Editorial

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Free light chains in the cerebrospinal fluid. Do we still need oligoclonal IgG?

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The diagnosis of multiple sclerosis (MS) is based on the demonstration of dissemination in space (DIS), i.e. involvement of different areas of the central nervous system (CNS), and dissemination in time (DIT), i.e. the occurrence of at least two relapses or, in the case of primary progressive MS, disability progression for at least 1 year. Magnetic resonance imaging (MRI) is the most important paraclinical test in patients with suspected MS that can demonstrate both DIS and DIT (i.e. simultaneous appearance of new and old lesions or appearance of new lesions over time). However, MRI findings lack pathological specificity, and an increasing proportion of MS patients is diagnosed after the first clinical episode suggestive of a demyelinating disease where the criterion of DIT might not be met. Demonstration of an inflammatory nature of the disease by means of CSF analysis, namely the presence of intrathecal humoral immune response, has been used for decades to support MS diagnosis, despite the fact that the importance of CSF analysis was de-emphasised in successive revisions of the McDonald MS diagnostic criteria [1]. The latest revision of these criteria, published ahead of print on December 21, 2017, revived the role of CSF analysis by stating that the presence of 2 or more oligoclonal bands (OCB) in the CSF absent from serum can substitute for the DIT criterion and thus enable determining the diagnosis of MS in patients with the first episode suggestive of a demyelinating disease (the so-called clinically isolated syndrome, CIS) if the DIS criteria are met [2].

Ample evidence from numerous studies shows that the diagnostic performance of oligoclonal bands (OCB) and kappa free light-chain (κFLC) tests for the laboratory support of MS diagnosis is roughly comparable. The measurement of κFLC is technically less demanding and less expensive compared to OCB detection; therefore, it has logically been proposed as the best single laboratory test to support the diagnosis of MS [3–5]. At the same time, others [6, 7] proposed to perform the OCB test selectively based on the results of κFLC measurements, using different algorithms.

Although oligoclonal IgG (o-IgG) is mentioned either explicitly [1] or implicitly [2] under the term "oligoclonal bands" in the original and most recent version of McDonald criteria, it is worth mentioning that this term has become somewhat ambiguous since not only o-IgG, but also oligoclonal FLC and IgM tests are in use in some laboratories. Oligoclonal κFLC test has been studied for its diagnostic value in MS and in exceptional cases o-κFLC bands have been found without o-IgG [8–10] and even with normal FLC quantitative values [8], although its overall diagnostic performance was comparable to o-IgG and κFLC index [11]. Oligoclonal IgM and lipid-specific oligoclonal IgM have been proposed as a promising prognostic MS biomarker [12], but its possible significance for the diagnosis of MS is currently unknown.

In this issue of *Clinical Chemistry and Laboratory Medicine*, two other articles appear dealing with FLC in the CSF in order to support the diagnosis of multiple sclerosis (MS) [5, 13]. Comparing quantitative FLC measurements with o-IgG test, the study of Gurtner et al. [5] was designed to find the best and cost-effective single MS predictor. A major step forward in this study is an extensive method validation for κFLC in the CSF matrix. The authors came to the conclusion that it is sufficient to measure κFLC in the CSF, alleviating the need for paired serum analysis; using the cut-off 0.611 mg/L, the test sensitivity and specificity were comparable to both o-IgG and κFLC intrathecal synthesis calculations. The study of Ganelin-Cohen et al. [13], performed on a cohort of paediatric MS patients, deals with a substantially more complicated method of SDS electrophoresis under non-reducing conditions and relative quantification of FLC monomers and dimers in CSF and serum. The authors found that the presence of one of the three FLC monomer-dimer patterns typical of MS (elevated levels of κFLC monomers and dimers and/or λFLC dimers) was both more sensitive and more specific for MS than the o-IgG test.

Is it possible to state that o-IgG test is no longer necessary for the laboratory support of MS diagnosis if FLC analysis is performed instead? This issue should be treated very cautiously. About 20 years ago, it was pointed out by Reiber that the pattern of intrathecal immunoglobulin

synthesis varies among different inflammatory neurological diseases and that MS is one of the diseases characterised by a dominant intrathecal IgG synthesis [14]. FLC, however, are synthesised in excess over heavy chains during immunoglobulin synthesis in general. Therefore, an increase in CSF FLC can be expected in the case of any intrathecal humoral immune response and as a result, FLC could be less specific for MS than o-IgG. o-IgG is not specific for MS either and can occur in other (especially chronic) inflammatory CNS diseases, e.g. neurosyphilis, neuroborreliosis, HIV infection and paraneoplastic CNS syndromes [14, 15]. It would be very interesting to study specifically the cases in which the results of o-IgG and κFLC tests are discrepant. Likewise, the method described in the article of Ganelin-Cohen et al. [13] should be compared not only to o-IgG, but also to quantitative FLC measurements in order to compare the diagnostic performance but also to further assess the presumed differences in anti-FLC antibody reactivities to monomeric and dimeric FLC forms [15].

According to international consensus, the preferred method of o-IgG detection is isoelectric focussing (IEF), whereas at least two bands in CSF not present in serum represent a conventional criterion of positivity [2, 15]. In this sense, both studies in this issue of *Clinical Chemistry and Laboratory Medicine* might underestimate the diagnostic accuracy of o-IgG test to some degree since four (instead of two) CSF-restricted IgG bands define positive CSF in the study of Gurtner et al. (fortunately, however, the results for an alternative cut-off of two bands are reported as well), while electrophoresis with subsequent immunofixation (instead of IEF) was used in the study of Ganelin-Cohen et al. [13].

Concerning the CSF FLC quantitation, other analytical platforms can be used for the Freelite™ test, and another nephelometric test (N Latex FLC™, Siemens) as well as various ELISA tests have been used in some studies, but these methods have not yet been validated for the CSF matrix in such a rigorous way as for Freelite™ on BN nephelometers in the study of Gurtner et al. [5]. Although the diagnostic performance may be similar for different methods, the cut-offs can be expected to differ substantially [11].

No intrathecal immunoglobulin (and FLC) synthesis is expected to occur under normal conditions, therefore the presence of low concentrations of immunoglobulins and FLC in normal CSF is a result of their diffusion from blood across the blood-CSF barrier. Unlike for IgG, however, the contribution of blood-derived FLC to total CSF FLC concentration is very low in the cases with intrathecal synthesis. The intrathecal fraction of CSF κFLC is greater than 80% in most MS patients [4]. This is probably the reason why CSF levels are equally sensitive as κFLC index or other formulas for intrathecal κFLC synthesis estimation if studies do not involve samples with grossly elevated serum FLC levels and/or severe blood-CSF barrier dysfunction. Although these abnormalities *per se* might cast doubt on MS diagnosis, exactly in such cases, correction for serum FLC and blood-CSF-barrier status (albumin quotient) should be used to prevent false-positive results. Unfortunately, the comparison of the diagnostic performance of simple CSF κFLC concentration, κFLC index and/or other estimates of intrathecal κFLC synthesis has only been reported in a minority of studies published so far.

Most CSF FLC studies concentrate on κFLC only since it has repeatedly been demonstrated that the diagnostic performance of CSF λFLC test is considerably inferior to κFLC. However, Voortman et al. [16] has recently shown that relatively lower CSF κ/λ FLC ratio increases the risk of converting to MS in CIS patients. Ganelin-Cohen et al. found that mixed or λ type MS patients had more active disease course [13]. Interestingly, the reasons for very high CSF κ/λ FLC ratios seen in about half of MS patients [11] were never explained and one might intuitively assume that such a dysbalance in favour of κ light-chain intrathecal synthesis might be unfavourable. However, the two studies mentioned above show that rather the reverse might be true.

Finally, it should be kept in mind that the purpose of CSF analysis in MS is, first, to prove the presence of intrathecal humoral immune response, i.e. an inflammatory component, and second, to differentiate other causes of CNS inflammation as far as possible. Therefore, more CSF tests – at least the cell count, differential cell count, total protein and/or albumin quotient [14, 15] – should be performed in any way in order to allow a more confident diagnosis and to help exclude other diseases mimicking MS. This is also reflected in the revised McDonald criteria [2]. The search for a particular MS-specific pattern of intrathecal humoral immune response enabling the differentiation of MS from other chronic inflammatory diseases is even more challenging, and the studies on FLC monomer-dimer patterns seem to be very promising in this context. So far only the MRZ reaction, i.e. intrathecal synthesis of IgG antibodies against at least two of three viruses (measles, rubella and varicella zoster), has proved to be more specific for MS than o-IgG test, but its sensitivity is much lower [14, 15]. Although the result of o-IgG test is usually presented as negative or positive only, the reproducibility and possible differential diagnostic importance of a more elaborate classification into five types

as repeatedly recommended by panels of CSF experts [15] should be addressed in future studies since such a classification is widely ignored by neurologists.

While economical considerations are highlighted in some studies [5, 6], it should be realised that CSF analysis is usually performed only once in patients with suspected MS and the costs of it are negligible compared to the costs of life-long MS treatment. From this point of view, it would be more useful if clinical chemists in co-operation with neurologists strove to set a panel of a few biomarkers with the best possible combined diagnostic (and, possibly, even prognostic) value in the cases of suspected MS.

More complicated tests such as FLC monomer-dimer analysis and lipid-specific oligoclonal IgM need reproduction in other laboratories to allow independent confirmation of their diagnostic and/or prognostic value. The authors of such methods should be prompted to give sufficient details to ensure independent method reproduction. Unfortunately, a detailed method description is usually given on the first occasion only, while it can be assumed that during further research substantial improvements and/or simplifications could be achieved in the laboratory from which the method had originated.

It can be concluded that quantitative CSF κFLC analysis has just entered clinical routine. The question for the near future is whether o-IgG test can provide any useful additional information; if not, it will probably be abandoned forever since it is both more expensive and more laborious than κFLC measurements. Perhaps the next revision of McDonald criteria could mention intrathecal κFLC synthesis as a suitable alternative to oligoclonal IgG. Nevertheless, in the up-to-date version of these criteria, it is explicitly stated that, "The qualitative demonstration of two or more CSF-specific oligoclonal bands more reliably indicates intrathecal antibody synthesis than do other tests, such as the IgG index. Positive results on these other tests should be interpreted with caution when testing for oligoclonal bands is negative or not done." Returning to the question in the title of the editorial, although it is well possible that the o-IgG test has just entered its swan-song season, performing the o-IgG test whenever CSF analysis is indicated in order to support MS diagnosis will probably remain mandatory, at least until the next revision of McDonald criteria appears.

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