanne)*. 2017 **4:**89.

[27]. Orvain C, Augusto JF, Besson V, *et al.* Thrombotic microangiopathy due to acquired ADAMTS13 deficiency in a patient receiving interferon-beta treatment for multiple sclerosis. *Int Urol Nephrol*. 2014 **46:**239-242.

[28]. Nishio H, Tsukamoto T, Matsubara T, Okada Y, Takahashi R, Yanagita M. Thrombotic microangiopathy caused by interferon beta-1b for multiple sclerosis: a case report. *CEN Case Rep*. 2016 **5:**179-183.

[29]. Cai P, Luo H, Xu H, *et al.* Recombinant ADAMTS 13 Attenuates Brain Injury After Intracerebral Hemorrhage. *Stroke*. 2015 **46:**2647-2653.