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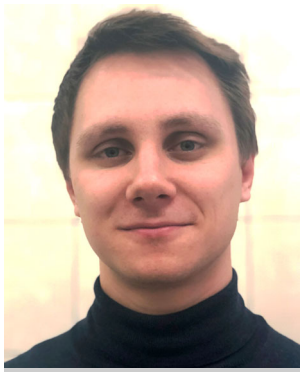
ORIGINAL ARTICLE

Concentrations of immunoglobulin free light chains in cerebrospinal fluid predict increased level of brain atrophy in multiple sclerosis

Vladimir Nazarov[1] · Gleb Makshakov[2,3] · Ivan Kalinin[2] · Sergey Lapin[1] · Elena Surkova[1] · Liya Mikhailova[1,4] · Boris Gilburd[4,5,6] · Alexander Skoromets[3] · Evgeniy Evdoshenko[2]


Vladimir Nazarov

Abstract

Recent studies showed that B cells play a major role in the pathogenesis of neurodegeneration in multiple sclerosis (MS). In this study, we aimed to determine the possible link between immunoglobulin free light chains (FLC) and brain atrophy in patients with MS. Ninety-two patients (32 males and 60 females) with MS were included. Kappa and lambda FLC concentrations in serum and cerebrospinal fluid (CSF) samples of MS patients were measured using ELISA assay. FLC quotients (Q-k and Q-λ, respectively) were calculated. In a cross-sectional group ($n = 92$), the MRI data were acquired within 6 months from the date of the lumbar puncture. Twenty patients from this cohort performed a follow-up MRI after 1 year of observation. Brain volumes were calculated with SIENAX and the brain atrophy (percentage brain volume change (PBVC)) was assessed with SIENA. Spearman's test was performed to assess correlations. We have shown statistically significant correlation of Expanded Disability Status Scale (EDSS) level with normalized brain volume (NBV, $r = -0.2721$, $p = 0.0062$), white matter volume (WMV, $r = -0.2425$, $p = 0.015$), and gray matter volume (GMV, $r = -0.216$, $p = 0.0309$). Multiple Sclerosis Severity Score (MSSS) score correlated with NBV ($r = -0.2521$, $p = 0.0352$) and WMV ($r = -0.315$, $p = 0.0079$). Neither EDSS, nor MSSS scores correlated with the age of patients and relapse rate during the first year and 5 years. In our study, we found statistically significant correlations of k-FLC in the CSF with NBV ($r = -0.311$, $p = 0.003$) and with GMV ($r = -0.213$, $p = 0.0423$). Q-k correlated only with NBV ($r = -0.340$, $p = 0.006$) and Q-λ were negatively correlated with WMV ($r = -0.366$, $p = 0.003$). We did not find correlations of k-FLC in CSF, λ-FLC in CSF, Q-k, and Q-λ with duration of MS course, EDSS, MSSS, number of relapses during the first year, and during the first 5 years of disease. Additionally, we subdivided the study population in accordance with level of k-FLC CSF, Q-k, and Q-λ on the 25th and 75th percentile subgroups (25-k-FLC_{CSF}/75-k-FLC_{CSF}; 25-λ-FLC_{CSF}/75-λ-FLC_{CSF}; 25-Q-k/75-Q-k; 25-Q-λ/75-Q-λ). We found statistically significant difference of NBV and GMV between 25-k-FLC_{CSF} and 75-k-FLC_{CSF} subgroups ($p = 0.0047$, $p = 0.0297$ respectively), NBV between 25-Q-k and 75-Q-k subgroups ($p = 0.038$), and NBV and WMV between 25-Q-λ and 75-Q-λ subgroups ($p = 0.0446$, $p = 0.0026$ respectively). PBVC in the prospective group showed negative correlation with kappa FLC in the CSF ($r = -0.4853$, $p = 0.0301$) and Q-k ($r = -0.6132$, $p = 0.0224$), but not with other clinical, epidemiological data. In this study, we showed a strong negative correlation of k-FLC, Q-k, and Q-λ with brain atrophy in MS patients. Additionally, patients with high concentration of FLC had lower brain volumes. We did not find correlations of FLC with the relapse rate, age of patients, and MS time course. In the prospective group, the rate of atrophy was correlated with k-FLC and Q-k. We suggest that level of intrathecal production of FLC can be a good prognostic biomarker for MS.

Keywords Cerebrospinal fluid · Brain atrophy · Multiple sclerosis · Free light chains

✉ Vladimir Nazarov
nazarov19932@mail.ru

[1] Center for Molecular Medicine,Laboratory for Diagnostics of Autoimmune Diseases, First Pavlov State Saint-Petersburg Medical University, Saint Petersburg, Russian Federation

[2] City Center of Multiple Sclerosis and Autoimmune Diseases, Municipal Clinical Hospital No 31, Saint Petersburg, Russian Federation

[3] Neurology Department, First Pavlov State Saint-Petersburg Medical University, Saint Petersburg, Russian Federation

[4] Laboratory of the Mosaics of Autoimmunity, Saint Petersburg University, Saint Petersburg, Russian Federation

[5] Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel

[6] Sackler Faculty in Medicine, Sheba Medical Center, Tel Aviv University, Tel Aviv, Israel

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Introduction

Multiple Sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system (CNS) with prominent neurodegeneration [1]. Histological and imaging studies revealed that neurodegeneration in MS proceeds in parallel with episodes of demyelination [2]. Over the last decade, a number of studies showed that brain neurodegeneration in MS patients occurs at earliest stages of MS and at significantly faster rate than in healthy persons (0.5–1% per year vs 0.1–0.3% respectively) [3].

Demyelination, axonal loss, and subsequent neuronal death lead to evident cerebral and spinal cord atrophy that is the cause of long-term disability in MS patients. Main culprits of neurodegeneration in MS remain to be identified, but it was shown that Wallerian degeneration after acute axon transection, mitochondrial dysfunction, oxidative stress, iron accumulation, toxic cytokines milieu, altered axonal ion homeostasis, and activation of microglia may play a role in neuronal death [4].

For a long time, it was thought that T cells are the most important player in the pathogenesis of MS. But today, it became clear that B cells also have prominent role not only in episodes of demyelination, but also in the neuronal death. It was substantiated by demonstration of B cells in white matter lesions of MS patients, their presence in the ectopic lymphoid follicles, increased concentration of B cell–related cytokines in cerebrospinal fluid (CSF) (e.g., CXCL13 and BAFF), restricted activation of the humoral immune response in the CNS, synthesis of myelin-specific autoantibodies, and also the good clinical response to the B cell depletion therapy [5, 6]. A number of recent studies also demonstrated that concentrations of B cells in the CSF correlate with gray matter atrophy [7].

Increased intrathecal production of immunoglobulins (Ig) was the first identified immunologic biomarker of MS. Kappa and lambda light chains (k-FLC and λ-FLC respectively) are the structural part of Ig molecules. Elevated CSF concentrations of k-FLC and λ-FLC were typically found in CIS and MS patients and their diagnostic significance in MS was consistently confirmed by the several research groups [8, 9]. FLC can be the early-term and long-term prognostic marker for MS [10]. Our group has found that MS patients with higher concentration of kappa FLC achieve irreversible disability faster than MS patients with lower FLC concentrations [11]. These data can reflect the possible link between FLC and neurodegeneration in MS.

Aim

The aim of this study is to determine the possible link between FLC and brain atrophy in MS patients.

Materials and methods

This study was approved by the local Ethics Committee of the Municipal Clinical Hospital No 31, City Center of Multiple Sclerosis and Autoimmune Diseases. All participants provided written informed consent.

In the first part of this study, we performed a cross-sectional analysis of clinical, radiological, and immunological data of the heterogeneous group of 92 patients, diagnosed with RRMS and secondary progressive MS (SPMS) according to the McDonald 2010 criteria [12]. Lumbar puncture and serum sampling were performed in all patients at the time of diagnosis of the disease. Immunological tests were carried out during the first week after sample acquisition.

All data were collected for the time of MRI study. Demography data included the gender and the age. Duration time of MS course (dated from the first relapse), number of relapses during the first year of disease, number of relapses during the first 5 years of disease, Expanded Disability Status Scale (EDSS) score, and Multiple Sclerosis Severity Score (MSSS) measured at the time of MRI was collected.

In a subgroup of patients ($n = 20$), the prospectively analyzed brain atrophy rate after 1 year of follow-up (prospectively studied subgroup).

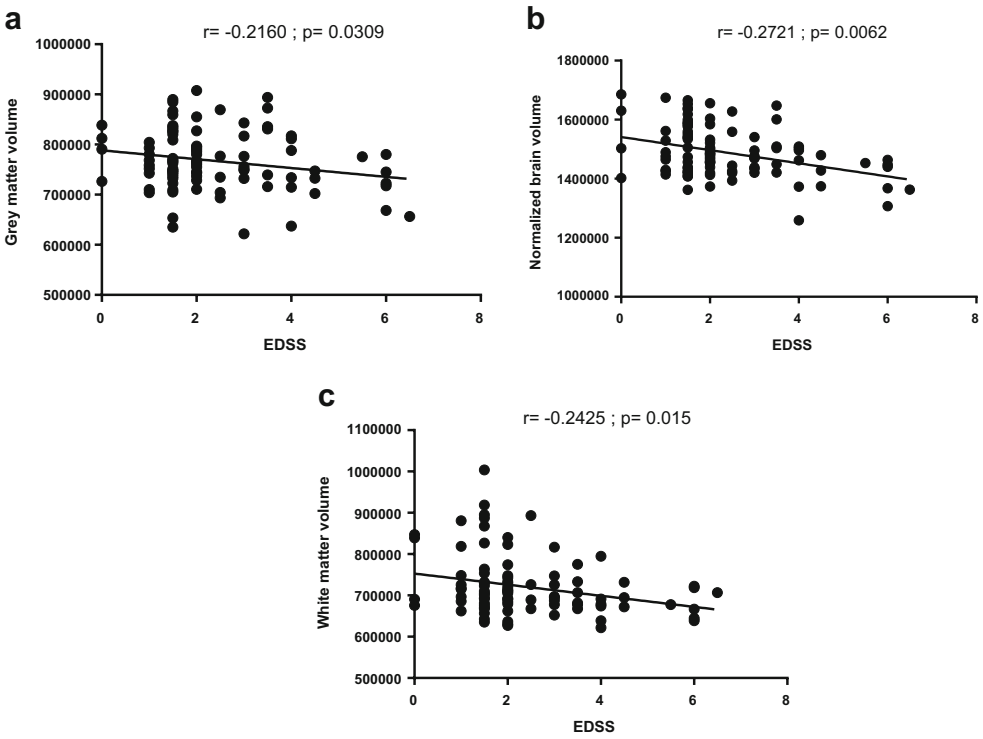
Demographical data are presented in Table 1.

Kappa and lambda FLC concentrations in serum and CSF samples of patients were measured using ELISA assay (Polignost Ltd., St. Petersburg, Russia) based on monoclonal anti-k and anti-λ antibodies directed against cryptic epitopes of FLC molecules. This ELISA test has shown good concordance with nephelometric measurement of FLC [8]. To exclude possible influence of serum FLC concentration on the CSF concentration, we calculated FLC quotients (Q-k and Q-λ, respectively): $Q\text{-FLC} = FLC_{CSF}/FLC_{SERUM}$. Calculation of FLC index that includes quotient of albumin as a marker of blood-brain barrier (BBB) integrity was considered to be unnecessary. This is explained by preservation of BBB, assessed by albumin quotient, in patients with definite MS [13, 14].

Table 1 Clinical and epidemiological data of studied MS patients

Clinical and epidemiological data of studied patients	Values
Median duration of MS course (months)	22 (0, 472)
Median number of relapses during the first year of disease (years)	1 (1, 4)
Mean number of relapses during the first 5 years of disease (years)	2.485 ± 1.395
Median EDSS score	2 (0, 6.5)
Mean MSSS score	4.825 ± 2.509
Gender (male/female)	32/60
Median age (years)	32.5 (18, 65)

MS, multiple sclerosis; *EDSS*, Expanded Disability Status Scale; *MSSS*, Multiple Sclerosis Severity Score



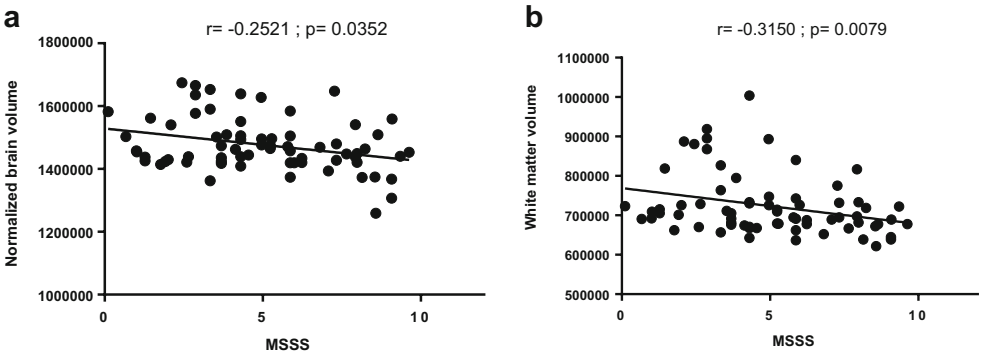
Picture 1 Correlation of EDSS score with brain volumes. Correlations of EDSS level with **a** normalized brain volume, **b** gray matter volume, and **c** white matter volume. EDSS Kurtzke's Expanded Disability Status Scale score

In all patients in this study, the normalized brain volume (NBV), gray matter volume (GMV), and white matter volume (WMV) were measured with automated brain morphometric technique called SIENAX, part of FSL package [15]. The MRI data were acquired within 6 months from the date of the lumbar puncture. 1.5 Tesla T1-weighted MRI sequences with 1-mm isometric voxel were used to estimate NBV, GMV, and WMV. A prospective group of patients performed a second MRI after 1-year follow-up on the same machine. In this subgroup, the two-time point percentage brain volume change (PBVC) was estimated with SIENA (part of FSL) [15]. Volumes are presented in cubic millimeter.

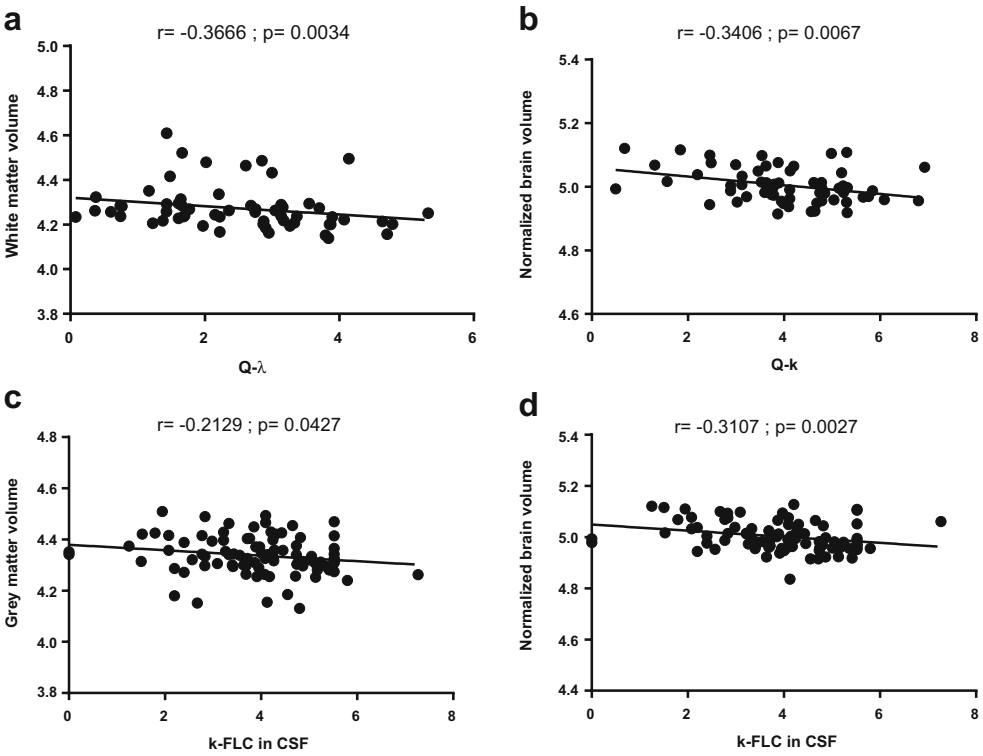
Numerical data were analyzed with GraphPad Prism 6 (GraphPad Software Inc., CA, USA). To test for normality, the Kolmogorov-Smirnov test was applied. All data demonstrated non-normal distributions have been analyzed using non-parametric Mann-Whitney *U* test. Data with normal distribution were compared with help of parametric statistical methods. A two-sided p value < 0.05 was considered to indicate statistical significance. The analysis of correlation was performed using non-parametric Spearman's r value.

Results

Associations between brain volumes (NBV, GMV, WMV) and clinical and epidemiological data were evaluated. We did not find statistically significant correlation of NBV, GMV, and WMV with number of relapses during the first year of disease and first 5 years of disease, but WMV and number of relapses during the first year of disease had a trend to statistical significance ($p = 0.06$). The age of patients correlated with NBV ($r = -0.3880$, $p = 0.0001$) and GMV ($r = -0.5239$, $p = < 0.0001$), but not with WMV.

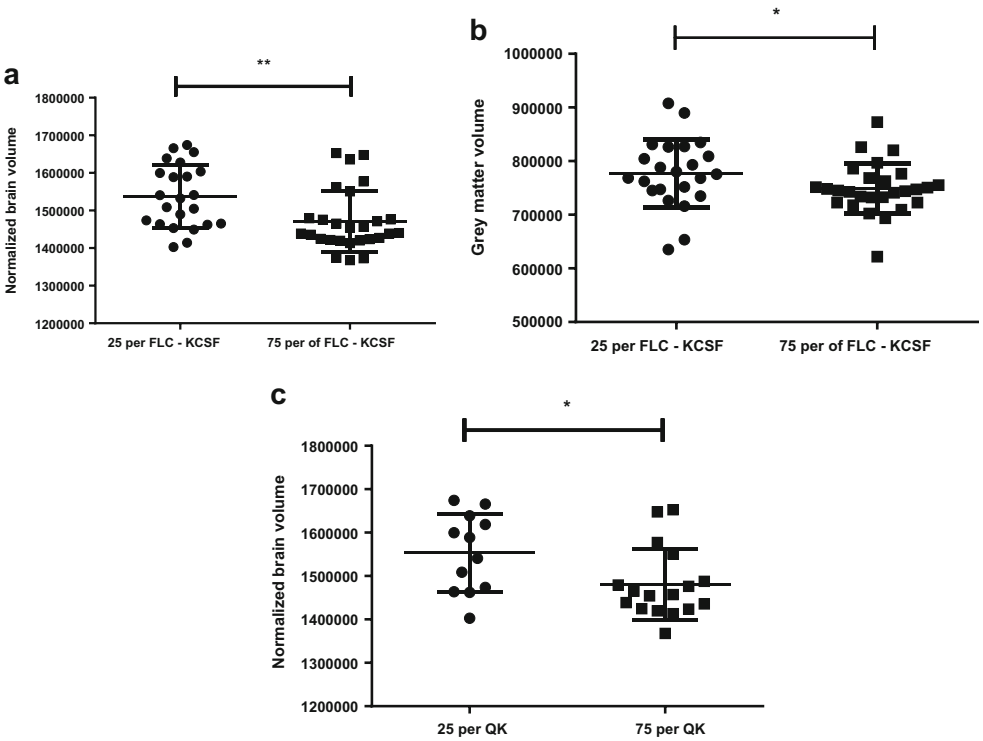


Picture 2 Correlations of MSSS score with brain volumes. Correlation of MSSS level with **a** normalized brain volume and **b** white matter volume. MSSS Multiple Sclerosis Severity Score

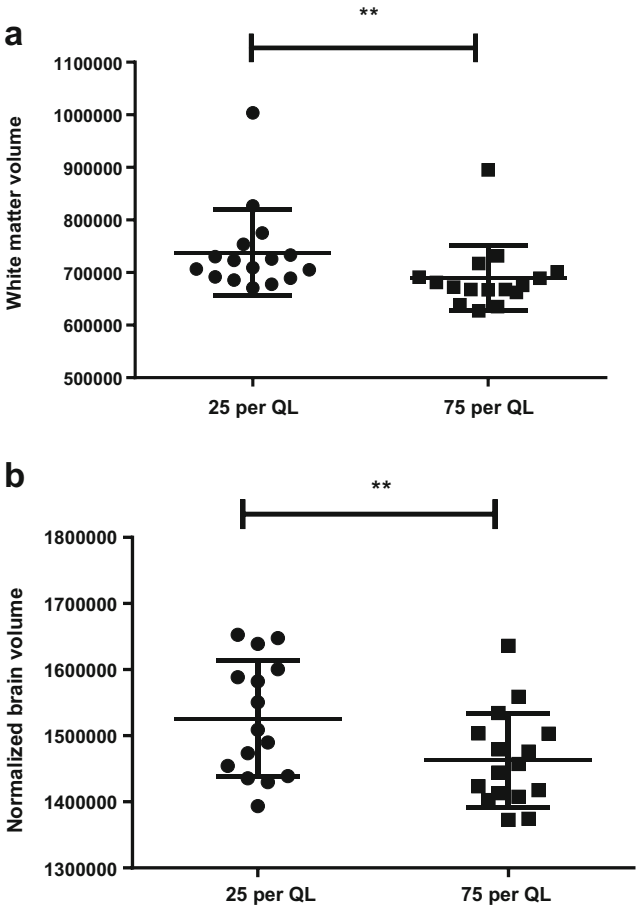


Picture 3 Correlations of FLC and Q-k with brain volumes (values on the picture are replaced by their ranks). Correlations of kappa FLC in CSF with **a** normalized brain volume and **b** gray matter volume. Correlations of Q-k with **c** normalized brain volume and correlation of Q-l with **d** white matter volume. K-FLC kappa free light chains, CSF cerebrospinal fluid, Q-k kappa free light chain quotients, Q-λ lambda free light chain quotients

We identified that brain volumes (NBV, WMV, and GMV) correlated with level of EDSS: NBV ($r = -0.2721$, $p = 0.0062$); WMV ($r = -0.2425$, $p = 0.015$); and GMV ($r = -0.216$, $p = 0.0309$). Additionally, MSSS level correlated with NBV ($r = -0.2521$, $p = 0.0352$) and WMV ($r = -0.315$, $p = 0.0079$), but not with GMV (Pictures 1 and 2). We did not find significant correlations of EDSS and MSSS with the MS relapse rate during the first year and 5 years and the age of patients (data not shown).



Picture 4 Difference of brain atrophy between 25th and 75th percentiles of k-FLC concentrations and Q-k. Difference of **a** normalized brain volume and **b** gray matter volume between 25 per FLC-K CSF and 75 per FLC-K CSF subgroups, and difference of **c** normalized brain volume between 25 per Q-k and 75 per Q-k subgroups. K-FLC kappa free light chains, CSF cerebrospinal fluid, Q-k kappa free light chain quotients



Picture 5 Difference of brain atrophy between 25th and 75th percentiles of λ-FLC concentrations and Q-λ. Difference of **a** normalized brain volume and **b** white matter volume between 25 per Q-l and 75 per Q-l subgroups. Q-λ lambda free light chain quotients

To detect possible links between FLC and brain volumes, we evaluated associations of k-FLC and lambda FLC in the CSF, Q-k, and Q-λ with NBV, GMV, and WMV. We identified statistically significant correlations of k-FLC in the CSF with NBV ($r = -0.311, p = 0.003$) and with GMV ($r = -0.213, p = 0.0423$), but not with WMV ($r = 0.043$, $p = 0.671$). Q-k correlated with NBV ($r = -0.340, p = 0.006$), but not with WMV ($r = -0.123$, $p = 0.367$) or GMV ($r = 0.014$, $p = 0.897$). We also identified that Q-λ were negatively associated with WMV ($r = -0.366$, $p = 0.003$), but not with BMV ($r = -0.113$, $p = 0.674$), GMV ($r = -0.034$, $p = 0.245$).We did not find correlations of k-FLC in CSF, λ-FLC in CSF, Q-k and Q-λ with time of MS course, EDSS, MSSS, age of patients, number of relapses during the first year of disease, and number of relapses during the first 5 years of disease (Picture 3).

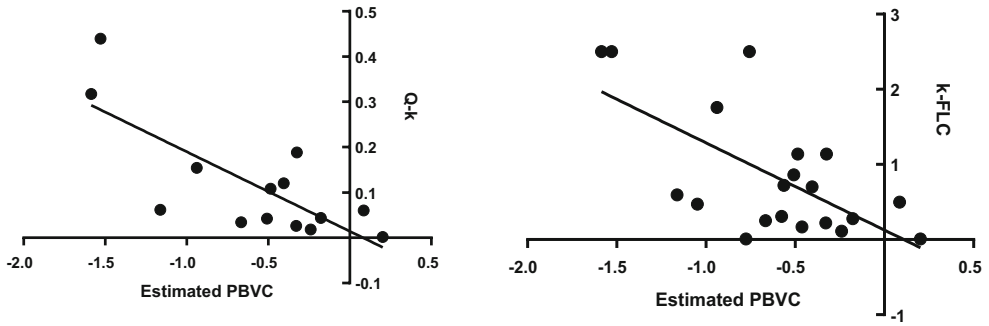
To further investigate the FLC correlation with brain volumes, the study population was subdivided in accordance with level of k-FLC_{CSF}, λ-FLC_{CSF}, Q-k, and Q-λ on 25th and 75th percentile subgroups (25-k-FLC_{CSF}/75-k-FLC_{CSF}; 25-λ-FLC_{CSF}/75-λ-FLC_{CSF}; 25-Q-k/75-Q-k; 25-Q-λ/75-Q-λ). After that, brain volumes were compared between 25th and 75th percentile subgroups of λ-FLC_{CSF}, k-FLC_{CSF}, Q-k, and Q-λ. The age and the time of MS course did not differ between percentile subgroups ($p = 0.45$ and $p = 0.76$ respectively).

We identified statistically significant difference of NBV and GMV between 25-k-FLC_{CSF} and 75-k-FLC_{CSF} subgroups ($p = 0.0047$, $p = 0.0297$ respectively), NBV between 25-Q-k and 75-Q-k subgroups ($p = 0.038$), and NBV and WMV between 25-Q-λ and 75-Q-λ subgroups ($p = 0.0446$, $p = 0.0026$ respectively) (Pictures 4 and 5).

In the prospective group of patients with MS, we identified negative correlation of brain volume changes with kappa FLC in the CSF ($r = -0.4853$, $p = 0.0301$) and Q-k ($r = -0.6132$, $p = 0.0224$), but not with other clinical (rate of relapses and EDSS level), epidemiological data (age of patients and time of MS course) and λ-FLC_{CSF} and Q-λ (Picture 6).

Discussion

Neuroinflammation and neurodegeneration are two related processes that are characteristic for patients with MS. B cells play the major role in neurodegeneration in MS. They reside in the brain parenchyma, cerebrospinal fluid, and leptomeninges. It is supposed that B cells can drive neurodegeneration by producing autoantibodies or other soluble toxic factors and also via activation of other cell population (e.g.,



Picture 6 Correlation of kappa FLC_{CSF} and Q-k with the change of brain volume of MS patients. K-FLC kappa free light chains, CSF cerebrospinal fluid, Q-k kappa free light chain quotients, PBVC percentage brain volume change

microglia); however, exact mechanisms are to be elucidated [4]. Plasmacytes and plasmablasts are specialized producers of k-FLC and λ-FLC. Animal model studies have shown that these cells can populate and reside inside the inflamed CNS. Concentrations of CSF FLC-k predict conversion to MS and have prognostic significance for future disability after 2 years following CIS and for long-term disease prognosis that was not dependent from the relapse rate or MRI activity [8].

The goal of this research was to evaluate possible links of FLC concentrations with brain atrophy.

The decrease of brain volume is a normal process in aging during which a healthy person loses 0.1–0.3% of brain matter annually [3]. These data explain identified correlation of NBV ($r = -0.3880$, $p = 0.0001$) and GMV ($r = -0.5239$, $p = < 0.0001$) with the age of patients in our study.

Acute demyelination of WM and GM can lead to axonal transection and subsequent Wallerian degeneration of neurons [2]. In this study, we did not show statistically significant correlation of inflammatory demyelinating activity of MS patients (number of relapses during the first year and first 5 years of the disease) with NBV, WMV, and GMV, which is in line with some research groups that also did not find the link between the relapse rate and brain atrophy [16, 17].

In patients with MS, demyelination, loss of neurons, and neuroinflammation are the main processes, leading to the atrophy of white and gray matter and disability progression. In this study, we identified statistically significant negative correlation of EDSS score with GMV, WMV, NBV, and MSSS with WMV and NBV. Interestingly, Fisniku et al. found that disability scores correlated with GMV, but not with WMV [18]. Correlation of disability scores with WMV in our study can be explained by short mean time of MS course duration in patients included to the research.

Although correlation between disability level, measured by EDSS and MSSS, and clinical inflammatory activity and age of patients was absent. These data suggest that neurodegeneration on the later stages is caused by a process that is independent of demyelination, and myelin loss is not linked with long-standing disability of MS patients.

In accordance with previously published data, high intrathecal concentrations of FLC predict worse early-term and long-term prognosis of MS patients [10]. In this study, we performed correlation analysis of brain volumes and FLC. We showed strong negative correlation of k-FLC with GMV and NBV, Q-k with NBV, and also negative correlation of Q-l with NBV and WMV. FLC did not correlate with other clinical and demography variables. These data were additionally confirmed by increased brain atrophy in patients with high concentration of FLC variables (75th percentile subgroups) that showed significant correlation with brain volumes. These data suggest that concentration of FLC may predict increased brain atrophy independently of the relapse rate, age of patients, and MS time course. Kappa FLC was shown to be a good prognostic factor for MS, but to our knowledge, this is one of the first studies that identified the link of FLC and the brain matter shrinkage. Nevertheless, it was shown that intrathecal production of immunoglobulin is linked with profound brain atrophy [19].

FLC can be a marker of B cell–mediated process that may not be related to acute demyelination, but can be associated with neuroinflammation and subsequent neurodegeneration. Interestingly, that only intrathecal concentration of kappa FLC correlated with GMV, but lambda FLC correlated with WMV atrophy. These data should be further evaluated.

We also found that in prospectively studied group concentrations of k-FLC and Q-k negatively correlated with rates of brain atrophy ($r = -0.4853$ and $r = -0.6132$, respectively) that reflects faster rates of atrophy in patients with high FLC concentrations. We have already showed the clinical link of high concentration of kappa FLC with achievement of irreversible disability [11]. Newly received data directly reflect association of elevated concentration of kappa FLC and Q-kappa with neurodegeneration in MS patients. Our data are compatible with the study of María S. Sáez et al. that showed correlation of kappa and lambda FLC in CSF with PBVC ($r = -0.72$ and $r = -0.65$, respectively) in clinically isolated syndrome [20]. The absence of statistically significant correlation of PBVC with lambda FLC in our study can be explained by MRI performance on the later stage of MS, with probably more significant influence of kappa FLC with increasing disease duration.

Presented results of the study encourage us to assume that level of intrathecal production of FLC in patients with MS can help to predict future neurodegeneration and the rate of brain atrophy. The main cause of neurodegeneration and brain atrophy in MS is still a matter of debate. Elevated concentration of FLC in CSF and their association with brain atrophy and disease progression can be a marker of unknown mechanisms of B cell–mediated neuronal death.

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Compliance with ethical standards

This study was approved by the local Ethics Committee of the Municipal Clinical Hospital No 31, City Center of Multiple Sclerosis and Autoimmune Diseases. All participants provided written informed consent.

Conflict of interest E. Evdoshenko has received honoraria for lectures and speaking in the past 2 years from Merck, Biogen, Roche, Johnson &

Johnson, Novartis, GlaxoSmithKline, Sanofi, Genzyme, Generium. G. Makshakov has received honoraria for lectures and speaking in the past 2 years from Roche, Janssen and Genzyme.

Other authors have no potential conflict of interests. This does not alter the authors' adherence to journal policies on sharing data and materials.

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