Improving the efficacy of cerebrospinal fluid testing for the diagnosis of multiple sclerosis

by a hyperbolic reference range according to Reiber (Reibergram) using the N Latex FLC kappa Assay

Dr. Andreas Rechner, Siemens Healthcare Diagnostics Products GmbH
Prof. Dr. Thomas Skripuletz, Department of Neurology, Hannover Medical School

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Multiple sclerosis (MS) is an autoimmune inflammatory neurological disease with an incidence of approximately 6560 new cases per year and a prevalence of 143,000 to 199,500 patients in Germany alone.[1] In 2013, 2.3 million people worldwide were estimated to have MS, with a much higher prevalence in North America and most European countries.[2] Early diagnosis and treatment can significantly improve management and slow the progression of this not-yet-curable disease.[3]

In the latest revision of the McDonald criteria for the diagnosis of multiple sclerosis,[4] the qualitative detection of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) allows the diagnosis of MS in selected patients as a substitute for fulfilling the requirement of dissemination in time (DIT). The presence of OCBs in CSF is an indicator of an intrathecal humoral immune response. The consensus recommendation in the McDonald criteria defines the presence of CSF OCBs as evidence of DIT, which is characterized by the development of an inflammatory activity in the central nervous system at different times.

According to latest findings, the qualitative detection of OCBs in CSF has a diagnostic sensitivity of up to 98% in MS patients.[5,6] However, it is a laborious method in the routine laboratory that requires skilled and experienced staff. The fully automated, nephelometric determination of kappa free light chain (κFLC) levels in CSF has been shown to be a suitable screening test to detect an intrathecal humoral immune response. Siemens Healthineers was first to introduce a CE-marked assay application for FLC testing in CSF samples.

Several studies used the Siemens Healthineers N Latex FLC kappa Assay to assess the presence of MS, applying different approaches and algorithms.[7-9] For example, Valencia-Vera et al. concluded: "κFLC determination is rapid and automatized, but it has no higher sensitivity and specificity than OCB in MS diagnosis. Nevertheless, when used in screening, it could reduce the number of manual OCB tests."

All approaches aim to compensate for the large inter-individual variation in blood-brain barrier function as well as the serum levels of κFLC. Reiber et al.[10] now propose the use of a hyperbolic reference range in CSF/serum quotient diagrams to further improve and standardize result interpretation of κFLC levels in CSF. For decades, similar diagrams have been used for the assessment of inflammatory processes in the central nervous system (CNS) and the detection of intrathecal synthesis of immunoglobulins. In this study, the N Latex FLC kappa Assay (site 1) and FREELITE assay (site 2) were used to derive a hyperbolic reference range from a total of 433 defined controls by fitting a hyperbolic function to the CSF/serum quotients of albumin plotted against the respective CSF/serum quotients of κFLC (Figure 1, Reibergram). A method-independent hyperbolic reference range was then established by adding ±3 times the mean coefficient of variation (CV).

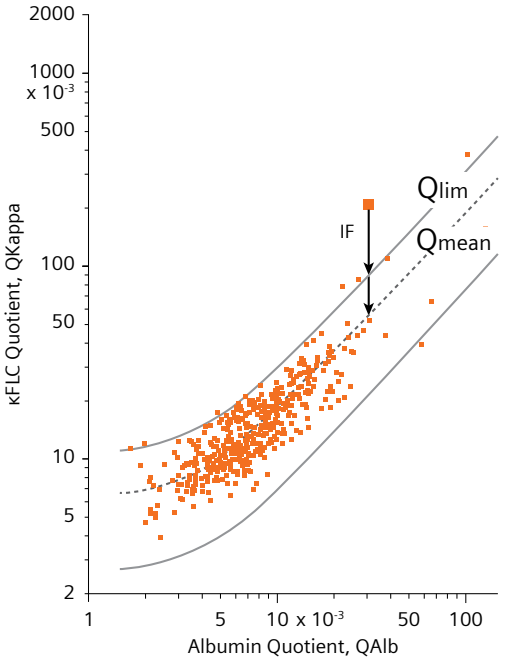


Figure 1. κFLC control data in a double logarithmic quotient diagram (Reibergram).

The hyperbolic functions are shown up to QAlb = 150 x 10^{-3}. Qmean (dashed line) is shown to characterize the optical asymmetry of Qmean in the double log diagram. The intrathecal fraction, IF, is represented either with reference to Qlim for diagnostic purposes as a relative fraction (KIF in %) or with reference to Qmean for statistical purposes, KIF(mean), eventually calculated as quantitative value Kloc(mean) in mg/L.

Applying the Reibergram for the diagnosis of MS appeared to have a comparable sensitivity to the reference method of OCBs in CSF. The evaluation of 95 MS patients (45 MS patients and 50 clinically isolated syndrome patients, later confirmed as MS patients) showed a sensitivity of 93% when using the suggested kFLC analytic approach (Figure 2).

"Reiber's κFLC diagram shows a great diagnostic performance to detect an intrathecal κFLC production in patients with MS."

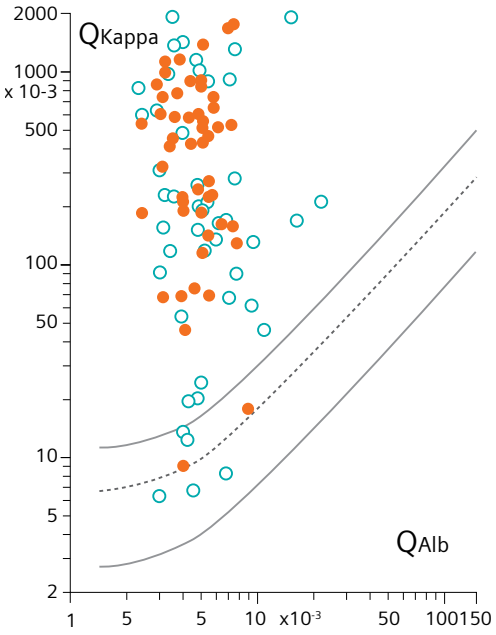


Figure 2. MS and CV patient data in the κFLC Reibergram.

Filled circles are multiple sclerosis patients (n = 45), and open circles represent clinically isolated syndrome patients, later found as MS (n = 50). FLC-K analysis detects 93% of a total of 95 MS patients.

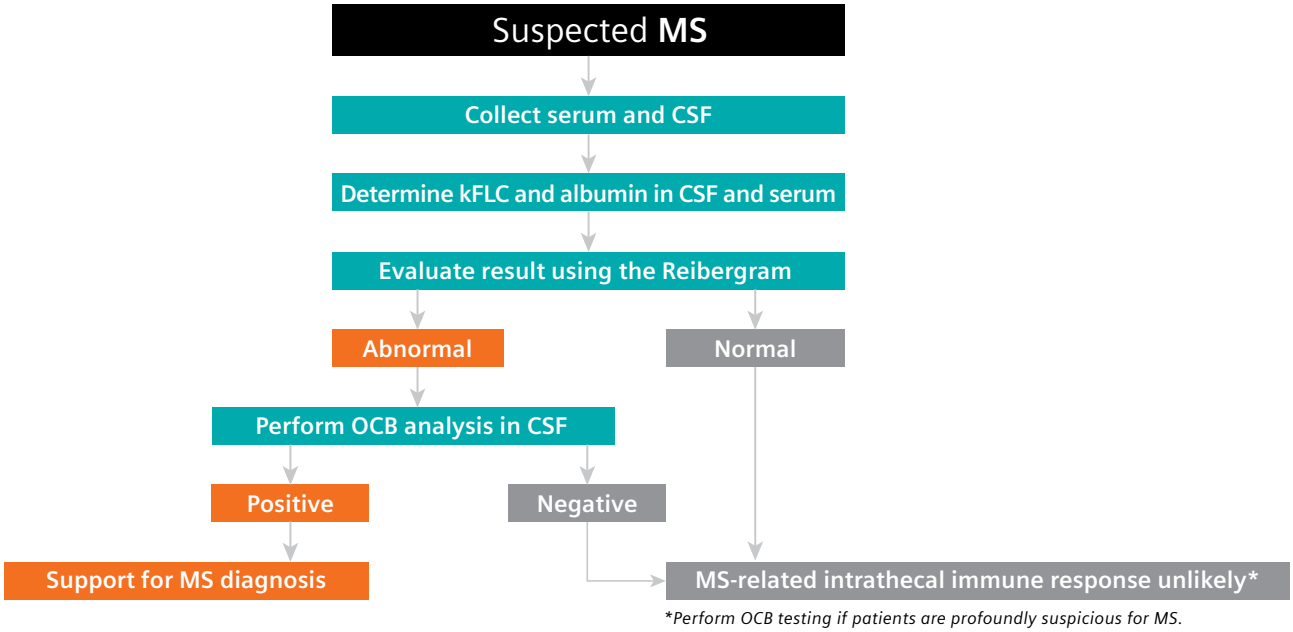
Another study used the N Latex FLC kappa Assay for the quantification of κFLC in CSF and serum to investigate this new algorithm by Reiber, which was able to identify 98 of 100 confirmed MS patients, just one patient less (99/100) than with OCB analysis.[11] In comparison, Reiber's approach appeared to be superior to other approaches for assessing MS using the κFLC results, as summarized in Table 1.

MS (McDonald 2017)			
Characteristics	**MS, n = 100**	**CIS to MS, n = 24**	**Stable CIS, n = 44**
Oligoclonal bands, n (%)	99/100 (99%)	21/24 (88%)	11/44 (25%)
Reiber's FLC diagram, n (%)	98/100 (98%)	21/24 (88%)	9/44 (20%)
Presslauer's FLC curve, n (%)	96/100 (96%)	19/24 (79%)	9/44 (20%)
Senel's FLC curve, n (%)	96/100 (96%)	19/24 (79%)	8/44 (18%)
FLC index >5.9, n (%)	96/100 (96%)	19/24 (79%)	7/44 (16%)
Intrathecal IgG synthesis, n (%)	59/100 (59%)	18/24 (75%)	2/44 (5%)
Intrathecal IgM synthesis, n (%)	33/100 (33%)	4/24 (17%)	2/44 (5%)
Intrathecal IgA synthesis, n (%)	11/100 (11%)	2/24 (8%)	0/44 (0%)
Age, median (range)	32 (16–73)	30.5 (15–73)	36 (16–53)
Females, n (%)	73/100 (73%)	15/24 (63%)	28/44 (64%)

Table 1. Demographic and laboratory characteristics of patients diagnosed with MS according to the McDonald criteria of 2017, patients clinically isolated syndrome (CIS) converted to MS during follow-up, and patients with stable CIS. Laboratory characteristics include the determination of oligoclonal bands, the kappa free light chains index, and the proportion of an intrathecal synthesis of FLC using the methods of Reiber,[10] Presslauer,[12] and Senel.[7]

The authors of this direct-comparison study consequently concluded that *"Reiber's κFLC diagram shows a great diagnostic performance to detect an intrathecal kFLC production in patients with MS."*

Although additional studies need to be conducted to confirm these results, the fully automated and quantitative determination of κFLC in CSF and serum could significantly reduce the number of time-consuming OCB analyses in CSF by providing evidence of dissemination in time for MS diagnostics according to the McDonald criteria of 2017. One possibility might be the following two-stage approach intended to improve the workflow in the laboratory:



*Perform OCB testing if patients are profoundly suspicious for MS.

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Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen, Germany
Phone: +49 9131 84-0
siemens-healthineers.com

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