Characteristics of fibrin/fibrinogen degradation products in multiple sclerosis following SARS-CoV-2 infection


Abstract. Background. The purpose of this study was to investigate plasma levels of fibrinogen and products of its degradation in patients with multiple sclerosis (MS) with and without a history of coronavirus disease 2019 (COVID-19). Materials and methods. We examined 97 patients with MS. Based on the presence of COVID-19, all cases were divided into two groups. MS group included 56 patients who did not suffer from COVID-19 previously. MS + COVID group consisted of 41 cases who had a laboratory-verified diagnosis of COVID-19. The group of healthy controls included 30 healthy volunteers. Spectrophotometric techniques were used to measure the concentrations of fibrinogen, D-dimer, and soluble fibrin monomer complexes (SFMCs). Size-exclusion chromatography was applied to analyze the composition of SFMC fractions. Results. We found that concentrations of fibrinogen, D-dimer, and SFMCs were remarkably increased in plasma of all MS patients compared with healthy controls. The levels of D-dimer, and SFMCs did not differ between two MS groups, while plasma fibrinogen concentration was significantly increased in MS + COVID patients compared to MS group. Moreover, the development of MS was accompanied by the changes in both quantity and quality of SFMC composition compared to that of healthy controls. Our results demonstrated accumulation of high-molecular-weight SFMCs in plasma of MS patients. Conclusions. The findings indicated that MS patients had changed hemostasis characteristics; however, more research is required to determine the connection between particular hemostatic factors, namely fibrinogen, D-dimer, and SFMCs, and the pathophysiology of MS.

Keywords: multiple sclerosis; SARS-CoV-2 infection; fibrinogen; D-dimer; soluble fibrin monomer complexes

Introduction

Multiple sclerosis (MS) is an inflammatory-mediated demyelinating disease of the central nervous system (CNS) characterized by neuroinflammation and neurodegeneration [1]. In the past decades, great efforts have been made in understanding the risk factors, and immune dysregulation mechanisms that are responsible for detecting MS and its progression. MS is thought to emerge as a result of a complex combination of genetic predispositions, environmental triggers, infectious events, and factors that lead to pro-inflammatory states, including smoking, obesity, etc. [2]. MS lesions are widely acknowledged to originate from an autoimmune process that affects the myelination of the neurons present in the CNS. This leads to the formation of demyelinated plaques, which cause injury to neurons and their axonal extensions [3]. Numerous studies have empha-
sized the existence of relationship between the activation of the coagulation cascade and neuroinflammation, indicating that coagulation factors may play a more comprehensive role involving neurodegeneration and neuroinflammation in addition to being essential for the activation of the hemostatic cascade [4–6]. In recent years, significant evidence has emerged implicating several of the major clotting factors, such as thrombin, or fibrinogen, in the pathogenesis of MS by triggering microglia activation and driving the progress of neuroinflammation [6].

Fibrinogen is a soluble 340 kDa dimeric glycoprotein that is synthesized in the liver by the hepatocytes. Each monomer made up of three polypeptide chains designated \( \alpha, \beta, \) and \( \gamma \) that are stabilized by disulfide bonds. Thrombin removes two small peptides, fibrinopeptides A and B, from the fibrinogen molecule, leading to exposure of multiple polymerization sites and initiating the formation of insoluble and stable fibrin clot [7]. In addition to its major function in blood clotting, fibrin(ogen) plays an essential role in cellular and matrix interactions, inflammation, wound healing, angiogenesis, etc. Various studies have highlighted the contribution of fibrin(ogen) to the pathophysiology of MS [8–11]. It was shown that the areas occupied by demyelinating lesions and characterized by axonal damage coincided with the areas of fibrin deposition in MS patients [12]. Furthermore, fibrin deposition may precede the formation of demyelinating lesions [13]. The role of fibrin in formation of demyelinating lesions may be associated with its proinflammatory function and ability to increase the release of several other immune mediators, which, in turn, can modulate immune process as well as both cell adhesion and migration [14]. The other way by which fibrin(ogen) can be involved in the pathogenesis of MS is its direct triggering microglia activation. Ample evidence suggests that fibrinogen induces release of reactive oxygen species in microglia causing damage to nerve cells, which play a crucial role in the induction of axon degeneration in inflammatory demyelination [15].

Given the important role of fibrin(ogen) in the pathogenesis of MS, we hypothesized that factors affecting the concentration of circulating fibrinogen could be considered independent risk factors for deterioration in MS patients. Since coronavirus disease 2019 (COVID-19) is associated with both quantitative and qualitative changes in circulating fibrinogen, intra- and extravascular fibrin deposition, and fibrin degradation [16], COVID-19 infection can increase the risk of CNS demyelinating lesions leading to exacerbation of MS. Despite the expectation of a deleterious effect of COVID-19 on MS manifestations, the results regarding the relationship between these two disorders are quite controversial [17] and further studies are required.

The current research aimed to investigate plasma levels of fibrinogen and products of its degradation in MS patients with and without COVID-19 history. This new information should help clarify the underlying mechanisms of MS pathogenesis, and hopefully lead to new findings regarding contribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection to deterioration in MS patients.

Materials and methods

This research was conducted in accordance with the ethical standards and provisions of the Declaration of Helsinki (1964), and applicable regulatory requirements. The study protocol was approved by the ethics committees of both Taras Shevchenko National University of Kyiv (Kyiv, Ukraine) and Bogomolets National Medical University (Kyiv, Ukraine). A total of 97 MS patients (gender distribution: 34 male and 63 female) were seen at the University Clinic of Bogomolets National Medical University between January 2021 and August 2022 were enrolled in this study. Average duration of disease was similar in all MS patients and ranged between 3 and 5 years. Participants were divided into two groups. MS group (n = 56) included MS patients who did not suffer from COVID-19 previously. MS + COVID group (n = 41) consisted of MS patients who had a laboratory-confirmed diagnosis of COVID-19 in the past 3–6-month period, but currently were negative, as determined by testing nasopharyngeal swabs. The healthy control (HC) group included 30 subjects, all of them were SARS-CoV-2-negative by nasopharyngeal swab at the time of blood sampling. We excluded all individuals who had cardiovascular and cerebrovascular diseases; were on hemostatic medications; had any acute or chronic disorders that can affect the hemostasis system. Written informed consent was obtained from all participants.

Whole blood samples were collected into vacutainer plasma tubes (tubes with K₂EDTA). Immediately after drawing, tubes were gently inverted, and centrifuged at 2,500 g for 15 minutes. Plasma aliquots were separated immediately and stored at −20 °C until use. Prior to analysis, frozen plasma samples were placed into a +37 °C water bath, thawed for five to ten minutes, and mixed by gentle inversion.

The qualitative detection of immunoglobulins M (IgM) and G (IgG) antibodies against SARS-CoV-2 in blood plasma was done by chemiluminescence immunoassay using the reagent kit Maglumi 2019-nCoV IgM/IgG (Shenzhen New Industries Biomedical Engineering Co., Ltd., China). The results are expressed in absorbance unit (AU/mL). Accoding to the operating instructions, a result less than 1.00 AU/mL was considered to be non-reactive, while a result greater than or equal to 1.00 AU/mL was considered to be reactive.

Fibrinogen concentration was measured spectrophotometrically according to the method [18]. Briefly, the fibrin clot formed after the addition of thrombin (2 NIH) was dissolved in 0.125% acetic acid. The optical density (OD) of samples was measured at wavelengths of 280 and 320 nm. The fibrinogen concentration (g/L) was calculated using the formula: (OD₂₈₀ – OD₃₂₀) × 255 / 1.506, where 255 represents the conversion factor of the fibrinogen concentration in the sample volume to its plasma concentration, and 1.506 represents the fibrin extinction coefficient at 280 nm.

The concentration of D-dimer was determined by a sandwich-type immunochemiluminescent assay using D-dimer Test Kit for CLIA Maglumi (Snibe Co., Ltd., China). All manipulations were carried out according to the...
The concentration of soluble fibrin monomer complexes (SFMCs) was determined using o-phenanthroline technique [18]. This method is based on the estimation of time needed to form fibrin particles after addition of 0.78% o-phenanthroline solution (1 : 1). Further purification of the formed complexes was performed.

SFMCs were isolated from blood plasma according to the procedure described previously [19]. The SFMCs were collected from 1 mL of blood plasma of each individual and kept at 4 °C till chromatographic analysis. Before chromatographic analysis, equal volume of SFMCs solutions obtained from five individuals of the same experimental group were randomly gathered to form pool of SFMCs, which was used in further analysis. Size-exclusion chromatography was successfully applied to SFMCs isolated from blood plasma according to the manufacturer’s instructions. The analyzer automatically calculated the concentration of D-dimers in the samples based on the calibration curve. The results were expressed in μg FEO/mL.

Table 1. Basic characteristics of the multiple sclerosis and healthy control groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HC</th>
<th>MS</th>
<th>MS + COVID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients, n</td>
<td>30</td>
<td>56</td>
<td>41</td>
</tr>
<tr>
<td>Age, years</td>
<td>41 ± 4</td>
<td>40 ± 5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>Gender: M/F, n</td>
<td>10/20</td>
<td>20/36</td>
<td>14/27</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>–</td>
<td>4.4 ± 1.5</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Anti-SARS-CoV-2 IgM, AU/mL</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Anti-SARS-CoV-2 IgG, AU/mL</td>
<td>0.18 [0.04–0.48]</td>
<td>0.12 [0.01–0.23]</td>
<td>4.70 [2.92–7.34]**</td>
</tr>
<tr>
<td>D-dimer, mg/mL</td>
<td>0.23 [0.22–0.30]</td>
<td>0.35 [0.29–0.41]</td>
<td>0.38 [0.32–0.47]</td>
</tr>
<tr>
<td>SFMCs, μg/mL</td>
<td>35 [33–36]</td>
<td>65 [58–70]</td>
<td>68 [62–70]**</td>
</tr>
</tbody>
</table>

Notes: age and disease duration in years are reported as mean ± SEM; for anti-SARS-CoV-2 IgG, fibrinogen, D-dimer, SFMCs, the median and interquartile range [P25–P75] are given; * — p < 0.05 vs. HC; ** — p < 0.01 vs. MS group.
analyze and compare the composition of SFMC fractions isolated from the bloodstream of MS patients and healthy individuals.

Our results showed that the examined SFMC pools contained a variety of protein molecules that had different molecular weight (up to 550 kDa). The difference between the results of fractionation of SFMC isolated from the plasma of MS patients, and HC was obvious (Table 2). As can be seen, the SFMC pool of healthy individuals consisted of 3 main fractions. The most abundant protein fraction in the SFMC pool of healthy controls was a fraction with a molecular weight of 330–340 kDa, which represented about 45% of total SFMCs. Another majority fraction, which represented 35% of the total SFMC content, corresponded to proteins whose molecular weight ranged from 100 to 110 kDa. Finally, 30% of the SFMC pool was represented by 260–280 kDa protein complexes.

The development of MS was accompanied by changes in both the quantity and quality composition of the SFMC pool compared to the results obtained for HC (Table 2). Thus, in the group of anti-SARS-CoV-2 IgG seronegative MS subjects, we did not observe protein fractions ranging between 260–340 kDa. On the other hand, protein complexes with a molecular weight of 540–550 kDa were identified in the SFMC pool, and this fraction represented about 70% of total SFMCs. The levels of the other two fractions, 100–110 and 140–160 kDa, were almost equal and represented 16 and 14% of the total SFMC pool, respectively.

In the group of anti-SARS-CoV-2 IgG seropositive MS subjects (MS + COVID group), the levels of 2 majority fractions, 120–130 and 140–160 kDa, were almost equal; they represented 49 and 42% of the total SFMC pool, respectively. Finally, a trace amount (9%) of SFMCs was represented by 330–340 kDa protein complexes (Table 2).

**Discussion**

COVID-19 poses a considerable threat to public health worldwide. The hallmark of COVID-19 pathogenesis is the cytokine storm, which may lead to multiorgan dysfunction and could influence inflammatory and degenerative processes in the CNS. Moreover, COVID-19 has been associated with hemostasis disbalance, which seems to be a crucial factor in neuroinflammation [16]. Thus, the COVID-19 pandemic became an ongoing global challenge, especially for people with autoimmune diseases, such as multiple sclerosis. Indeed, there is a lot of evidence showing a relationship between COVID-19 and MS [17, 20–22]. However, current findings are sometimes controversial, and more studies are needed to collect data on greater numbers of patients with SARS-CoV-2 infection since these cohorts are not yet large enough to make specific conclusions for patients with MS.

The results of our previous study demonstrated that SARS-CoV-2 IgG seropositive donors had increased levels of circulating fibrinogen, D-dimer, and SFMCs [19]. Given the important role of fibrinogen in the pathogenesis of MS, we hypothesized that SARS-CoV-2 infection could be a risk factor for deterioration in MS patients who suffered from COVID-19.

The major differences between the coagulation parameters of healthy volunteers and MS patients were as follows: 1) circulating fibrinogen concentration was increased by 45 and 50% in patients of MS and MS + COVID groups, respectively, compared to the controls; 2) the concentration of D-dimer was increased by 50 and 65% in plasma of patients from MS and MS + COVID groups, respectively, compared to control subjects; and 3) the concentration of SFMCs was 2-fold higher in both MS groups than in the controls. Interestingly, according to the results obtained, a SARS-CoV-2 infection had a limited effect on the studied coagulation parameters in MS patients, causing statistically significant changes in only one of them, namely plasma fibrinogen concentration.

Our study confirms the findings of other researchers [8–11] that fibrinogen levels are elevated in MS patients. The severity of MS is assumed to correlate with fibrinogen level. This clotting factor is involved in the MS pathogenesis by triggering microglia activation and neuroinflammation [23]. Since fibrinogen is an acute-phase protein, its accumulation in the bloodstream of MS patients may occur due to systemic inflammation, which takes place under this autoimmune disorder [11, 23]. Even more pronounced hyperfibrinogenemia in anti-SARS-CoV-2 IgG seropositive MS patients could develop due to thrombotic complications resulting from COVID-19 [24].

In this study, we demonstrated increased circulating D-dimer and SFMC values for both MS patient groups. The presented findings may be explained by the activation of the coagulation cascade under MS conditions. As a result of fibrinogen cleavage, accumulated fibrin mesh may be ul-

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**Table 2. SFMC fractions derived from the plasma of multiple sclerosis patients and healthy controls, %**

<table>
<thead>
<tr>
<th>Molecular weight, kDa</th>
<th>HC</th>
<th>MS</th>
<th>MS + COVID</th>
</tr>
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<tbody>
<tr>
<td>540–550</td>
<td>–</td>
<td>70 ± 6</td>
<td>–</td>
</tr>
<tr>
<td>330–340</td>
<td>45 ± 4</td>
<td>–</td>
<td>9 ± 3*</td>
</tr>
<tr>
<td>260–280</td>
<td>30 ± 2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>140–160</td>
<td>–</td>
<td>14 ± 3</td>
<td>42 ± 3*</td>
</tr>
<tr>
<td>120–130</td>
<td>–</td>
<td>–</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>100–110</td>
<td>35 ± 4</td>
<td>16 ± 3*</td>
<td>–</td>
</tr>
</tbody>
</table>

**Notes:** values are expressed as mean ± SEM; * — p < 0.05 vs. HC; # — p < 0.05 vs. MS group.
timely disintegrated by plasmin into its degradation products, including D-dimer. The results of other studies have shown the elevated level of D-dimer in cerebrospinal fluid from MS patients. Moreover, it was proposed to use cerebrospinal fluid D-dimer as routine clinical marker of disease activity in MS patients [25].

The increased circulating SFMC in MS patients appeared to be also related to the activation of the coagulation cascade [8]. After thrombin cleaves the fibrinogen molecule, the fibrin monomers are formed, and during the early phase of thrombus formation, these monomers achieve stability by creating SFMCs with fibrinogen breakdown products. As the process of thrombosis continues, more fibrin monomers are created, and more complexes are formed. When the concentration of fibrin polymers reaches a threshold level, they combine with factor XIII to form stable clots. We hypothesized that SFMCs could also be used as a promising biomarker of intravascular hypercoagulation state, since these molecular complexes appear in plasma before actual “clot” formation occurs, in its early stages.

Subsequent examination of the composition of SFMC fractions isolated from the plasma of MS patients showed the formation of protein complexes with molecular weights different from those of healthy individuals. We think that abnormalities in the coagulation process under pathological conditions may be the reason for the variation in the SFMC composition. In MS patients, the formation of high-molecular-weight complexes (550–560 kDa) may be linked to increased plasma coagulation. The appearance in the bloodstream of high-molecular-weight complexes seems to be one of the promising diagnostic criteria for both coagulation and vascular thrombosis in MS patients.

**Conclusions**

Taken all together, our findings provided evidence for the role of hypercoagulation in MS pathophysiology. The development of MS is significantly associated with elevated levels of such coagulation factor as fibrinogen (385 [367–410] in MS group vs. 267 [210–303] in HC, p < 0.01). The increased concentrations of circulating fibrinogen degradation products, namely D-dimer (0.35 [0.29–0.41] in MS group vs. 0.23 [0.22–0.30] in HC, p < 0.01) and SFMCs (65 [58–70] in MS group vs. 35 [33–36] in HC, p < 0.01), could be an indicator of hypercoagulation state in MS patients. The appearance of high-molecular-weight SFMCs (550–560 kDa) in the bloodstream of MS patients may be due to the imbalance in coagulation system. On the other hand, the results of our study did not confirm the changes of studied hemostatic markers in MS patients after SARS-CoV-2 infection.

It seems crucial to determine the exact links between MS pathogenesis and coagulation pathway dysregulation. Finding out how particular hemostatic factors, namely fibrinogen, D-dimer, and SFMCs, relate to the progression of neurodegeneration and neuroinflammation under MS will be the next task ahead. Further experiments on understanding the role of studied coagulation factors as promising biomarkers of MS severity may lead to improved diagnostic options, not only for demyelinating diseases, such as MS, but also for other neurodegenerative conditions.

**References**


Резюме. Виявлено, що рівні фібриногену та D-димеру в плазмі всіх пацієнтів із розсіяним склерозом (РС) супроводжували зміни як кількості, так і якості складу РФМК порівняно зі здоровими особами. Наші результати продемонстрували накопичення високомолекулярних РФМК у плазмі всіх пацієнтів із РС, які мали чи не мали COVID-19 в анамнезі. Пацієнти із розсіяним склерозом (РС) та COVID-19 продемонстрували значну кількість РФМК порівняно з здоровими особами. У цих групах змінилися характеристики гемостазу, однак навіть у пацієнтів із РС та COVID-19 порівняно з груповим контрольним. У здорових особистісних показники гемостатичних факторів, а саме фібриноген, D-димер та РФМК були значно підвищені в плазмі всіх пацієнтів із РС порівняно зі здоровими особами. Уявлення D-димеру та РФМК відіграють важливу роль в хворих на РС після інфікування SARS-CoV-2.

Ключові слова: розсіяний склероз; інфекція SARS-CoV-2; фібриноген; D-димер; розчинні фібрин-мономерні комплекси; гемостаз; скlerоз; COVID-19.