Analysis of CSF D-Dimer to Identify Intrathecal Fibrin-Driven Autoimmunity in Patients With Multiple Sclerosis

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Abstract

Background and Objectives
Proteins of the coagulation system contribute to autoimmune inflammation in patients with multiple sclerosis (MS). On blood-brain barrier (BBB) disruption, fibrinogen enters the CNS and is rapidly converted to fibrin, unfolding pleiotropic autoimmune mechanisms. Fibrin accumulation leads to subsequent proteolytic degradation that results in D-dimer generation. The primary objective of this study was to determine intrathecal levels of D-dimer in CSF as a measure of intrathecal coagulation cascade activation and to evaluate its diagnostic utility in patients with MS in contrast to healthy subjects. Key secondary objectives included analysis of CSF D-dimer in differential diagnoses of MS and its relation to routine clinical markers of disease activity.

Methods
Patients admitted for the assessment of suspected MS were prospectively recruited from October 2017 to December 2020. Blood plasma and citrated CSF samples were analyzed using a highly sensitive luminescent oxygen channeling immunoassay. Intrathecal generation of D-dimer was analyzed by adjusting for CSF/serum albumin (Q_{alb}) and CSF/plasma D-dimer quotients ($Q_{D\text{-}dimer}$), and corresponding CSF fibrinogen levels were determined. Final diagnoses after full evaluation and clinical data were recorded.

Results
Of 187 patients, 113 patients received a diagnosis of MS or clinically/radiologically isolated syndrome. We found increased intrathecal CSF D-dimer generation levels ($Q_{D\text{-}dimer}/Q_{alb}$-index) for patients with relapsing-remitting MS (RRMS; n = 71, median 4.7, interquartile range [IQR] 2.5–8.0) when compared with those for disease controls (n = 22, median 2.6, IQR 2.1–4.8, $p = 0.031$). Absolute CSF D-dimer values correlated with CSF fibrinogen levels (r = 0.463; $p < 0.001$) and CSF leukocytes (r = 0.273; $p = 0.003$) and were elevated in MS patients with contrast enhancement (CE) compared with MS patients without CE on MRI (n = 48, median 6 ng/mL, and IQR 3–15.25 vs n = 41, median 4 ng/mL, and IQR 2–7; $p = 0.026$). Exploratory subgroup analyses indicated a correlation of intrathecal inflammatory activity and CSF D-dimer levels.

Discussion
D-dimer in CSF can be reliably determined and correlates with markers of CNS inflammation and CSF fibrinogen levels. Adjusted for BBB dysfunction, CSF D-dimer may allow the identification of intrathecal coagulation cascade activation in patients with MS.

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Glossary

%CV = coefficient of variation; **AQP4** = aquaporin-4; **BBB** = blood-brain barrier; **CE** = contrast-enhancing lesions; **CIS** = clinically isolated syndrome; **EDSS** = Expanded Disability Status Scale; **IQR** = interquartile range; **IVMP** = IV methylprednisolone; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **PPMS** = primary progressive MS; **RIS** = radiologically isolated syndrome; **RRMS** = relapsing-remitting MS.

Classification of Evidence

This study provides Class I evidence that CSF D-dimer levels are elevated in patients with RRMS.

Components of the coagulation system have been identified as major drivers of CNS inflammation.[1-3] On blood-brain barrier (BBB) disruption, fibrinogen enters the CNS and is rapidly converted into insoluble fibrin.[4,5] This initiates pleiotropic detrimental immune mechanisms,[6] such as macrophage recruitment and microglial activation,[7] which result in inflammatory lesion formation and neurodegeneration.[3,4,8] Accordingly, high levels of both fibrinogen and fibrin were identified in demyelinating lesions in MS, and fibrinogen in CSF was correlated with cortical lesion load at early MS disease stages.[5,7,9] The fibrin accumulation leads to a subsequent plasmin-driven proteolytic degradation of cross-linked fibrin mesh into D-dimer and other soluble fibrin degradation products.[10,11] Consistently, accumulations of intrathecal D-dimer were immunolocalized to MS plaques, and fibrin degradation products were found elevated in the plasma of patients with MS.[12,13]

Additional deregulatory effects on the immune response were described for prothrombin, factor (F) X, and F XII in both blood plasma and the CNS.[2,14-16] Here, effects derive from receptor-specific cellular and molecular interactions of F XII and downstream factors, independent of their proteolytic functions in the hemostatic coagulation cascade.[5,6,17] In addition, thrombin activation correlates with CNS inflammation and focal BBB disruption.[18]

Presence of elevated D-dimer levels in CSF has been described for patients with subarachnoid hemorrhage, intracerebral hemorrhage, and neoplastic disease.[19-22] In MS, measurement of D-dimer in CSF as a downstream fibrinolytic pathway product could allow to monitor intrathecal coagulation cascade activation and provide insight into underlying pathophysiologic mechanisms. This study aims to determine CSF levels of D-dimer in patients with MS and in differential diagnoses to evaluate their utility as a biomarker for intrathecal fibrin-driven autoimmunity and coagulation cascade activation in the CNS.

Methods

Study Population

This single-center prospective study was performed at the Department of Neurology, Goethe-University, Frankfurt am Main. Patients admitted to the hospital for the routine or emergency assessment of suspected MS were recruited from October 2017 to December 2020. For study enrollment, patients had to be older than 18 years and had to be scheduled for a clinically indicated lumbar puncture. Visibly blood-stained samples due to (traumatic) lumbar punctures were excluded from the study. Subjects were followed up for the duration of their hospital stay, and final diagnosis and clinical, imaging, and laboratory findings were recorded. Medication plans were evaluated for all patients.

The MR imaging data of brain and spine obtained during the hospital stay and the respective radiologic reports were reviewed regarding the presence of contrast-enhancing lesions (CE). This was defined as either cerebral or spinal contrast enhancement of gadolinium-based contrast agent on an MRI performed 7 days before or after the CSF sample acquisition. If no contrast agent was given, patients were separated from analyses regarding CE.

The 2017 revisions of the McDonald criteria were used in clinical routine to define MS diagnoses.[23] For this analysis, patients were grouped into the following diagnosis categories: relapsing-remitting MS (RRMS), clinically isolated syndrome (CIS), radiologically isolated syndrome (RIS), and primary progressive MS (PPMS). Differential diagnoses comprised antimyelin oligodendrocyte glycoprotein (MOG)-antibody or anti-aquaporin-4 (AQP4)-antibody diseases, isolated demyelinating events without suspicion of chronic inflammatory CNS disease based on the McDonald criteria, *other acute CNS inflammation, CNS neoplasia, non-CNS neurologic diseases*, and *other diseases* (not assignable to the categories). If a full diagnostic workup during the hospital stay (including clinical examination, brain MRI, and laboratory and CSF findings) did not reveal signs of an underlying organic disease, patients were considered as *disease controls*.

Standard Protocol Approvals, Registrations, and Patient Consents

A signed written informed consent to participate in the study was mandatory by patients or their legally authorized representative before study inclusion. Ethical approval for the study was granted by the institutional review board of the ethics

committee at the University Hospital Frankfurt (project-number: 173/19). All research was performed in accordance with relevant guidelines and regulations and in accordance with the Declaration of Helsinki.

Primary, Secondary, and Exploratory Outcome Parameters

The primary aim of this study was to compare levels of intrathecal D-dimer production (i.e., levels of D-dimer in the CSF corrected for plasma D-dimer and BBB dysfunction) between patients with RRMS and disease controls. Secondary goals included the levels of absolute D-dimer generation (i.e., without adjustment for BBB dysfunction) between patients with RRMS and disease controls and the association of adjusted and absolute CSF D-dimer levels with the presence of CE on MRI and with CSF fibrinogen levels and CSF leukocytes. Exploratory aims included the investigation of adjusted and absolute CSF D-dimer levels in differential diagnoses of MS and analysis of CSF D-dimer in association with clinical (Expanded Disability Status Scale [EDSS]) and other laboratory parameters in patients with MS.

Plasma and CSF Sample Acquisition

During lumbar puncture, an extra 2.6 mL of CSF was collected directly into a citrate plasma tube (S-Monovette 2.6 mL LH-GEL+, Sarstedt AG & Co. KG). Visibly blood-stained CSF samples due to traumatic punctures were dismissed (n = 15). Blood plasma was collected into an identical citrate plasma tube. Both citrated samples were centrifuged at 3,000 rpm for 10 minutes, immediately pipetted, and frozen at −20°C. All citrated CSF and citrated blood samples were processed and frozen within 60 minutes of collection. Every 4 weeks, the collected samples were moved to a −80°C freezer, where they remained frozen until the final laboratory measurements. On the single day of CSF D-dimer measurements, citrated CSF samples were heated to +37°C using a ThermoMixer C (Eppendorf AG, Hamburg, Germany).

CSF Analysis

In CSF, manually counted cell counts for leukocytes and erythrocytes per mm^3, CSF/serum albumin quotient (Q_{alb}), and intrathecal immunoglobulin G (IgG) synthesis were assessed. The Q_{alb} as a marker of BBB dysfunction was interpreted based on the age-adjusted upper reference limit, as introduced by Reiber et al.[24] (Q_{alb} = 4 + age/15). A CSF/plasma D-dimer ratio ($Q_{D\text{-}dimer}$) was calculated and related to Q_{alb} to adjust for possible influx of D-dimer from the blood stream along with albumin and to correct for differing plasma D-dimer values ($Q_{D\text{-}dimer}/Q_{alb}$ – index). The $Q_{D\text{-}dimer}/Q_{alb}$ – index was multiplied with 10ˆ3 for the purpose of readability. Fibrinogen in CSF was analyzed at a dilution of 1:51 and 1:201 using commercially available ELISA according to manufacturer's protocol (RK024A, Hyphen Biomed, France). Intra-assay imprecision was between 0.4% and 7.4 % coefficient of variation (%CV) using 3 different CSF pools of 10 replicates each.

D-Dimer Analysis and Quality Control of Luminescent Oxygen Channeling Immunoassay Technology

The quantitative determination of D-dimer in citrated CSF was performed using a Luminescent Oxygen Channeling Immunoassay (INNOVANCE LOCI) based on an Atellica COAG 360 System (Siemens Healthineers, Erlangen, Germany). The calibration curve showed a linear relationship between the plotting signal and the analyte concentration (hs D-Dimer INNOVANCE LOCI) with only minimal deviation from the manufacturer's target value (max. 9.4%). Dilution series were performed to assess measurability of D-dimer in very low concentrations (1:10 [22 ng/mL], 1:20 [11 ng/mL], 1:50 [4 ng/mL], 1:100 [2 ng/mL], and 1:200 [1 ng/mL]). Intra-assay imprecision of the assay was between 0.66% and 0.73 %CV using 3 individual samples (high control, low control, and plasma pool) in 20 replicates. Duplicate testing in 24 randomly selected patients confirmed the accuracy of the test results. All laboratory tests were performed in a single run within 1 day and under identical circumstances. The quantitative determination of D-dimer in citrated blood plasma was performed with a HemosIL D-Dimer HS 500 latex-enhanced immunoassay on an ACL TOP 700 testing system (Instrumentation Laboratory, Bedford, MA).

Statistical Analysis

Baseline differences between groups were tested by the independent samples t test for parametric data, the Pearson χ^2 test for categorical data, and the Mann-Whitney U test for nonparametric data. Descriptive statistics were used to present baseline characteristics, results of outcome measurements, and interquartile ranges (IQRs). A Spearman rank-order correlation was run to determine relationships in nonparametric, paired data with a monotonic relationship. Patients with missing data were removed from analysis. For all analyses, the level of significance was set at $p < 0.05$. The primary end point comprised 1 single analysis. The results of the secondary end points were corrected for multiplicity of testing using Bonferroni correction. The exploratory analyses did not undergo correction.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Patient Characteristics

In total, 187 patients with a mean age of 38.0 years (SD ± 13.2, median 35.0 years) were recruited, 65.8% of whom were female individuals (Table 1). Based on final diagnoses at hospital discharge, we identified 71 patients with RRMS, 23 with CIS, 8 with RIS, and 11 with PPMS. In addition, 14 patients experienced an isolated demyelinating event (e.g., isolated myelitis or optic neuritis), and = 4 experienced an anti-MOG/AQP4 disease. Patients finally experiencing other differential diagnoses of MS were grouped as those with CNS neoplasia (n = 5, including cases with meningeosis carcinomatosa and astrocytoma), other acute CNS inflammation (n = 5,

Table 1 Baseline Parameters of the Patient Cohort and CSF Characteristics

	Age (y ±SD)	Female individuals (%)	CSF leukocytes median (IQR) and % above 5/mm³	CE on MRI (%)	OKB positivity (%)	CSF $Q_{D\text{-}dimer}/Q_{alb}$ index (IQR)	CSF fibrinogen in ng/mL (IQR)
All, n = 187	38.0 (±13.2)	123 (65.8%)	3 (1.0–9.0) 36.0% (67)	51 (37.0%)	111 (62.7%)	3.7 (2.2–7.7)	630 (370–1,010)
RRMS, n = 71	34.5 (±9.7)	58 (81.7%)	7 (3.0–15.0) 60.6% (43)	38 (53.5%)	68 (95.8%)	4.7 (2.5–8.0)	640 (430–950)
CIS, n = 23	33.8 (±11.2)	16 (69.6%)	3 (1.0–6.0) 26.1% (6)	6 (27.3%)	17 (73.9%)	3.4 (1.8–7.4)	590 (350–1,360)
RIS, n = 8	34.9 (±11.8)	6 (75%)	4 (2.25–5.75) 28.6% (2)	1 (12.5%)	6 (75.0%)	4.9 (2.4–9.6)	530 (240–1,420)
PPMS, n = 11	49.1 (±11.6)	4 (36.4%)	2 (2.0–13.0) 45.5% (5)	3 (27.3%)	11 (100.0%)	3.3 (1.5–13.9)	920 (380–2,000)
Anti-MOG/AQP4, n = 4	40.8 (±10.4)	3 (75%)	14 (1.25–52.25) 50.0% (2)	3 (75%)	2 (50.0%)	11.7 (3.9–35.0)	980 (550–1,840)
Isolated demyelinating event, n = 14	34.3 (±11.7)	7 (50.0%)	2.5 (1.0–5.0) 14.3% (2)	5 (35.7%)	2 (14.3%)	3.9 (2.5–8.0)	510 (300–830)
CNS neoplasia, n = 5	50.6 (±13.3)	3 (60.0%)	2 (0.5–24.5) 40% (2)	2 (40.0%)	1 (20.0%)	9.4 (3.9–144.7)	720 (440–8,390)
Acute CNS inflammation, n = 5	35.2 (±9.7)	0	35 (2.0–362.0) 60.0% (3)	3 (60.0%)	0	97.8 (5.9–339.4)	5,280 (1,640–7,320)
Non-CNS neurologic diseases, n = 7	57.0 (±15.4)	1 (14.3%)	0 (0–1.0) 0	0	0	3.0 (1.1–9.6)	790 (630–1,290)
Other diseases, n = 17	48.4 (±19.0)	7 (41.2%)	1 (1.0–4.0) 17.6% (4)	0	5 (35.7%)	2.4 (1.3–10.4)	630 (420–1,160)
Disease controls, n = 22	34.8 (±9.7)	18 (81.8%)	1 (0–2.0) 0	0	1 (5.3%)	2.6 (2.1–4.8)	360 (280–740)

Abbreviations: AQP4 = aquaporin 4; CE = contrast-enhancing lesions; CIS = clinically isolated syndrome; Ig = immunoglobulin; IQR = interquartile range; NA = not applicable; OKB = oligoclonal band; PPMS = primary progressive MS, MOG = myelin oligodendrocyte glycoprotein; Q_{alb} = CSF/serum albumin quotient; RIS = radiologically isolated syndrome; RRMS = relapsing-remitting MS.
The mean values are given with SD. Percentages are provided in relation to all patients of the respective subgroup.

including cases with viral and bacterial meningitis, neurosarcoidosis, and M. Behçet), non-CNS neurologic diseases (n = 7, e.g., peripheral neuropathies), and other diseases (n = 17, not assignable to the other categories, including cases with epilepsy, myopathy, and vascular disorders). In total, 22 patients without signs of an organic disease after extensive diagnostic workup (e.g., unspecific or somatoform symptoms) were considered as disease controls.

## Primary, Secondary, and Exploratory Outcomes

The adjusted $Q_{D\text{-}dimer}/Q_{alb}$ – index as the primary outcome parameter confirmed higher intrathecal D-dimer production in patients with RRMS (median 4.7, IQR 2.5–8.0) compared with that in disease controls (median 2.6, IQR 2.1–4.8, $p = 0.031$) (Figure 1A). The distribution of the adjusted $Q_{D\text{-}dimer}/Q_{alb}$ – index values among the 11 study subgroups is summarized in Table 1. In this study, disease controls showed the lowest $Q_{D\text{-}dimer}/Q_{alb}$ – index and the lowest absolute CSF D-dimer values (Figure 1).

Secondary outcome parameter analysis revealed no difference for the adjusted $Q_{D\text{-}dimer}/Q_{alb}$ – index in RRMS patients with CE on MRI (n = 38, median 5.0, IQR 2.4–8.4) compared with that in patients without CE on MRI (n = 16, median 5.4, IQR 3.4–15.1, $p = 0.28$). Similarly, in all 113 patients with MS (comprising patients with RRMS, CIS, RIS, and PPMS), there was no difference in the $Q_{D\text{-}dimer}/Q_{alb}$ – index between patients with CE on MRI (n = 48, median 5.3, IQR 2.4–9.5) when compared with that in patients without CE on MRI (n = 40, median 4.4, IQR 2.6–8.1, $p = 0.57$).

For absolute CSF dimer, there was a trend toward higher values in patients with RRMS (median 4 ng/mL, IQR 3–8) compared with those in disease controls (median 3 ng/mL, IQR 2–4.25, $p = 0.063$, Figure 1B). In all 113 patients with MS, the presence of CE on MRI was associated with higher absolute CSF D-dimer levels (n = 48, median 6 ng/mL, IQR 3–15.25) when compared with the absence of CE on MRI (n = 41, median 4 ng/mL, IQR 2–7, $p = 0.026$, not significant after correction, Figure 2). Analysis of CSF fibrinogen revealed a strong correlation with CSF D-dimer values in the 113 MS patients with RRMS, CIS, RIS, or PPMS (r = 0.463; $p < 0.001$, corrected, Figure 3). Absolute CSF D-dimer also correlated with CSF leukocytes in the 113

Figure 1 D-Dimer and Fibrinogen Subgroup Analysis
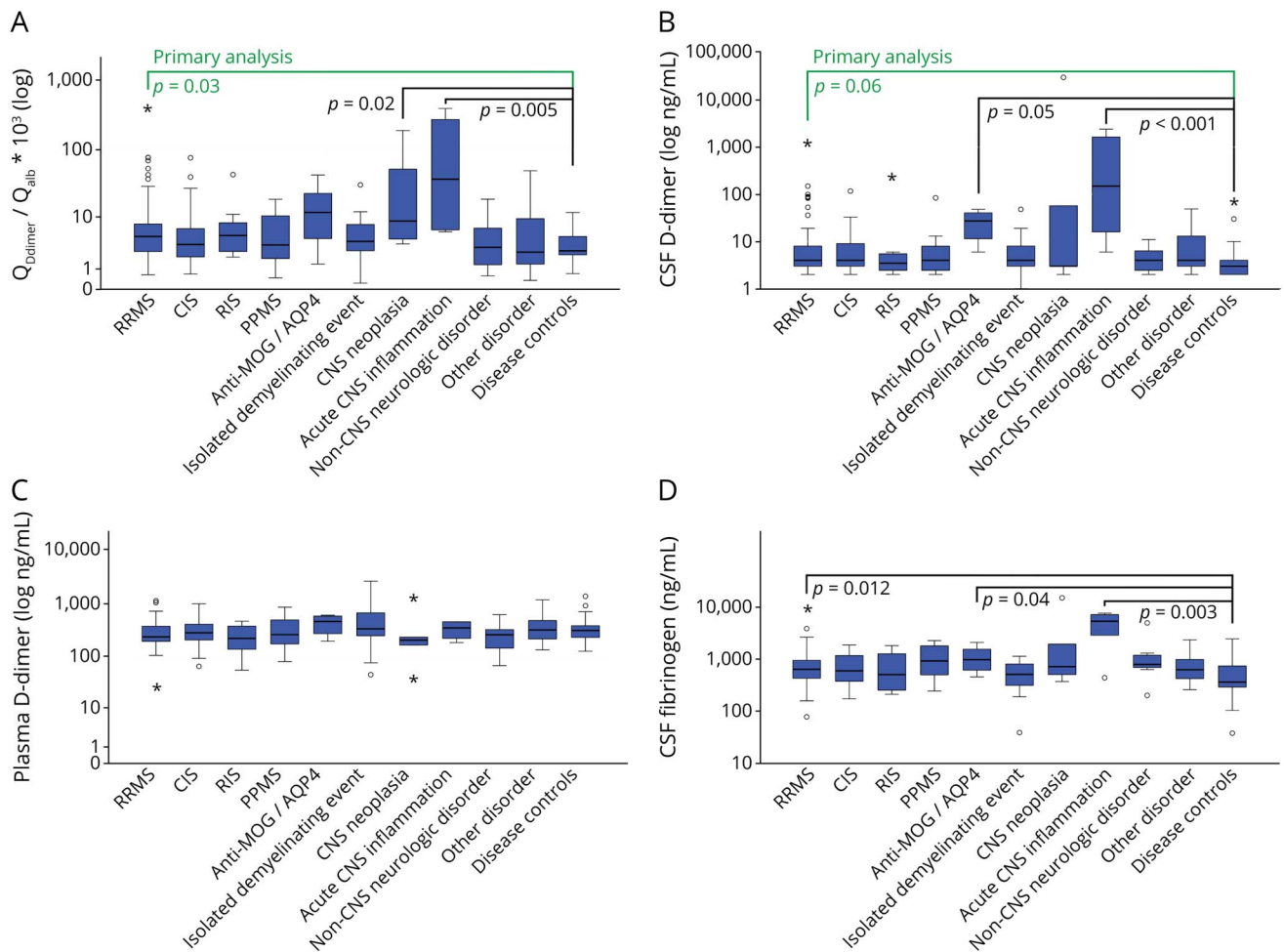
Boxplot diagrams demonstrating (A) the $Q_{D-dimer}/Q_{alb}$ index (Y – axis) in the different study subgroups (X – axis), (B) the absolute D-dimer values in the CSF (Y – axis [ng/mL]) in the different study subgroups (X – axis), (C) the D-dimer values in the plasma (Y – axis [ng/mL]) in the different study subgroups (X – axis), and (D) the fibrinogen values in the CSF (Y - axis [ng/mL]) in the different study subgroups (X-axis), each on a logarithmic scale. Circles and asterisks indicate mild and extreme outliers. AQP4 = aquaporin 4; CIS = clinically isolated syndrome; dis. = disease; inflamm. = inflammation; isol. dem. event = isolated demyelinating event; MOG = myelin oligodendrocyte glycoprotein; neur. = neurologic; PPMS = primary progressive MS; Q_{alb} = CSF/serum albumin quotient; $Q_{D-dimer}$ = CSF/plasma D-dimer quotient; RIS = radiologically isolated syndrome; RRMS = relapsing-remitting MS.

patients with MS (r = 0.273; p = 0.003, corrected). However, for $Q_{D-dimer}/Q_{alb}$ – index, this correlation with CSF leukocytes was not significant (r = 0.146; p = 0.13). The number of CSF erythrocytes did not correlate with absolute CSF D-dimer (r = 0.04; p = 0.65), and there was no correlation of intrathecal IgG synthesis and CSF D-dimer values (p = 0.97).

Exploratory analysis of the $Q_{D-dimer}/Q_{alb}$ – index was performed for all subgroups (Figure 1A). In this study, the $Q_{D-dimer}/Q_{alb}$ – index was higher in patients with other acute CNS inflammation (p = 0.005) and CNS neoplasia (p = 0.023) when compared with that in disease controls (Table 1). We found no correlation between $Q_{D-dimer}/Q_{alb}$ – index and EDSS score (0–4.5, documented before treatment) (r = 0.07; p = 0.445). This did not change when dichotomizing patients with EDSS ≥3.0 and patients with EDSS <3.0 (p = 0.19). The

$Q_{D-dimer}/Q_{alb}$ – index in patients with MS did not correlate with presence of oligoclonal bands (p = 0.42) or with intrathecal IgG synthesis (r = 0.07; p = 0.47).

Analysis of absolute CSF D-dimer among the subgroups revealed highest values in patients with other acute CNS inflammation (Figure 1B). Similarly, patients with anti-MOG/AQP4 diseases showed higher absolute CSF D-dimer values when compared with disease controls (p = 0.005). Patients with CNS neoplasia showed a very broad distribution of CSF D-dimer levels, depending on the presence of meningeosis or a solid tumor (median 3 ng/mL, IQR 2.5–15,028). Patients with non-CNS neurologic diseases and patients with other diseases showed low CSF D-dimer values. The CSF D-dimer in patients with MS did not correlate with the presence of oligoclonal bands (p = 0.54) or with intrathecal IgG synthesis (r = 0.09; p = 0.35).

**Figure 2** Contrast-Enhancing Lesions on MRI
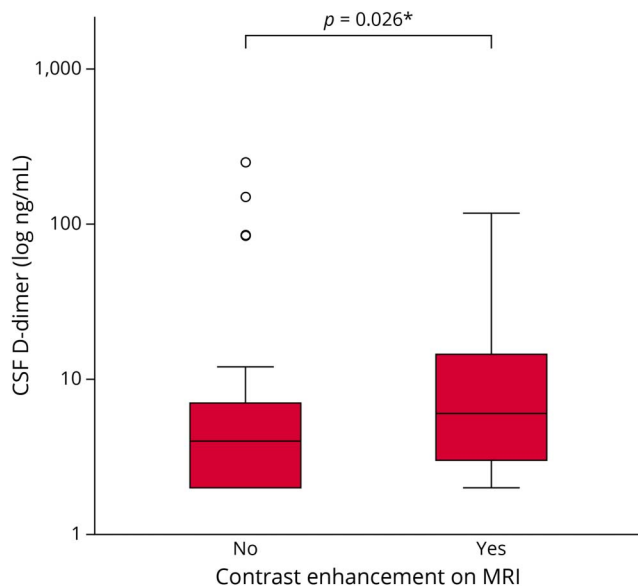
A boxplot diagram demonstrates the D-dimer values in the CSF (Y – axis; [ng/mL]) for 113 patients with MS, comprising patients with relapsing-remitting MS (n = 71), clinically isolated syndrome (n = 23), radiologically isolated syndrome (n = 8), and primary progressive MS (n = 11), with and without contrast enhancement (X – axis) on MRI on a logarithmic scale. Circles and asterisks indicate mild and extreme outliers. *Not significant after Bonferroni correction for multiplicity of testing.

We found no differences for plasma D-dimer (median 273 ng/mL, IQR 194–438) between the subgroups (Figure 1C). The CSF fibrinogen was increased in patients with RRMS when compared with that in disease controls (p = 0.012, Figure 1D)

Figure 3 Scatterplot
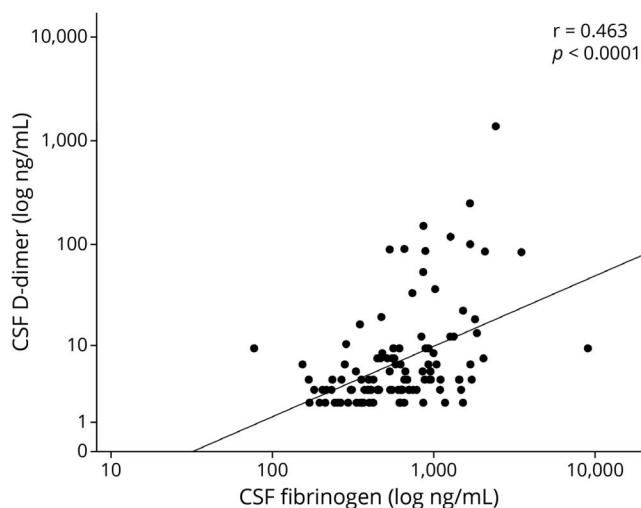
Scatterplot demonstrating the 2-sided Spearman correlation of D-dimer levels in the CSF (X – axis; logarithmic [ng/mL]) with fibrinogen levels in the CSF (X – axis; logarithmic [ng/mL]) in 113 patients with MS (71 patients with relapsing-remitting MS, 23 with clinically isolated syndrome, 8 with radiologically isolated syndrome, and 11 with primary progressive MS). Abbreviation: r = Spearman correlation coefficient.

and was not significantly elevated in all 113 MS patients with CE on MRI (n = 48, median 657 ng/mL, IQR 480–1,017) when compared with that in patients without CE on MRI (n = 41, median 594 ng/mL, IQR 357–1,099, p = 0.39). The CSF fibrinogen closely correlated with albumin quotient (Q_{alb}) as an indicator of BBB disruption (r = 0.660; p < 0.001). In addition, CSF fibrinogen correlated with intrathecal IgG synthesis (r = 0.265; p = 0.005). The CSF fibrinogen in patients with MS did not correlate with EDSS score (r = 0.07; p = 0.48), with CSF leukocytes (r = 0.11; p = 0.26), or the presence of oligoclonal bands (p = 0.45).

No patients in this study had received MS disease–modifying drugs before lumbar puncture. Previous administration of IV methylprednisolone (IVMP) within 7 days of lumbar puncture in 19 patients experiencing MS (median 5 ng/mL, IQR 3–13) did not affect CSF D-dimer compared with patients without IVMP (median 4 ng/mL, IQR 3–9; p = 0.50). One patient (group: other diseases) was on treatment with an anti-F Xa anticoagulant and showed low absolute CSF D-dimer of 2 ng/mL.

Classification of Evidence

Primary question of the investigation was the measurability of adjusted CSF D-dimer in patients with MS using a highly sensitive luminescent oxygen channeling immunoassay and to analyze its diagnostic utility in differentiating RRMS from disease controls and active from nonactive disease stages. This study provides Class I evidence that CSF D-dimer levels are elevated in patients with RRMS.

Discussion

In this study, we demonstrated that D-dimer can be reliably measured in CSF using a high-sensitivity assay. When adjusting for BBB dysfunction with a $Q_{D\text{-}dimer}/Q_{alb}$ index, increased intrathecal CSF D-dimer values could be shown for patients with RRMS when compared with those for disease controls. Highest absolute values for CSF D-dimer were found in acute infectious and antibody-mediated autoimmune diseases (including meningoencephalitis and anti-MOG/AQP4 autoimmune diseases). Analysis of CSF fibrinogen in the same patients showed similar results and a strong correlation with CSF D-dimer levels. The findings support the hypothesis that CNS inflammation is associated with fibrinogen influx through a dysfunctional BBB and a subsequent intrathecal coagulation cascade activation. In patients with MS, CSF D-dimer may in perspective constitute a measure of this intrathecal fibrin-driven autoimmunity.

Previous experience on the measurement of D-dimer in the CSF was limited to intracranial bleeding, tumor, and major CNS infection, and no standardized laboratory testing exists.[20-22,25] Those studies relied on ELISA with a lower detection limit for D-dimer in CSF of approximately 50 ng/mL. Because considerably lower values for D-dimer were to be

expected in CSF of patients with MS, measurements in this study were performed using high-sensitive chemiluminescence technology (LOCI). The lower detection limit for D-dimer was determined in dilution series to be at least 1 ng/mL, with intra-assay imprecision very well within acceptable limits. To the best of our knowledge, this is the first study to reliably describe CSF D-dimer in patients with MS, or in fact in any patients, in such low concentrations.

The presented findings may enrich the pathophysiologic understanding of how proteins of the coagulation system, especially fibrinogen, are embedded into CNS autoimmunity.[5] After BBB disruption, fibrinogen is washed into the CNS and subsequently cleaved by thrombin to fibrin, which fuels autoimmunity that can be allocated to MS plaques.[5,17,26] This passive fibrinogen influx during CNS inflammation was well-documented in this study by the close correlation of CSF fibrinogen levels and Q_{alb} (as a measure of BBB disruption) in patients with MS, as described in previous literature.[9,27] Subsequent activation of

the coagulation cascade in the CNS results in accumulation of fibrin mesh, which is ultimately disintegrated by plasmin into its degradation products, including D-dimer (Figure 4). Similar to other coagulation factors, fibrinogen itself is not expressed within the nervous system.[5] Thus, the presence and extent of D-dimer in the CNS has to be connected to the fibrinogen influx and its subsequent processing in case of BBB disruption.[24] Noteworthy, because fibrin deposition is long sustained in CNS lesions,[5] presence of D-dimer in the CNS may also indicate a preceding BBB dysfunction, even after the BBB is restored.

Aside fibrinogen, thrombin is another driver of the intrathecal fibrinogenic pathway and was shown to be increased in the CNS and to be associated with axonal damage, demyelination, and fibrin deposition in EAE.[18] In addition, an acute increase of other drivers of fibrinogenesis—F X and prothrombin—was demonstrated in the plasma of patients with RRMS and SPMS.[16] Because those coagulation factors are likely not produced in the CNS, their presence and

Figure 4 Model of the Coagulation Pathways Leading to the Formation of D-Dimer During Blood-Brain Barrier Disruption
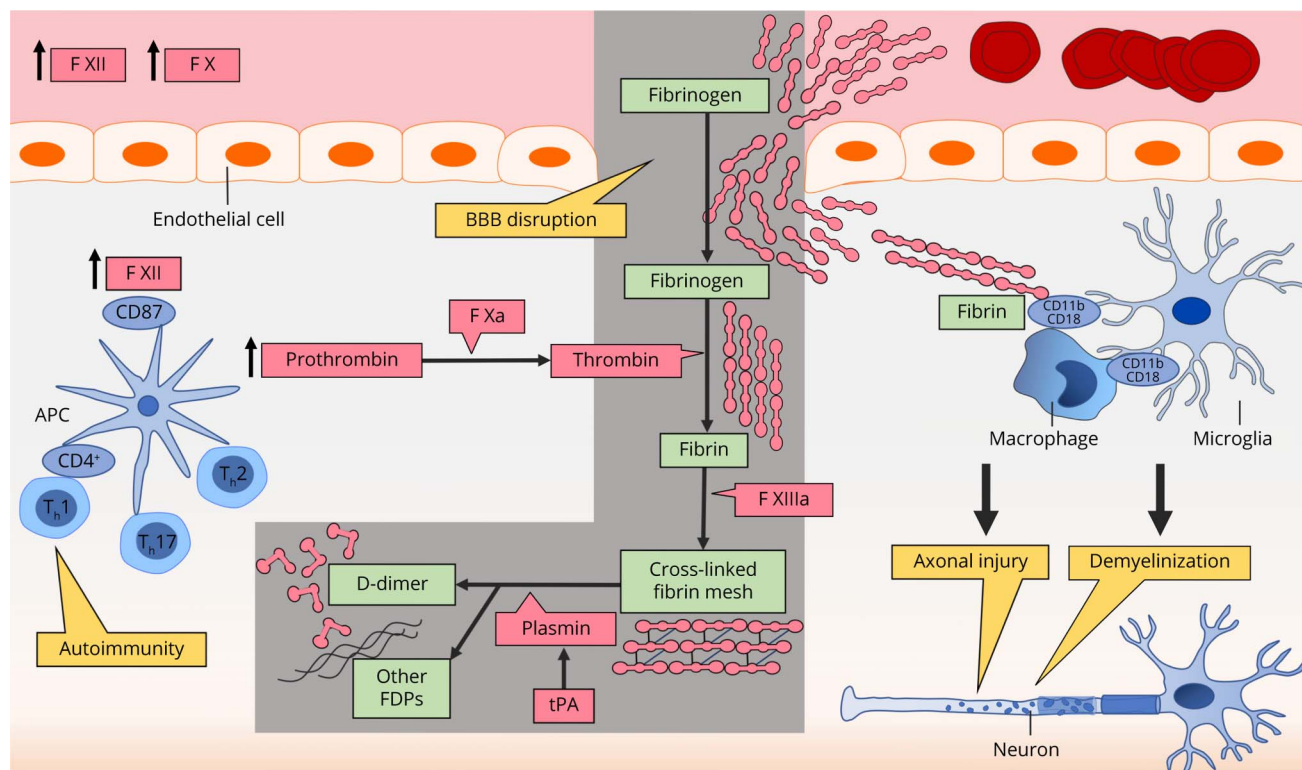
During a blood-brain barrier (BBB) disruption, fibrinogen enters the CNS and is rapidly converted to fibrin by thrombin.[5,7] Fibrin then immerses in pleiotropic roles of immune system activation by binding to the integrin receptor CD11b/CD18 of macrophages and microglia, leading to peripheral T-cell recruitment, axonal injury, demyelinization, and scar formation.[3,5,6,8] The fibrinolytic pathway (gray shaded) continues with the formation of cross-linked fibrin mesh by activated factor XIII (F XIIIa). The fibrin mesh is then degraded to soluble D-dimer and other fibrin degradation products (FDPs) by plasmin, itself activated by tissue-type plasminogen activator (tPA).[10,11] Prothrombin and Factor X (F X) are elevated in the plasma of patients with MS.[16] In the CNS, they engage in the process modulated by activated Factor X (F Xa) of cleaving thrombin from prothrombin. Thrombin then exerts its starter function in the fibrinogenic pathway by converting fibrinogen to fibrin. In parallel, Factor XII (F XII) drives an adaptive cellular CD87-dependent autoimmune response mediated by dendritic cells (APC) and was shown to be elevated in the plasma of patients with MS during a relapse.[2] Parts in this figure were edited based on data held under a free Creative Commons Attrition 3.0 license (commons.wikimedia.org/wiki/File:Microglia.svg). APC = antigen-presenting cell; BBB = blood-brain barrier; F = factor; FDP = fibrin degradation products; tPA = tissue-type plasminogen activator.

activation are also a consequence of previous inflow through the dysfunctional BBB.

We conclude that any observed intrathecal coagulation cascade activation (e.g., depicted by CSF D-dimer increase) seems to be dependent on the substrates that cross, or have earlier crossed, a dysfunctional BBB. The measurement of fibrinogen in CSF allows insight into the extent of BBB disruption in patients with RRMS and correlates well with the presence of MS pathology.[4,5,7,28] Acute BBB disruption (CSF/serum albumin quotient) in patients with MS is known to correlate with CNS inflammation injury and was recently linked to increased intrathecal neurofilament light chain, chitinase 3-like 1, metalloproteinase 2, and interleukin-6 levels.[29,30] However, although levels of fibrinogen in CSF correlate with levels of acute (or past) BBB dysfunction, they do not necessarily reflect the extent of fibrinogenic pathway activation, e.g., processing of fibrinogen into fibrin, which effectively exerts proinflammatory effects.[5] By contrast, D-dimer in CSF is not only a substrate passing the BBB but also a product of the coagulation cascade. Hence, analysis of D-dimer in CSF can provide additional information on the amount of activated fibrinogen in the CSF (i.e., fibrin generation and further processing) and may allow to further quantify the degree of coagulation cascade activation, which is also modulated by, e.g., F Xa, prothrombin, and thrombin levels. Additional adjustment for Q_{alb} and $Q_{D-dimer}$ is likely necessary to account for passive D-dimer inflow from the blood and measure intrathecal D-dimer generation more accurately.

In this study, we demonstrated increased CSF D-dimer and CSF fibrinogen values for MS patients with contrast-enhancing lesions on MRI, in whom both an acute BBB disruption and a high inflammatory burden in the CNS can be assumed. This correlation was lost after adjusting for permeability of the BBB ($Q_{D-dimer}/Q_{alb}$ – index). Moreover, the correlation of CSF leukocytes and D-dimer mostly diminished after adjustment for BBB dysfunction. A considerable BBB disruption (to be assumed with CE on MRI and/or leukocytosis) seems to overhaul the effect of inflammatory intrathecal D-dimer production. Thus, correcting for Q_{alb} diminishes previously observed differences in values that either cross the BBB (fibrinogen) or are highly dependent on previous BBB crossing of other substrates (D-dimer).

The increasingly evident interaction of coagulation system proteins and CNS inflammation entails the challenge to identify novel therapeutic targets. Already, fibrin-targeting immunotherapy has been explored with encouraging effects on CNS autoimmunity and neurodegeneration.[31] In addition, F XII and other upstream factors of the coagulation cascade may be promising targets.[2,6,16] Although the utilization of warfarin or novel anticoagulants with selective F Xa blockade showed appealing results,[14,15] safer, more site-specific options are necessary to prevent unwarranted risk of bleeding.

Limitations of this study include the pilot character of CSF D-dimer measurements with chemiluminescence technology, which has not been formally standardized for the viscosity and lipoprotein composition of CSF. However, dilution series with control reagent and quality control runs showed a solid intra-assay precision of the CSF D-dimer values in this study, and patient samples proved to be consistent with available data based on ELISA.[20,22] In this study, we found no correlation of functional disability as assessed by EDSS score at admission with markers of the coagulation cascade—neither of adjusted CSF D-dimer nor with CSF fibrinogen. Notably, it must be considered that the study comprised numerous MS patients with spinal (n = 12) or cerebral (n = 20) contrast-enhancing lesions as a marker of activated CNS inflammation but only no to mild disability (EDSS ≤3.0), causing a high variance within the sample.

Future studies shall assess additional markers of coagulation cascade activation in the CSF such as thrombin and prothrombin and relate the parameters of fibrin-derived autoimmunity to other prognostic markers of MS disease activity, such as cytokines,[28,32] chemokines related to B-cell activity,[9] and markers of neuroaxonal degeneration (e.g., neurofilament light chain[33]) to deepen the understanding of the interconnection of coagulation system and neuroimmunologic mechanisms.

In summary, D-dimer in the CSF can be reliably determined in high-sensitivity assays and correlates well with CSF fibrinogen levels. In MS, D-dimer bears diagnostic potential as a biomarker for disease activity (including CE) and as a surrogate to monitor intrathecal coagulation cascade activation in future therapeutic studies.

Disclosure
The authors MSP, JHS, YY, HS, LF, EH, WM, KK, and FW declare that there is no conflict of interest to report. CF reports speaker honoraria and honoraria for participating on advisory boards from Novartis, Teva, Merck, Sanofi Genzyme, Alexion, Bristol-Myers Squibb, and Roche, and has received research support from Sanofi Genzyme and Novartis. Go to Neurology.org/NN for full disclosures.

Publication History

Appendix Authors

Name	Location	Contribution
Martin A. Schaller-Paule, MD	Department of Neurology, University Hospital Frankfurt, Goethe-University, Frankfurt am Main, Germany	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
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Wolfgang Miesbach, MD	Department of Haemostaseology and Hemophilia Center, Medical Clinic 2, Institute of Transfusion Medicine, University Hospital Frankfurt, Germany	Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design
Frank Weber, MD	Neurological Clinic Cham, Cham, Germany	Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design
Konstantin Kirchmayr, MD	punktmed Center for Vascular Medicine and Haemostaseology, Nuremberg/Amberg, Germany	Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
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