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Relationship Between Elevated Cerebrospinal Fluid Levels of Plasminogen Activator Inhibitor 1 and Neuronal Destruction in Patients With Neuropsychiatric Systemic Lupus Erythematosus

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Objective. **A homeostatic imbalance between coagulation and fibrinolysis might occur intrathecally in neuropsychiatric systemic lupus erythematosus (NPSLE). However, there are no published data on levels of fibrinolytic factors in the cerebrospinal fluid (CSF) of patients with NPSLE. The present study was undertaken to assess CSF levels of fibrinolytic molecules, including urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA), D-dimer, and plasminogen activator inhibitor 1 (PAI-1), in SLE patients with clinically verified neuropsychiatric involvement and to compare these levels with those in SLE patients without neuropsychiatric involvement and in healthy subjects.**

Methods. **Levels of uPA, tPA, and PAI-1 were assessed in CSF from 94 patients with SLE (33 who had NPSLE, 56 who did not have NPSLE, and 5 who were positive for antiphospholipid antibody [not included in the NPSLE or non-NPSLE group]) and from 53 age-matched controls. Patients were evaluated clinically, with magnetic resonance imaging of the brain, analyses of neuronal/glial degradation products in CSF, and neuropsychiatric testing.**

Results. **In the group of patients with NPSLE, intrathecal PAI-1 levels were significantly elevated compared with levels in SLE patients without overt neuropsychiatric involvement ($P < 0.05$) and in healthy controls ($P < 0.001$). In contrast, intrathecal levels of uPA did not differ significantly. Intrathecal levels of PAI-1 correlated significantly with CSF levels of interleukin-6 (IL-6) ($r = 0.34$, $P < 0.001$) and IL-8 ($r = 0.33$, $P < 0.001$). Importantly, increased PAI-1 and D-dimer levels were observed in SLE patients who had pathologically elevated levels of glial fibrillary acidic protein, neurofilament triplet protein, and tau protein in CSF.**

Conclusion. **Intrathecal release of PAI-1 is increased in patients with NPSLE. This results in impaired fibrinolysis, which might contribute to neuronal and astrocytic damage in NPSLE.**

Neuropsychiatric involvement is one of the major causes of morbidity and mortality in systemic lupus erythematosus (SLE) and carries a poor prognosis. The reported prevalence of neuropsychiatric involvement in SLE varies widely among studies (from 14% to 75%) (1–3). Neuropsychiatric manifestations of SLE are highly diverse, including common features such as headache and mood disorders to rarer events such as psychosis (4).

The causes of neuropsychiatric SLE (NPSLE) are still largely unknown. However, it is acknowledged that

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NPSLE pathogenesis is a complex process incorporating both immunologic factors and other factors. Levels of neuronal and astrocytic degradation products, such as neurofilament triplet protein (NFL) and glial fibrillary acidic protein (GFAP), are highly elevated in the cerebrospinal fluid (CSF) of SLE patients with neuropsychiatric involvement (5), suggesting that there is neuronal and astrocytic damage in this disease. Production of autoantibodies against brain structures (6,7), deposition of immune complexes in the central nervous system (CNS) (8), and intrathecal release of proinflammatory cytokines, e.g., interleukin-6 (IL-6) and IL-8 (9), may directly contribute to nervous system tissue injury. Besides immunologic processes, abnormalities of hemostasis might play a role in the pathogenesis of NPSLE, since one of the typical histopathologic changes found at autopsy in NPSLE patients is the presence of multifocal microinfarcts, seen particularly in the cerebral cortex and brain stem (10).

During systemic inflammation, homeostatic balance shifts toward coagulation by means of synthesis of tissue factor (an initiator of the coagulation protease cascade), inhibition of the anticoagulant pathway, and impairment of fibrinolysis by up-regulation of plasminogen activator inhibitor 1 (PAI-1), which is a major inhibitor of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) (11). Such an imbalance might also exist locally, for example in the CNS. It has been demonstrated that tissue factor is markedly up-regulated in the CNS during persistent viral infection (12). Intrathecal PAI-1 levels have been found to be significantly higher in patients with bacterial meningitis than in healthy controls, and consequently, homeostasis imbalance in the CNS was associated with the development of brain infarction (13). It seems reasonable to assume that in NPSLE there is a similar imbalance between coagulation and fibrinolysis, driven by local inflammatory processes. However, to date there have been no published reports on intrathecal fibrinolytic status in patients with NPSLE.

In the present study, we investigated whether intrathecal fibrinolysis is impaired in NPSLE. We measured tPA antigen, uPA, D-dimer, and PAI-1 in CSF from healthy controls, SLE patients without overt neuropsychiatric involvement, and patients with NPSLE. Our data suggest that PAI-1 concentrations are significantly increased in NPSLE patients, and that levels of PAI-1 are related to neurodegeneration as well as to markers of inflammation.

PATIENTS AND METHODS

Patients. Ninety-four patients who fulfilled the American College of Rheumatology (ACR) revised criteria for the classification of SLE (14) were included in the study. All patients (81 female and 13 male; mean ± SD age 42 ± 14 years [range 17–75]) were seen at the Department of Rheumatology, Sahlgrenska University Hospital. Patients were consecutively enrolled in this cross-sectional study. Disease duration varied from new diagnosis to 33 years (mean ± SD 6 ± 7 years). Patients underwent a thorough clinical examination by an experienced staff rheumatologist and neurologist. Evaluation of neuropsychiatric signs and symptoms included lumbar puncture, neuropsychiatric testing, and magnetic resonance imaging (MRI) of the brain.

The proposed definition of NPSLE that is incorporated in the ACR criteria for SLE (14) appears inadequate, given that only 2 elements—psychosis and seizures—are included. We defined NPSLE as the presence of at least 2 of the following 7 items, occurring in association with clinical evidence of disease progression: 1) recent-onset psychosis, 2) transverse myelitis, 3) aseptic meningitis, 4) seizures, 5) pathologic changes seen on brain MRI, 6) severely abnormal cognitive dysfunction documented by neuropsychiatric testing (15), and 7) the presence of oligoclonal IgG bands in the CSF. Our criteria stress the inflammation process in the CNS more than do the 1999 ACR nomenclature and case definitions for NPLSE (16). It has previously been shown that antiphospholipid antibody positivity might influence systemic coagulation status (17). We therefore excluded patients with this condition from the experiments comparing levels of coagulation-related molecules in SLE patients with and those without neuropsychiatric involvement. Patients with non-SLE causes of neurologic events, such as cerebral infection, metabolic derangement (e.g., uremia and liver encephalopathy), and hypertension-triggered encephalopathy, as well as patients with neurologic events that were deemed to be side effects of drugs, were also excluded.

Based on the above criteria, patients were divided into 3 distinct groups: patients with NPSLE (n = 33), patients with SLE complicated by the antiphospholipid syndrome (n = 5), and patients with SLE who did not meet our criteria for NPSLE (n = 56). There were 15 SLE patients (16%) who did not meet our criteria for NPSLE (and thus were included in the non-SPSLE group) but who did fulfill the ACR 1999 criteria for NPSLE (all of the patients who had NPSLE according to our criteria also fulfilled the ACR criteria). These 15 patients with diagnostic discrepancy had highly elevated CSF levels of PAI-1 (mean ±SEM 0.383 ± 0.04 ng/ml) in comparison with healthy controls (0.06 ± 0.01 ng/ml).

Control subjects. CSF samples from 53 subjects (41 female and 12 male; mean ± SD age 42 ± 17 years) without a history of neurologic disorder were used as controls. These subjects had sought emergency care at the emergency unit of Sahlgrenska University Hospital, due to headache. Examination of all control subjects had revealed normal neurologic status, and the findings of subsequent computed tomography and CSF examinations excluded neurologic disease. All CSF samples were aliquoted and stored at –80°C until assayed.

The Medical Ethics Committee at Göteborg University approved the study, and informed consent was obtained from all patients and control subjects after written and verbal information about the study was provided.

Routine CSF analyses. Albumin and IgG levels in CSF and serum were measured by immunonephelometry, with a Beckman Immage Immunochemistry system (Beckman Instruments, Munich, Germany). The albumin ratio was calculated as the ratio of CSF albumin (mg/liter) to serum albumin (gm/liter) and was used as an indicator of blood–brain barrier function (18). The normal albumin ratio is <6.8 in individuals ≤ 45 years of age and 10.2 in individuals >45 years of age (19). The IgG index was calculated as the IgG ratio (ratio of CSF IgG [mg/liter] to serum IgG [gm/liter]) divided by the albumin ratio and was used as an indicator of intrathecal IgG production (18). The normal value is <0.63 (19). All CSF samples were also analyzed by isoelectric focusing with silver staining to detect oligoclonal IgG bands.

MRI analyses. Neuroimaging with multiplanar MRI was performed to evaluate the extent and localization of brain lesions. T2-weighted axial proton-density images of the brain were obtained using a Gyroscan T5-II (Philips, Munich, Germany). Findings considered to be pathologic were small punctate focal lesions in white matter, cortical atrophy, periventricular white matter hyperintensity, diffuse white matter changes, discrete gray matter lesions, diffuse gray matter hyperintensities, cerebral edema, and new infarct (20). Other causes of CNS dysfunction, such as preexisting infarctions and space-occupying lesions, were not considered as pathologic findings for the purposes of this study.

Neuropsychiatric assessment. Neuropsychiatric testing was performed by a professional neuropsychologist, and cognitive dysfunction was rated as absent, mild, moderate, or severe. This was a subjective rating performed after reviewing the 1999 ACR nomenclature defining cognitive impairment (16). The interpretation of test results and the identification of impairment for clinical decision-making were based on normative data appropriate for age, education, sex, and ethnic group. The investigators conducting the MRI analyses were blinded with regard to the results of neuropsychiatric testing.

Reagents and procedures. Levels of tPA antigen, uPA, PAI-1 antigen, and D-dimer were determined using specific sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the protocols recommended by the manufacturer (Haemochrom Diagnostica, Essen, Germany). The detection threshold was 0.25 ng/ml for tPA antigen and PAI-1 antigen, and 10 ng/ml for D-dimer.

Levels of tau protein were determined using a sandwich ELISA (Innotest hTAU-Ag; Innogenetics, Ghent, Belgium) constructed to measure both phosphorylated and nonphosphorylated tau protein (21). NFL and GFAP concentrations in the CSF were measured using ELISA kits, as previously described (22,23); NFL and GFAP standard was purified from primate brain.

Cell line B13.29, which is dependent on IL-6 for growth, has been described previously (24). For IL-6 determinations, the more sensitive subclone B9 was used (25). B9 cells were harvested from tissue culture flasks, seeded onto microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 5,000 cells per well, and cultured in Iscove's medium supplemented with 2-mercaptoethanol (5×10^5 moles/liter), 5% fetal calf serum (Sera Lab, Sussex, UK), penicillin (100 units/liter), and streptomycin (100 gm/ml), and CSF or serum samples were added. Tritiated thymidine was added after 68 hours of culture, and cells were harvested 4 hours later. The samples were tested in 2-fold dilutions and compared with a recombinant human IL-6 standard (Genzyme, Cambridge, MA). B9 cells were previously shown not to react with several recombinant cytokines, including IL-1, IL-2, IL-3, IL-5, granulocyte–macrophage colony-stimulating factor, tumor necrosis factor (TNF), and interferon (25). In a neutralization assay, only weak reactivity was observed upon addition of monoclonal antibody specific for human IL-6 (Genzyme); preincubation of 10 μg/ml of this antibody with either recombinant IL-6 or CSF containing naturally produced IL-6 (1 hour at 37°C) reduced proliferative responses of B9 indicator cells by, on average, at least 95%. IL-8 was quantified by ELISA, according to the protocol recommended by the manufacturer (R&D Systems, Abingdon, UK). Levels of TNFα were estimated using the Predicta ELISA kit (Genzyme). IL-1β levels were estimated by ELISA (Quantikine; R&D Systems, Minneapolis, MN). IL-10 was quantified by ELISA using IL-10–specific monoclonal antibodies (4 μg/ml; PharMingen, San Diego, CA) for coating and biotinylated IL-10–specific monoclonal antibodies (4 μg/ml; PharMingen) as primary antibodies. Detection levels for IL-1β, IL-6, IL-8, IL-10, and TNFα in CSF were 4 pg/ml, 5 pg/ml, 8 pg/ml, 15 pg/ml, and 2 pg/ml, respectively. All values below detection levels were considered negative.

Statistical analysis. Statistical comparisons of PAI-1, uPA, and D-dimer levels in CSF from SLE patients without overt neuropsychiatric involvement, patients with NPSLE, and healthy controls were performed using unpaired *t*-tests, since the sample size in each group was >30. The Mann-Whitney U test was used for comparisons of NFL, GFAP, and tau protein levels in CSF from the SLE patient groups. Results are presented as the mean ± SEM. Pearson's correlation was used to calculate correlation coefficients. P values less than 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA).

RESULTS

Characteristics of the study patients. Clinical characteristics of the SLE patients who did not have neuropsychiatric involvement (n = 56), the patients with NPSLE (n = 33), and the SLE patients with the antiphospholipid syndrome (n = 5) are shown in Table 1. Neuropsychiatric features in patients who did not meet our criteria for NPSLE and in those who did meet these criteria are presented in Table 2.

Table 1. Clinical data on the SLE patients included in the study*

Parameter	No NPSLE (n = 56)	APS (n = 5)	NPSLE (n = 33)
No. male/female	8/48	0/5	5/28
Age, years	41.1 ± 1.7	42.4 ± 8.9	45.6 ± 2.7
Disease duration, years	6.9 ± 1.1	2.5 ± 1.2	8.7 ± 1.9
Pathologic findings on MRI, no.	19	4	24†
Positive anti-dsDNA, no.‡	33	4	23
Albumin ratio§	4.7 ± 0.2	10.6 ± 0.1	6.8 ± 0.9¶
IgG index§	0.5 ± 0.01	0.6 ± 0.1	0.7 ± 0.1
Pleocytosis, 10^6/liter	1.6 ± 0.2	11.2 ± 7.3	11.4 ± 7.2
Oligoclonal bands, no. +/−#	10/42	0/5	18/15†
Pharmacologic treatment, no.			
Prednisolone (<10 mg day/ >10 mg/day)	32/4	3/2	15/4
Antimalarial agents	6	0	1
AZA	12	2	4
AZA and CSA	2	0	2
AZA and antimalarial agents	0	0	0
MTX	2	1	4
CSA	4	0	1
CYC	0	1	11
CYC and CSA	0	0	1
Antihypertensive agents	11	1	11
Low-dose aspirin	15	1	8
Warfarin	0	2	4

* Except where indicated otherwise, values are the mean ± SEM. APS = antiphospholipid syndrome; MRI = magnetic resonance imaging; anti-dsDNA = anti–double-stranded DNA; AZA = azathioprine; CSA = cyclosporin A; MTX = methotrexate; CYC = cyclophosphamide.
† $P < 0.001$ versus systemic lupus erythematosus (SLE) patients without neuropsychiatric SLE (NPSLE).
‡ Data not available or examination not performed on 3 SLE patients without NPSLE and 1 patient with NPSLE.
§ See Patients and Methods.
¶ $P < 0.01$ versus SLE patients without NPSLE.
# Data not available or examination not performed on 1 SLE patient without NPSLE.

Elevated PAI-1 levels in CSF from patients with NPSLE. CSF levels of PAI-1, uPA, and D-dimer in patients with NPSLE are shown in Figure 1. In CSF both from controls and from SLE patients, tPA was not detectable (data not shown). Patients with NPSLE had significantly higher CSF levels of PAI-1 compared with SLE patients who did not meet our criteria for NPSLE (mean ± SEM 0.53 ± 0.11 ng/ml versus 0.32 ± 0.04 ng/ml; $P < 0.05$) and healthy controls (mean ± SEM 0.06 ± 0.01 ng/ml; $P < 0.001$) (Figure 1A). CSF levels of PAI-1 were also clearly elevated in patients with SLE without neuropsychiatric involvement compared with healthy controls ($P < 0.001$). In contrast, levels of uPA were significantly decreased in SLE patients without neuropsychiatric involvement compared with healthy controls (0.087 ± 0.007 ng/ml versus 0.137 ± 0.011 ng/ml; $P < 0.001$), but no significant difference from levels in controls was found in patients with NPSLE (0.108 ± 0.011 ng/ml; $P = 0.067$) (Figure 1B).

Intriguingly, intrathecal D-dimer levels were significantly lower in SLE patients without NPSLE than in healthy controls (11.8 ± 4.3 ng/ml versus 26.9 ± 5.9 ng/ml; $P < 0.05$) (Figure 1C). There was trend toward elevation of D-dimer levels in CSF from NPSLE patients (37.7 ± 18.6 ng/ml) in comparison with SLE patients without neuropsychiatric involvement ($P = 0.078$), suggesting that intrathecal procoagulation might be more profound in the former condition.

Strong association of intrathecal PAI-1 levels with markers of inflammation and neuronal damage in CSF from SLE patients. We assessed the association of PAI-1, uPA, and D-dimer levels with levels of neuronal and astrocyte degradation products and certain proinflammatory molecules in CSF from the 94 patients with SLE (Table 3). Most of the significant correlations observed were between PAI-1 levels and levels of markers of neuronal damage, i.e., tau protein ($r = 0.53$, $P < 0.001$) and NFL ($r = 0.57$, $P < 0.001$). PAI-1 levels were also significantly correlated with levels of IL-1β ($r = 0.22$, $P < 0.05$), IL-6 ($r = 0.34$, $P < 0.001$), and IL-8 ($r = 0.33$, $P < 0.001$). D-dimer levels were significantly correlated with levels of PAI-1 ($r = 0.52$, $P < 0.001$), IL-6 ($r = 0.51$, $P < 0.001$), IL-8 ($r = 0.41$, $P < 0.001$), and NFL (0.23, $P < 0.05$), and with pleocytosis ($r = 0.44$, $P < 0.001$) and albumin ratio ($r = 0.51$, $P < 0.001$). In contrast, intrathecal uPA levels were relatively weakly

Table 2. Clinical neuropsychiatric manifestations in the SLE patients who did and those who did not meet the study criteria for NPSLE*

Feature	No NPSLE (n = 56)	NPSLE (n = 33)
Acute confusional state	1	1
Anxiety disorder	3	1
Aseptic meningitis	0	1
Cerebrovascular disease	7	4
Cognitive dysfunction	16	3
Demyelinating syndrome	0	2
Headache	17	7
Mood disorder	9	6
Movement disorder	0	1
Myelopathy	0	2
Psychosis	1	3
Seizure disorder	1	5

* Values are the number of patients. The 5 systemic lupus erythematosus (SLE) patients with the antiphospholipid syndrome are not included. NPSLE = neuropsychiatric SLE.

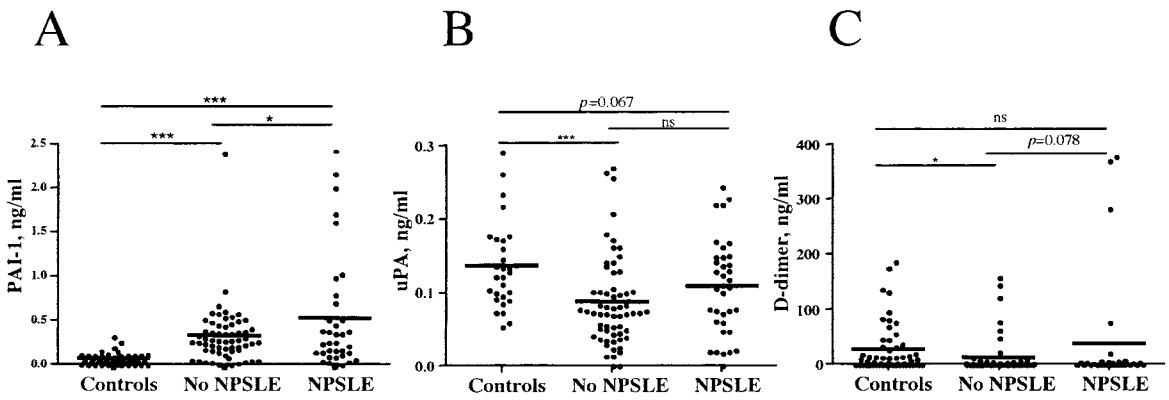

Figure 1. Levels of plasminogen activator inhibitor 1 (PAI-1) (**A**), urokinase plasminogen activator (uPA) (**B**), and D-dimer (**C**) in the cerebrospinal fluid of healthy controls, systemic lupus erythematosus (SLE) patients without overt neuropsychiatric involvement, and patients with neuropsychiatric SLE (NPSLE). Bars show the group means. * = $P < 0.05$; *** = $P < 0.001$. NS = not significant.

correlated with levels of tau protein (r = 0.23, $P < 0.05$), IL-1β (r = 0.19, $P < 0.05$), and IL-10 (r = 0.21, $P < 0.05$). (Table 3).

Increased PAI-1 and D-dimer levels in SLE patients with pathologically elevated levels of GFAP, NFL, and tau protein. The 94 SLE patients were divided into groups based on levels (normal or elevated) of the breakdown products GFAP, NFL, and tau protein in CSF. PAI-1 levels were significantly higher in the SLE patients with elevated CSF levels of GFAP than in patients with normal levels of GFAP (mean ± SEM 0.64 ± 0.13 ng/ml versus 0.28 ± 0.03 ng/ml; $P < 0.05$), and in patients with elevated CSF levels of NFL than in those with normal levels of NFL (0.71 ± 0.13 ng/ml versus 0.26 ± 0.02 ng/ml; $P < 0.001$) (Figures 2A and B), suggesting that CSF levels of PAI-1 are associated with neuronal and astrocytic damage. There was a tendency toward an increase in PAI-1 levels in the group with elevated CSF levels of tau protein in comparison with the group with normal tau protein levels (0.72 ± 0.18 ng/ml versus 0.29 ± 0.03 ng/ml) (Figure 2C). Similar correlations were observed regarding D-dimer levels in CSF: significantly higher levels of D-dimer were found in SLE patients with elevated CSF levels of NFL (29.8 ± 20.9 ng/ml versus 12.1 ± 4.4 ng/ml; $P < 0.01$) and tau protein (58.9 ± 40.8 ng/ml versus 9.2 ± 4.0 ng/ml; $P <$

Table 3. Correlation between intrathecal levels of PAI-1, uPA, and D-dimer and levels of markers of inflammation and neuronal damage in cerobrospinal fluid from patients with SLE*

	PAI-1		uPA		D-dimer	
Marker	r	*P*	r	*P*	r	*P*
uPA	−0.16	0.06				
D-dimer	0.52	<0.001		NS		
Albumin ratio	0.34	<0.01		NS	0.51	<0.001
Pleocytosis	0.06	<0.01		NS	0.44	<0.001
IL-1β	0.22	<0.05	0.19	<0.05		NS
IL-6	0.34	<0.001		NS	0.51	<0.001
IL-8	0.33	<0.001		NS	0.41	<0.001
IL-10		NS	0.21	<0.05		NS
TNFα		NS		NS		NS
Tau protein	0.53	<0.001	0.23	<0.05		NS
GFAP		NS		NS		NS
NFL	0.57	<0.001		NS	0.23	<0.05

* The systemic lupus erythematosus (SLE) patients studied included 33 with neuropsychiatric SLE (NPSLE) and 61 without NPSLE. PAI-1 = plasminogen activator inhibitor 1; uPA = urokinase plasminogen activator; NS = not significant; IL-1β = interleukin-1β; TNFα = tumor necrosis factor α; GFAP = glial fibrillary acidic protein; NFL = neurofilament triplet protein.

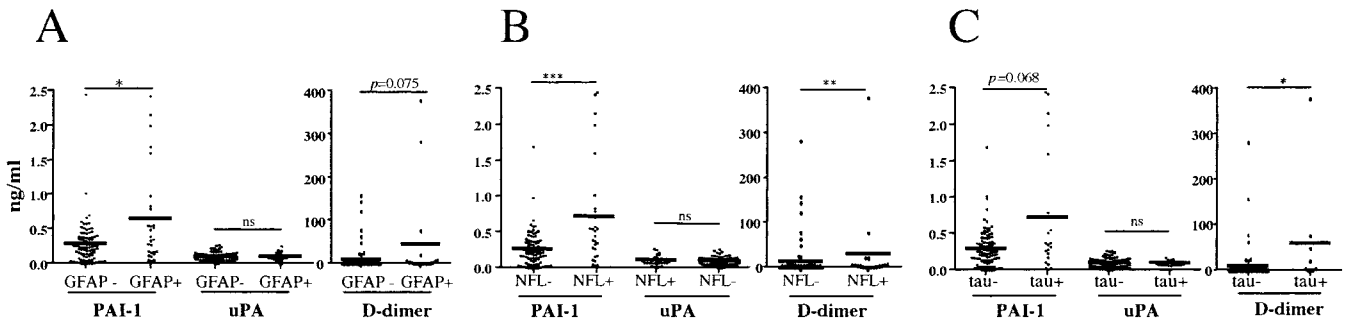

Figure 2. Intrathecal levels of PAI-1, uPA, and D-dimer in SLE patients with normal levels and those with pathologically elevated levels of glial fibrillary acidic protein (GFAP) (**A**), neurofilament triplet protein (NFL) (**B**), and tau protein (**C**). Cutoff values for designation of pathologic elevation were 750 ng/liter, 250 ng/liter, and 400 ng/liter for GFAP, NFL, and tau protein, respectively. Bars show the group means. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$. See Figure 1 for other definitions.

0.05) compared with those with normal levels of these degeneration products (Figures 2B and C). D-dimer levels also tended to be higher in patients with elevated levels of GFAP, but this difference did not reach statistical significance.

DISCUSSION

In this study, we showed that CSF PAI-1 levels in patients with NPSLE were significantly increased compared with levels in SLE patients without overt neuropsychiatric involvement or healthy controls. In contrast, differences in intrathecal levels of uPA were not detected. D-dimer levels in CSF were significantly reduced in SLE patients without overt neuropsychiatric involvement compared with healthy controls. Intrathecal levels of PAI-1 and D-dimer correlated significantly with levels of proinflammatory cytokines and neuronal damage markers.

Although the blood–brain barrier may be somewhat affected in SLE, this is not a likely explanation for the increased PAI-1 levels in patients with NPSLE, since we did not observe a difference in CSF levels of uPA between healthy controls and NPSLE patients. Moreover, the fact that tPA was undetectable in the CSF of patients with NPSLE suggests that PAI-1, having similar molecular mass as tPA, is not able to penetrate the blood–brain barrier at all. It has became increasingly clear that systemic inflammation leads to substantial suppression of fibrinolysis, by up-regulation of PAI-1 in the circulation. Would intrathecal inflammation also induce such a hemostatic imbalance in the CNS? Indeed, we found a strong association between intrathecal PAI-1 levels and levels of proinflammatory cytokines, including IL-6, IL-8, and IL-1β. Potential sources of PAI-1 in the plasma are endothelial cells (26) and hepatic cells (27). In contrast, the origin of PAI-1 in the brain of normal subjects is unknown (28). It has been shown that neoplastic astrocytes secrete PAI-1 (29), and, in Alzheimer's disease, microglial cells express PAI-2 (30). PAI-1 synthesis is regulated by various cytokines, such as TNFα (31), IL-1β (32), IL-6 (33), and transforming growth factor β (34). This suggests that in NPSLE, intrathecally elevated PAI-1 levels might be regulated by release of these proinflammatory cytokines.

The fibrin turnover metabolite D-dimer, an indicator of a procoagulation state, is a 16-kd small molecule. Intrathecal D-dimer in healthy individuals might reflect balanced local coagulation and fibrinolysis. However, in our SLE patients without neuropsychiatric involvement, up-regulation of PAI-1 and down-regulation of uPA led to inhibition of fibrinolysis, subsequently resulting in significantly decreased intrathecal levels of D-dimer (Figure 1C). In contrast, we observed a tendency toward elevation of D-dimer levels in CSF from NPSLE patients, which might be explained by penetration of D-dimer through the blood–brain barrier, since a significant correlation between D-dimer levels and the albumin ratio in CSF from SLE patients was demonstrated ($r = 0.51$, $P < 0.001$). A procoagulation state caused by local inflammation in the CNS might be an alternative explanation, since D-dimer levels were also associated with levels of proinflammatory cytokines (IL-6 and IL-8).

A very intriguing finding of the present study was that PAI-1 and D-dimer levels in CSF were strongly associated with the levels of the neuronal and astrocytic

damage markers GFAP, NFL, and tau protein. Several lines of evidence demonstrate that fibrin deposition in brain tissue leads to local amplification of the inflammatory process and subsequent tissue damage. First, it has been shown that fibrin deposition inhibits peripheral nerve remyelination, and fibrinogen depletion accelerates remyelination (35). Second, tPA deficiency leads to impaired fibrinolysis, which exacerbates neurodegeneration and demyelination in neuroinflammatory conditions (36,37). Finally, CSF levels of PAI-1 are associated with later cerebral infarction in patients with bacterial meningitis (13). All of these findings suggest that a local imbalance of homeostasis, resulting in fibrin deposition, may contribute to neuronal injury in NPSLE. Obviously, impaired fibrin removal by up-regulation of PAI-1 might greatly contribute to this imbalance of homeostasis in the CNS.

What are the clinical implications of our findings? The mean intrathecal level of PAI-1 was only 1.7-fold higher in patients with verified NPSLE than in SLE patients without overt neuropsychiatric involvement. In addition, elevated PAI-1 concentrations have been found in patients with other neurologic diseases (38), suggesting that they are a nonspecific marker of neurologic disease. Therefore, our results do not suggest that PAI-1 analysis should be used for diagnostic purposes in NPSLE. However, the strong association between neuronal injury and intrathecal homeostasis imbalance, to which PAI-1 release is a contributor, suggests that active anticoagulation treatment might be considered in patients with NPSLE, even in the absence of the antiphospholipid syndrome.

In conclusion, this study demonstrates that intrathecal levels of PAI-1 are clearly increased in patients with NPSLE compared with both SLE patients without overt neuropsychiatric involvement and healthy controls, indicating that intrathecal fibrinolysis might be impaired in NPSLE. The significant correlation of PAI-1 levels with levels of NFL and GFAP suggests a potential role of intrathecal PAI-1 release in the pathogenesis of neuronal and astrocytic damage in NPSLE.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Jin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Trysberg, Blennow, Tarkowski, Jin.

Acquisition of data. Kwieciński, Kłak, Trysberg, Blennow, Tarkowski, Jin.

Analysis and interpretation of data. Kwieciński, Kłak, Trysberg, Blennow, Tarkowski, Jin.

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