DOI 10.1007/s11055-018-0617-1
Neuroscience and Behavioral Physiology, Vol. 48, No. 6, July, 2018

The Role of Assay of Free Immunoglobulin Light Chains in the Diagnosis of the Onset of Multiple Sclerosis

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Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 117, No. 2, Iss. 2, Multiple Sclerosis, pp. 60–65, February, 2017.

Objective. To assess the diagnostic value of assaying immunoglobulin (IgG) light chains at the onset of multiple sclerosis (MS). **Materials and methods.** A total of 226 patients took part in the study; group 1 ($n = 111$) were patients with clinically isolated syndrome (CIS) with development to MS within the first two years; group 2 ($n = 49$) were patients with CIS who did not progress to MS in the first two years; group 3 ($n = 20$) consisted of patients with inflammatory diseases of the central nervous system. The reference group (group 4, $n = 46$) consisted of patients with noninflammatory CNS diseases. The following immunological indicators were assessed in the patients: the clonal nature of cerebrospinal fluid (CSF) IgG and the concentrations of free κ and λ light chains in the CSF and their ratio. **Results.** Synthesis of free light chains was significantly greater in group 1 than groups 2 and 4. Free light chain synthesis in group 3 was significantly greater than that in groups 2 and 4 but was not significantly different from that in group 1. Free light chain production was significantly greater in patients with oligoclonal IgG synthesis than in patients without oligoclonal synthesis. The level of production of free light chains in patients of group 1 was significantly greater than that in group 2, regardless of whether or not oligoclonal IgG was produced. The most valuable diagnostic markers were the concentration and the coefficient of CSF:serum concentrations of κ light chains. Use of these parameters along with assessment of the clonality of IgG synthesis produced a 50% reduction in number of false negative results. Independently of other factors, increases in κ chain levels led to a 9.718-fold increase in the probability of a diagnosis of MS. **Conclusions.** Assay of free light chains as a lab marker increases the accuracy of diagnoses of MS and provides an indirect evaluation of the risk that CIS will progress to complete MS in the next two years.

Keywords: multiple sclerosis, clinically isolated syndrome, free light chains, oligoclonal bands, biomarkers.

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the CNS. The pathogenesis is based on inflammatory damage to the myelin sheaths of nerve fibers in the CNS [1]. The first episode of demyelination, presenting as a typical clinical picture, is called clinically isolated syndrome (CIS). According to current criteria for the diagnosis of MS (McDonald, 2010), the transfer from CIS to complete MS (CMS) can be established on the basis of the development of a second exacerbation and by detection of new or gadolinium-contrasting plaques on MRI scans [2]. The time to the second exacerbation varies strongly in different patients [3]. In some patients, CIS does not undergo transformation to CMS and the preceding episode of neurological deficit remains the only one in the patient's lifetime [4]. A number of studies have demonstrated improvement in the

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TABLE 1. General Characteristics of Patients

Indicator	Group 1 (n = 111), n (%)	Group 2 (n = 49), n (%)	Group 3 (n = 20), n (%)	Group 4 (n = 46), n (%)
Age of onset, years ($M \pm SD$)	31.3 ± 9.41****	33.8 ±1 0.9*	34.6 ± 12.5**	39.8 ± 13.35
Range, years	15–64	15–62	20–60	18–68
Gender (F)	77 (69)	38 (78)	14 (70)	33 (72)
OCB-positive	98 (88)‡	21 (43)	15 (75)†	0 (0)
Range of diseases in groups 3 and 4			CNS tuberculosis (n = 1), CNS vasculitis on the background of unidentified systemic connective tissue disease (n = 4), toxoplasmosis of the CNS (n = 1), neuroAIDS (n = 2), Vogt–Koyanagi–Harada syndrome (n = 1), autoimmune limbic encephalitis (n = 2), paraneoplastic encephalitis (n = 2), Sjögren's syndrome with CNS involvement (n = 1), neurosarcoidosis (n = 2), acute disseminated encephalomyelitis (n = 1), isolated CNS angiitis (n = 1), acute cerebrovascular accident (n = 2)	Marie–Foix–Alajouanine ataxia, unidentified autonomic nervous system disorders (n = 4), subacute combined myelodegeneration (n = 1), central pontine myelinolysis (n = 1), schizophrenia (n = 1), epilepsy (n = 2), tension headache (n = 2), traumatic brain injury (n = 2), mixed pathology (n = 17), sequelae of cerebrovascular diseases (n = 14), vertigo (n = 1)

****$p < 0.0001$, comparison of age between groups 1 and 4; *$p < 0.05$, comparison of groups 2 and 4; **$p = 0.05$, comparison of groups 1 and 3; ‡comparison of numbers of OCB-positive patients in group 1 and 2, χ^2 ($p < 0.0001$); †χ^2 test for comparison of the numbers of OCB-positive patients in groups 2 and 3 ($p = 0.034$).

prognosis of the course of MS and increases in the time to the second exacerbation when treatment starts at the CIS stage [5]. The search for biomarkers predicting conversion of CIS into MS may help not only in the differential diagnosis of this disease, but also in assessing the risk of progression to CMS, as well as determining the therapeutic tactics.

The main immunological marker of MS is an increase in the intrathecal production of immunoglobulins (IgG) and their clonal restriction, which is characterized by the detection of oligoclonal bands (OCB) of IgG on isoelectric focusing of cerebrospinal fluid (CSF) [6–9]. Assay of IgG OCB is the "gold standard" for the lab diagnosis of MS [6]. Detection of IgG OCB during CIS has been shown to predict the future transformation of CIS into CMS [10]. However, as the results of this method are qualitative rather than quantitative, the test is subjective [11].

Another marker for intrathecal activation of B lymphocytes in MS is provided by increases in the concentrations of free κ and λ light chains (κ-FLC, λ-FLC) in the CSF [12, 13]. FLC are fragments of different IgG fractions synthesized in parallel with OCB. FLC have been shown to operate as mediators in inflammatory reactions and can bind the surface receptors of various cells, and also have antigenic specificity [14]. In contrast to OCB, assay of FLC in the CSF can be performed in patients with CIS or MS, using a highly sensitive and specific immunoenzyme analysis (IEA). The aim of the present work was to assess the diagnostic value of assaying IgG FLC at the onset of MS.

Materials and Methods. A total of 226 patients at the St. Petersburg City MS Center took part in the study. The study was approved by the local ethics committee of City Clinical Hospital No. 31. All patients signed voluntary informed content to take part in the study. Data collected from 2012 to 2016 were analyzed retrospectively. Patients were divided into four groups. Group 1 (n = 111) included patients with CIS, in whom the progression to true recurrent-remitting MS (RRMS) was confirmed in terms of the McDonald 2005 and 2010 criteria within two years. Group 2 (n = 49) included patients with CIS in whom diagnoses of RRMS were not made within two years of the onset of CIS. Group 3 (n = 20) included patients with inflammatory diseases of the CNS. The reference group, group 4 (n = 46), consisted of patients with noninflammatory diseases of the CNS. All patients with CIS underwent lumbar puncture during the first year from onset of the first symptoms to conversion to full RRMS. Patients in groups 3 and 4 attended the Center for consultations for suspected MS. In all these cases, brain MRI scans showed hyperintense plaques similar to those seen in demyelinating disease. Further investigations excluded the diagnosis of RRMS, as other inflammatory or noninflammatory diseases were found. The characteristics of the groups of patients are given in Table 1.

Concentrations of κ-FLC and λ-FLC were determined in CSF (κl-FLC and λl-FLC) and serum by imunoenzyme analysis (IEA) with anti-κ and anti-λ monoclonal antibodies to cryptic epitopes of FLC (from Polignost, St. Petersburg, Russia). The effects of serum FLC concentrations were assessed by determining the CSF:serum coefficients (Q) and concentrations of κ-FLC and λ-FLC (Q-κ and Q-λ, respectively): Q-FLC = FLC_{CSF}/FLC_{SERUM}. The permeabil-

TABLE 2. Concentrations and Coefficients of Concentrations of FLC in CSF (Me [Q_1; Q_4])

Indicator	Q-κ	Q-λ	Group 3	Group 4
κl-FLC (μg/ml)	0.45 [0.225–0.965]	0.11 [0.004–0.27]	0.45 [0.189–0.595]	0.05 [0.032–0.076]
λl-FLC (μg/ml)	0.08 [0.039–0.313]	0.034 [0.015–0.09]	0.16 [0.052–0.585]	0.03 [0.014–0.075]
Group 1	0.039 [0.016–0.099]	0.008 [0.003–0.024]	0.032 [0.019–0.069]	0.004 [0.003–0.005]
Group 2	0.01 [0.0035–0.037]	0.005 [0.003–0.008]	0.02 [0.003–0.07]	0.003 [0.002–0.006]

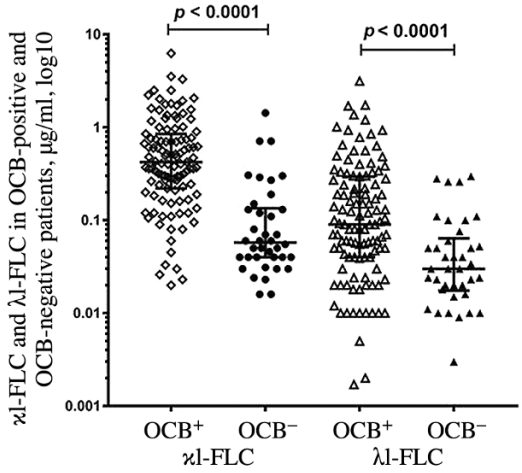

Fig. 1. Comparison of κl-FLC and λl-FLC concentrations in groups 1 and 2 (data combined) among OCB-positive and OCB-negative patients.

ity of the blood-brain barrier (BBB) was assessed in terms of the coefficient for albumin: $Q_{ALB} = ALB_{CSF}/ALB_{SERUM}$. FLC contents in the CSF were interpreted considering the permeability of the BBB in terms of indexes for the κ and λ chains (I-κ and I-λ, respectively) in relation to albumin: $I_{FLC} = Q_{FLC}/Q_{ALB}$.

After collection, CSF and serum samples were stored at –80°C. OCB were detected by isoelectric focusing in agarose gels followed by immunoblotting. The results were analyzed in accordance with international recommendations [15, 16].

Data were analyzed in the statistics program GraphPad-Prism 7 (Graph Pad Software Inc., CA, USA). The normality of distributions was tested using the Kolmogorov–Smirnov test. Parametric (Student's *t* test) or nonparametric (Mann and Whitney *U* test) methods were used depending on the type of distribution. The χ^2 test was used to compare groups of qualitative properties. Numerical data are presented as median and interquartile range. The criterion for statistical significance was $p < 0.05$ for all tests.

Results. The proportion of OCB-positive patients in group 2 (43%) was significantly less than those in groups 1 (88%, $p < 0.0001$) and 3 (75%, $p = 0.0340$).

Values of κl-FLC, λl-FLC, Q-κ, and Q-λ were significantly greater in groups 1 and 2 than in group 4. Serum FLC values were no greater than normal in all four groups (data not presented). In group 1, all measures of FLC were significantly greater than those in group 2. Comparison of the concentrations in groups 2 and 4 showed that only the κl-FLC concentration and Q-κ were significantly different from those in group 4. Group 3 also showed significantly greater values for all measures of FLC than group 4. Measures of FLC synthesis were not significantly different from those in group 1, though they were significantly greater than those in group 2 (Table 2).

As OCB and FLC production in MS is due to intrathecal activation of B lymphocytes, we evaluated the link between OCB production and FLC concentrations. FLC concentrations and their coefficients were significantly greater in OCB-positive patients (Fig. 1).

FLC concentrations in groups 1 and 2 were evaluated separately for OCB-positive and OCB-negative patients. A significant difference was found in κl-FLC concentrations but not in λl-concentrations between OCB-positive patients in groups 1 and 2 ($p = 0.02$ for κl-FLC and $p = 0.1031$ for λl-FLC). Analysis of results in OCB-negative patients also showed a significant difference in the κl-FLC concentrations, and in this case also a difference in λl-FLC concentrations between groups 1 and 2 ($p = 0.0016$ for κl-FLC and $p = 0.0344$ for λl-FLC) (Fig. 2).

ROC analysis showed that the best diagnostic characteristics in terms of sensitivity and specificity were κl-FLC and Q-κ (Table 3, Fig. 3).

In group 2, among OCB-negative patients, increased κl-FLC concentrations (>0.103 μg/ml) were seen in 50% of cases ($n = 6/12$), while no such patients were found in group 2. Considering the fact of increased FLC synthesis without production of OCB, we compared the sensitivities of OCB and FLC assays separately and the presence of at least one of them using the χ^2 method. The sensitivity of determining OCB was 88%, which was not significantly different from the sensitivity obtained using both methods (94%, $p = 0.0934$) (either OCB-positive status or increased FLC). The sensitivity of assaying κl-FLC was 88% and was also not significantly different from that obtained using both methods (94%, $p = 0.001$).

Discussion. The search for and assessment of the significance of clinical, laboratory, and instrumented diagnostic markers is currently among the priorities in MS research.

TABLE 3. ROC Analysis of Measures of FLC Concentrations.

Indicator	Sensitivity, %	Specificity, %	AUC	LR (likelihood ratio)	Reference values
κl-FLC, μg/ml	88.4	90.9	0.9374	9.718	>0.103
Q-κ	88.1	88.9	0.9441	7.929	>0.00875
λl-FLC, μg/ml	42.7	90.9	0.7327	4.699	>0.127
Q-κ	45.2	91.7	0.7100	5.429	>0.0125
I-κ	40.0	92.3	0.7677	5.200	>0.0032
I-λ	4.0	92.3	0.5677	0.520	>0.00029

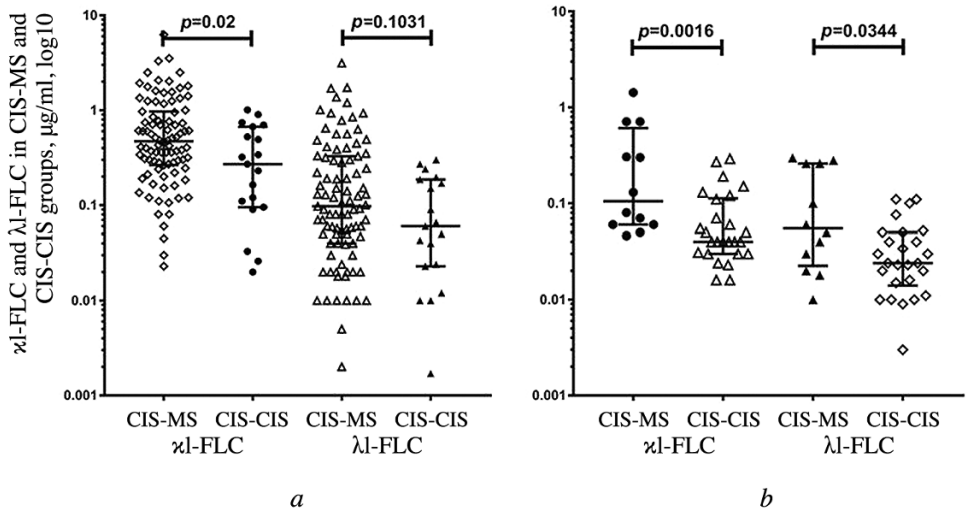

Fig. 2. Comparison of κl-FLC and λl-FLC concentrations in groups 1 and 2 with different OCB status.

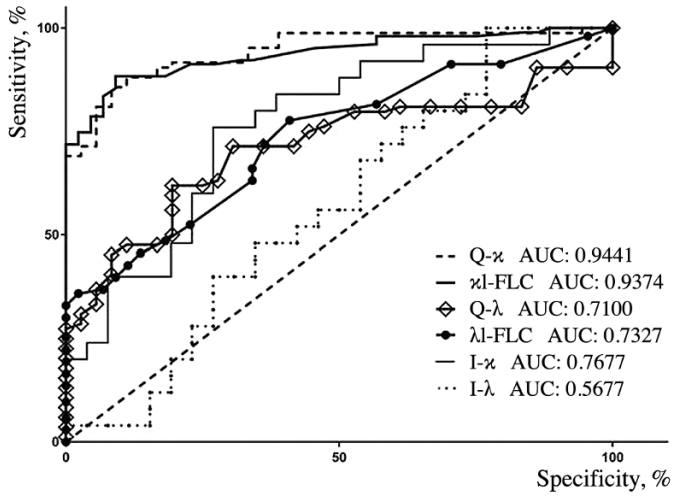

Fig. 3. Analysis of ROC curve for indicators of FLC synthesis in groups 1 and 4.

Analysis of the distribution of OCB in our population showed that 88% of patients of group 1 displayed intrathecal OCB synthesis, which is consistent with data from other authors on the sensitivity of this marker [9, 10].

The levels of intrathecal FLC production in groups 1 and 2 were significantly greater than that in group 4, which reflects the presence of intrathecal inflammation in patients with CIS and MS. Our data are also comparable with results from other studies of the diagnostic significance of FLC in CIS and MS [17, 18]. FLC levels were also found to be significantly greater in patients of group 1 than those of group 2. This may be because of more severe inflammatory changes preceding transformation to MS. Our study also compared the levels of FLC synthesis in patients with CIS and patients with various inflammatory diseases of the CNS, which were mostly OCB-positive. FLC concentrations in inflammatory diseases varied significantly, though there were no significant differences compared with group 1. This is consistent with data from other studies, though κ-FLC concentrations in MS in some studies were higher in viral and bacterial CNS infections [17]. We also showed that κl-FLC concentrations in OCB-positive patients in group 1 were significantly higher than those in OCB-positive patients in group 2.

In this study, the λl-FLC concentration in group 1 was no greater than that in group 2, reflecting the tendency, typical of MS, to intrathecal synthesis mainly of κ-FLC. FLC may also be an indicator of intrathecal inflammatory disease activity independently of OCB. We found a statistically significant difference between the κl-FLC and λl-FLC concentrations between groups 1 and 2 in OCB-negative patients. This fact may reflect staging in the development of MS, when patients with CIS do not yet display oligoclonal Ig synthesis but have significant intrathecal inflammation. This phenomenon requires further investigation.

Analysis of the ROC curve for indicators of FLC synthesis demonstrated that κl-FLC and Q-κ had the greatest levels of diagnostic informativeness, which is also consistent with results from other studies [11]. The sensitivity of κl-FLC was comparable with the sensitivity of OCB. Use of the indexes I-κ and I-λ, which consider the levels of BBB penetration assessed using Q_{ALB}, did not lead to any increase in the sensitivity of these measures. It should be noted that some authors have noted significant improvements in diagnostic indicators of FLC when taking the level of BBB permeability into account [17]. In addition, we have demonstrated that use of κl-FLC as an additional marker for the group provided a 50% reduction in the number of false negative results for analyses of OCB.

During this study we also showed that κl-FLC and Q-κ have quite high sensitivity and specificity for the diagnosis of CIS, while use of FLC as a laboratory marker can increase the accuracy of the diagnosis of MS for OCB-negative patients and can provide for indirect assessment of the risk of progression from CIS to CMS in the following two years.

This study was supported by a Russian Science Foundation grant (Application No. 16-15-10203).

The authors have no conflicts of interests.

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