Clinica Chimica Acta 497 (2019) 153–162

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Diagnostic relevance of free light chains in cerebrospinal fluid – The hyperbolic reference range for reliable data interpretation in quotient diagrams

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ARTICLE INFO

Keywords:
Cerebrospinal fluid
Free light chain kappa
Reference range
Grouped data statistics
Hyperbolic function
Multiple sclerosis
Clinically isolated syndrome
CIS

ABSTRACT

Background: Free light chains, type kappa (FLC-K), in cerebrospinal fluid (CSF) were compared to oligoclonal IgG in many studies for sensitive detection of immune reactions in brain. The missing consensus about CSF data interpretation prevents reliable conclusions. This can be overcome by a theory-based hyperbolic reference range in CSF/serum quotient diagrams.

Methods: Mean Quotients for FLC-K, Q_{Kappa}, and albumin, QAlb, of grouped, biochemically defined controls (N = 433) are fitted with the hyperbolic function $Q_{Kappa}(mean) = a/b\ (QAlb^2 + b^2)^{0.5} - c$ by a generally applicable procedure excluding outliers.

Results: With Q_{Kappa}(mean), the coefficient of variation CV (22.5%) and the reference range (Q_{Kappa}(mean) ± 3 CV) we got the discrimination line $Q_{Kappa}(lim) = (3.27(QAlb^2 + 33)^{0.5} – 8.2) \times 10^{-3}$ in a FLC-K Reibergram. Intrathecal FLC-K was found in 8% of another control group without OCB (N = 388) but was missed in 7% of patients with definite Multiple sclerosis (N = 95). In MS the mean intrathecal fraction was threefold larger for FLC-K (95%) compared to total IgG (36%). Similar mean quantities of intrathecal FLC-K contradict an immunological conversion between a Clinically isolated syndrome and MS.

Discussion: The hyperbolic reference range is superior to linear FLC-K Index (10 to 15% false negatives) and exponential curves (30% false positive interpretations for controls) in the analytical range of MS data, with excellent data fit for up to ten-fold larger QAlb values. Dynamics of the small molecule FLC-K contribute to the understanding of molecular size dependent barrier functions.

1. Introduction

1.1. Laboratory supported diagnosis of inflammatory neurological diseases

The diagnosis of inflammatory neurological diseases [1,2] is based on the detection of an intrathecal humoral immune response. The qualitative analysis of oligoclonal IgG [2,3] is so far the most sensitive method, which allows the detection of an intrathecal IgG fraction as small as 0.5% of the total CSF IgG [3] and has a diagnostic sensitivity in Multiple sclerosis of up to 98% [4]. But this gold standard for the sensitive detection of intrathecal IgG [5] lacks specificity and represents in the routine laboratory a notoriously capricious method: The demanding isoelectric focusing is not easily automated and needs individual, experienced interpretation, based on method-related criteria [5].

The quantitative protein analysis in CSF, like the detection of intrathecal total IgG, IgA and IgM, can provide by the combined analysis specific, disease-related data patterns [1,6] but is far less sensitive. The

Abbreviations: CIS, Clinically isolated syndrome; CV, Interindividual coefficient of variation; FLC-K, free light chain kappa; FLC-L, free light chain lambda; IgG_{IF}, intrathecal fraction of total IgG; IgG_{Loc}, locally, intrathecal synthesized total IgG; I_{Kappa}, FLC-K Index; K_{IF}, intrathecal fraction of FLC-K; K_{Loc}, locally, intrathecal synthesized FLC-K; OCB, Oligoclonal IgG bands; Q_{Kappa}, CSF/serum concentration quotient of FLC-K; QAlb, CSF/serum concentration quotient of albumin; Q_{Kappa}(Lim), upper limit of the reference range; Q_{Kappa}(mean), mean of the reference range; Q_{Kappa}(low), lower border of the reference range

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https://doi.org/10.1016/j.cca.2019.07.027
Received 14 June 2019; Received in revised form 24 July 2019; Accepted 24 July 2019
Available online 25 July 2019
0009-8981/

still frequently used IgG Index has a very restricted reliability due to its false positive and false negative interpretations [6].

1.2. Development of free light chain analysis

With the development of fully automated nephelometric or turbidimetric protein analysis the kappa type of free light chains (FLC-K) in CSF (Ref. in [7,8]) gained new interest [9]. FLC-K, which circulates in blood and CSF predominantly as a monomer (22.5 kDa), gained more diagnostic relevance in neurology compared to its immunological partner, the predominantly dimeric lambda type of the free light chains (FLC-L) [8].

Many studies report a promising high sensitivity of FLC-K analysis for detection of an intrathecal humoral immune response, in particular in MS, but suffer from a missing consensus for a reference range of the normal blood-derived protein fraction in CSF. The common deficit in most of these investigations is their restricted data base, which does not include blood-CSF barrier dysfunctions that exceed a QAlb of 25×10^{-3}.

There are mainly three groups of approaches:

A) Interpretations of the absolute FLC-K concentration in CSF with cut-off values between 0.4 mg/l [7,10], 0.5 mg/l [7,11] and 0.61 mg/l [12] ignore a large inter-individual variation for age, FLC-K blood concentration and blood CSF barrier function [3].
B) Interpretations of the CSF/serum FLC-K quotient, Q_{Kappa}, refer to the albumin CSF/serum concentration quotient, QAlb. This Index takes into account the variations by the individual barrier function. The proposed cut-off values of $I_{Kappa} = Q_{Kappa}/QAlb$ vary between 3.6 [13], 5.9 [14], 6.07 [11], and 12 [7]. In addition to these huge discrepancies without any consensus, the use of a linear fit (Index) for the control groups ignores the nonlinearity of the relation between two blood-derived molecules of different size, e.g., QIgG/QAlb [15]. Therefore the FLC-K Index creates again the same problems with false negative and false positive interpretations as shown for total IgG [6].
C) A third group of studies suggests nonlinear curve fits for the reference range, eventually with a mathematical function. In this group with the most sophisticated approach we find concepts based on the physiological and biophysical knowledge [15–17], albeit insufficiently applied [9,11] but also arbitrary solutions without a physiological rational [14,18].

1.3. Targets of the investigation

The empirical data of free light chain kappa were grouped for small QAlb intervals [15] and the mean values of these groups were fitted with a hyperbolic function, based on the empirically and theoretically founded concept [15,17]. With the curve for Q_{Kappa}(mean) and the mean of the CV-values we create the FLC-K quotient diagram (Reiber-gram) to compare the sensitivity of intrathecal FLC-K, OCB, linear Index and nonlinear reference ranges published so far. Based on the hyperbolic reference range we calculate the absolute amount of intrathecal FLC-K synthesis or the relative fraction of total FLC-K concentrations in CSF. This allows to compare the intrathecal immunological response of FLC-K and total IgG. This concept provides also a quantitative criterion for comparison of the immune reaction in patients with a Clinically isolated syndrome (CIS) and definite MS.

This study is primarily focused on the development of the empirically and theoretically correct reference range for FLC-K analysis and the possible pathophysiological and clinical applications. The answer to the question whether OCB can be replaced by FLC-K analysis in general, or in a restricted spectrum of diseases, is left to the reevaluation of the many earlier more clinically oriented studies.

2. Methods

2.1. Control patients

Controls had to fulfill the following set of biochemical data: No intrathecal IgG, IgA and IgM ($Ig_{IF} \leq 0$) [1,3], no oligoclonal IgG in CSF, normal cell counts in CSF (lymphocytes < 5/μl), no blood contamination and lactate in CSF ≤ 2.5 mmol/l. The detection limit in the FLC-K assay for CSF should be 0.04 mg/l to allow the calculation of a reliable quotient, i.e., samples with concentrations reported as $CSF_{Kappa} < 0.1$ mg/l were omitted from interpretations.

2.2. Ethics approval

The original studies have been approved by the local Ethics Committee, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic (Ref. No. 319/2014 and Ref. No. 400/2017). The general approval of the local ethics committee, Kantonsspital Aarau, Switzerland was obtained for the use of anonymized routine left-over samples for method development, validation or quality control.

2.3. Sources of data

This retrospective study uses data sets of the routine analysis from two independent laboratories with different methods (Table 1). All samples were analyzed fresh in the routine laboratory or retrospective from frozen samples to avoid a preanalytical bias. Possible instabilities could cause lowered concentrations preferentially of serum samples.

Table 1
Definition of analytical groups.

Group	N	Control/disease	Method	Reagent
1a	353	No outliers	BN Prospec nephelometer	Siemens
1b	256	Including outliers		Latex kits
2a	170	No outliers	SPAplus turbidimeter	Freelite
2b	132	Including outliers		Binding Site
3a	11	No outliers	(1 + 2)	(1 + 2)
3b	30	including outliers	(1 + 2)	(1 + 2)
4 MS	45	Multiple sclerosis	SPAplus turbidimeter	Freelite
5 CIS	50	Clin. isolated syndrome		Binding Site

1 = Bernasconi/Mundwiler group, 2 = Zeman/Kušnierová group. 3 = All extreme data, 3a for QAlb = 1.6–2,2 × 10^{-3} ($N = 11$) and 3b QAlb = 20–128 × 10^{-3} (N = 30), no oligoclonal IgG, combined from the analytical groups 1a and 2a. 4 = Definitive MS at time of puncture, 5 = CIS patients with a later diagnosis of definite MS. Group 4 and 5 are diagnosed by the group 2.

1. Data from the Bernasconi/Mundwiler laboratory
   From a set of $N = 599$ patient data, received in the routine laboratory consecutively between 2011 and 2017, we obtained a selection of $N = 367$ biochemically defined controls or $N = 353$ qualified controls after elimination of outliers (control group 1a in Table 1). This group was divided into subgroups with QAlb intervals of N ≈ 20 (Table 2). Control group 1b in Table 1 contains subsequently analyzed control data in the routine laboratory 2017–2018, including FLC-K outliers ($N = 256$ patients [19]).
2. Data from the Zeman/Kušnierová laboratory are compiled from several studies published earlier [8,11] resulting in $N = 170$ qualified controls without outliers (group 2a). Control group 2b (Table 1) were subsequently analyzed control data in the routine laboratory, including FLC-K outliers ($N = 132$).
3. Group 3 (Table 1) contains control patients with extreme, pathologically increased QAlb values, collected together with groups 1a and 2a. These data were not used in the statistical evaluation (Table 2) and possible outliers were not eliminated.
4. Group 4 (Table 1) definitive Multiple sclerosis at time of first puncture.
5. Group 5, Clinical isolated syndrome (CIS) which was later diagnosed as MS.
   Group 4 and 5 were analyzed and diagnosed by the Zeman/Kušnierová laboratory [7,10].

2.4. Qualified control groups

The total groups of biochemically defined controls were divided into equal subgroups of about $N = 20$ patients ($N = 11$–17 patients in group 2a) for the smallest possible QAlb intervals (Table 2). After calculation of preliminary medians, means, standard deviations (SD) and the mean coefficients of variation (CV = SD/mean × 100 [%]), the outliers ($> Q_{Kappa}$(mean) ± 3 × CV) were excluded from the subgroups. By iteration of this process we established the final mean/median and mean CV values (Table 2).

Table 2
Evaluation of the FLC-K data set for controls without outliers (Siemens assay, group 1a, total N = 353).

N	$Q_{Alb} \cdot 10^3$			$Q_{Kappa} \cdot 10^3$			
	Range	Mean	Med	Mean	Med	SD	CV %
17	2.0–2.99	2.57	2.53	7.4	7.3	2.24	30
20	3.0–3.6	3.35	3.36	8.5	7.8	1.9	23
21	3.6–4.05	3.79	3.74	8.9	8.2	1.8	20
24	4.1–4.47	4.30	4.29	9.75	9.4	2.23	23
21	4.5–4.8	4.65	4.65	9.58	9.1	1.5	16
20	4.8–5.2	4.99	4.95	11.2	10.5	2.57	23
23	5.2–5.6	5.41	5.40	11.7	11.4	2.48	21
30	5.7–6.2	5.86	5.84	11.8	11.1	2.95	25
23	6.2–6.8	6.47	6.42	13.6	13.2	3.26	24
25	6.8–7.2	6.98	7.0	13.7	13.1	3.7	27
27	7.2–7.9	7.5	7.46	14.63	13.6	3.9	27
27	7.9–8.6	8.21	8.13	14.8	14.2	3.2	22
24	8.7–9.8	9.26	9.31	17.0	16.5	2.84	17
25	9.9–11.9	10.7	10.6	19.3	19.4	3.04	16
18	12–14.8	13.6	13.85	23.0	22.2	5.4	23
8	17.1–19.3	18.4	18.9	30.0	31.5	(8.7)	(27.6)
						Mean	22.5

Data are evaluated as a grouped statistics method for mean and median quotient values, the standard deviation, SD × 10^3, and the interindividual coefficient of variation (CV = SD/Qmean × 100 in %). Data in parenthesis are not integrated in the calculation of mean CV.

2.5. Analytical methods

2.5.1. Albumin, IgG, IgA, IgM

Proteins were analyzed by nephelometry (Laboratories 1 and 2: Siemens reagents, BN ProSpec, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

2.5.2. Oligoclonal bands

OCB's were detected by isoelectric focusing in agarose gels and immunofixation (Laboratories 1 and 2: Hydragel 9 CSF isofocusing, Sebia). Two extra bands in CSF compared to serum (OCB ≥ 2) were the interpretation criteria for intrathecal oligoclonal IgG synthesis.

2.5.3. FLC-K analysis

Groups 1a, b. The nephelometric N Latex FLC kappa Siemens Assay uses the same calibration curve (range 0.034–1.09 mg/l) for CSF and serum samples, but modifies the default dilutions correspondingly (initially undiluted for CSF and 1: 100 for serum). The analyzer automatically dilutes CSF samples (1:5, 1:20, etc) in order to keep the measured values in the reliable range of the calibration curve.

Groups 2a,b. The SPA_{PLUS} turbidimetric analyzer, using Freelite™ kits (The Binding Site Ltd.) was described [8]. A common six-point calibration curve is used for CSF and serum samples. Lowest and highest standards are approximately 0.40 mg/l and 18.0 mg/l with a calibration curve including the origin (0 mg/l). CSF is analyzed undiluted or manually re-diluted 1:10. Serum analysis is performed at 1:10 dilution or reanalyzed after automatic dilution by the instrument.

This turbidimetric method (laboratory 2) has concentration-dependent inaccuracies with a tendency to false high quotients. QAlb values $< 6 \times 10^{-3}$ (group 2a) have not been used for the data set in Fig. 1. Few procedural changes could improve this method: A better choice of the sample dilutions in the assay to match CSF and serum concentration location on the calibration curve and a better control of the calibration curve to ensure acceptable recovery for a serially diluted sample.

2.6. Data fitting with a hyperbolic function

Concentration quotients (Q) of blood-derived proteins in CSF are characterized in quotient diagrams with reference to the albumin quotient, QAlb, by the general hyperbolic function [15]:

$$Q = a/b\,[QAlb^2 + b^2]^{0.5} - c \quad (1)$$

The meaning of the parameters a, b, c for this mathematical function are shown in Fig. 1. These parameters depend on the size of the molecule [15].

2.7. Manual fitting procedure for the Qmean curve

The asymptote y = a/b x - c (Fig. 1) is determined preliminary as an approximation to the slope of the empirical curve in the range of largest QAlb values. In the range of low concentrations of x ($x \rightarrow 0$) we can estimate the value of (a-c) (Fig. 1) and calculate the value of a with c from the asymptote. These preliminary parameter values yielded a preliminary hyperbolic curve (like Fig. 1) with the function Q_{kappa}(mean) corresponding to Eq. (1). By iteration of this process for different values of the parameters a, b, c the best fitting function for the Qmean curve of FLC-K, can be found with the method of least squares, but using relative concentration differences, [(Qmeasured - Qtheor)/Qmeasured]. This is needed to take the increasing standard deviation with increasing QAlb (Fig. 1) into account.

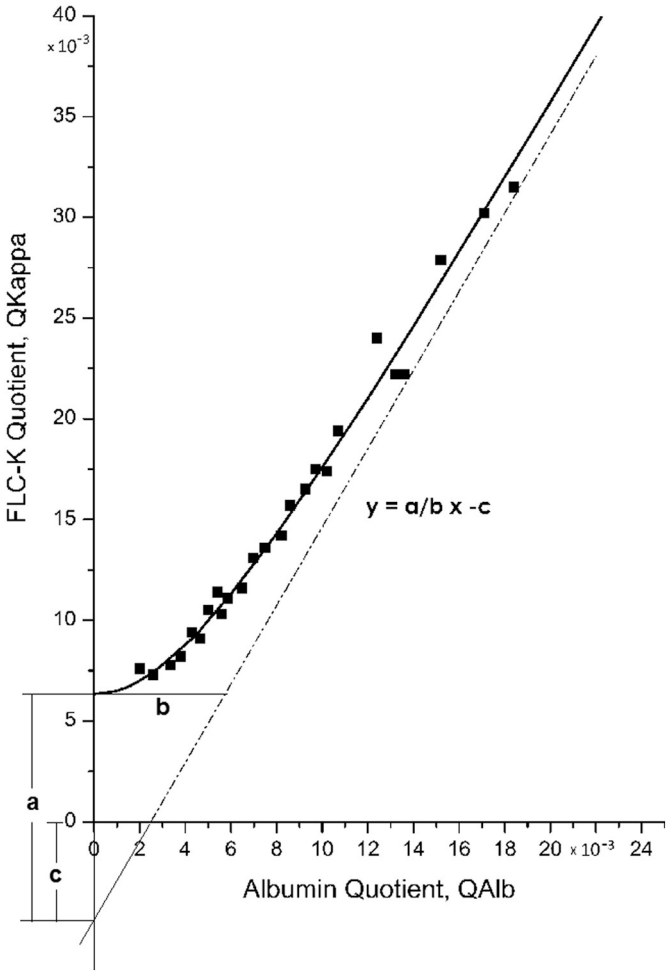

Fig. 1. Fitting procedure for the hyperbolic function. The squares represent means from grouped controls without outliers, (groups 1a in Table 2, part of the values of group 2a, s. methods and group 3a, total $N = 433$). Preliminary values for the parameters a-c and the slope of the asymptote s = a/b were used for the iteration procedure with the function $Q_{Kappa} = a/b[QAlb^2 + b^2]^{0.5}$- c, to find the best fitting parameters a,b,c with a particular least square calculation (methods).The theoretically defined limit of the hyperbolic function is reached at QKappa = 0.5 or QAlb = 256×10^{-3} (Table 3).

2.8. Construction of the complete reference range

With the mean CV value (Table 2) and the definition of the reference range as Qmean ± 3CV the complete curves for the upper limit (Qlim) and the lower border (Qlow) of the reference range (Table 3 in Results) could be established. This is shown in a linear diagram in Fig. 2 in Results.

3. Results

3.1. Hyperbolic function of the mean FLC-K reference curve, Q_{Kappa}(mean)

The mean values of FL-K quotients, Q_{kappa}, from the subgroups of controls are shown in Fig. 1 with the best fit for a hyperbolic function.

$$Q_{Kappa}(\text{mean}) = 1.95\,[QAlb^2 + 5.74^2]^{0.5} - 4.85\ (\times 10^{-3}) \quad (2)$$

characterized by the ordinate interval of a-c = 6.35×10^{-3} and the asymptote y = 1,95 x – 4,85 (Fig. 1 and Table 3). The grouped analysis of the control group with the smallest values of QAlb in the range 1,66 to 2.3×10^{-3} shows that the slope of the mean curve is approaching zero as expected for a hyperbolic function (Fig. 1). The normal Q_{Kappa}(mean) (Eq. (2)) for a given normal QAlb value (e.g., QAlb = 5×10^{-3}) can be calculated as $Q_{Kappa}(\text{mean}) = 1.95\,[5^2 + 33]^{0.5}$ -4,85($\times 10^{-3}$) = 10.0×10^{-3}. This yields a normal ratio of Q_{Kappa}/QAlb of 2 to 1.

3.2. Reference range of Q_{Kappa} in the quotient diagram

The mean interindividual coefficient of variation (CV = SD/Qmean × 100 in %) for Q_{Kappa} is CV = 22,5% (group 1a) and 21% (group 2a), correspondingly.

The upper and lower border line of the reference range is calculated with the confidence interval of Q_{mean} ± 3 CV or Q_{mean} ± 67.5%. This range provides a maximal diagnostic specificity for the detection of an intrathecal synthesis (connected with a lower sensitivity) The corresponding functions are calculated for the upper limit $Q_{lim} = Q_{mean} \times 1.675$ and for the lower curve $Q_{low} = Q_{mean} \times 0.325$ (Table 3) and shown in Fig. 2 with linear axes. Values above the reference range (Q_{mean} + 3 CV) are pathologic with a probability of 99.5%. Values below Qlow may represent an analytical fault.

Table 3
Parameters of hyperbolic functions, $Q_{Kappa} = a/b\ [QAlb^2 + b^2]^{0.5}$ -c (Fig. 1).

	a-c	a/b	a [×10³]	b² [×10⁶]	c [×10³]	QAlb (0.5)	QAlb (1)
Q_{Kappa} (lim)	10,6	3.27	18.8	33	8.2	< 155×10^{-3}	308×10^{-3}
Q_{Kappa}(mean)	6,35	1.95	11.2	33	4.85	< 256×10^{-3}	515×10^{-3}
Q_{Kappa} (low)	2,54	0.78	4.48	33	2	< 643×10^{-3}	> 1000×10^{-3}

The functions are shown in FLC-K Reibergrams for the upper border line Qlim, the mean Qmean, and the lower border line Qlow in Fig. 2. The theoretical limit of the valid range for the Q_{Kappa} functions in the QAlb quotient diagram is defined for Q_{Kappa} < < 0.5 = 500×10^{-3}. As example for Q_{Kappa}(mean) = 500×10^{-3} we get for QAlb (0.5) = $[(504{,}85/1.95)^2 – 33]^{0.5} = 256 \times 10^{-3}$. Q_{Kappa} = 1 is reached at a QAlb(1) with values calculated as approximation from the asymptote. Corresponding calculations are performed for Qlim and Qlow.

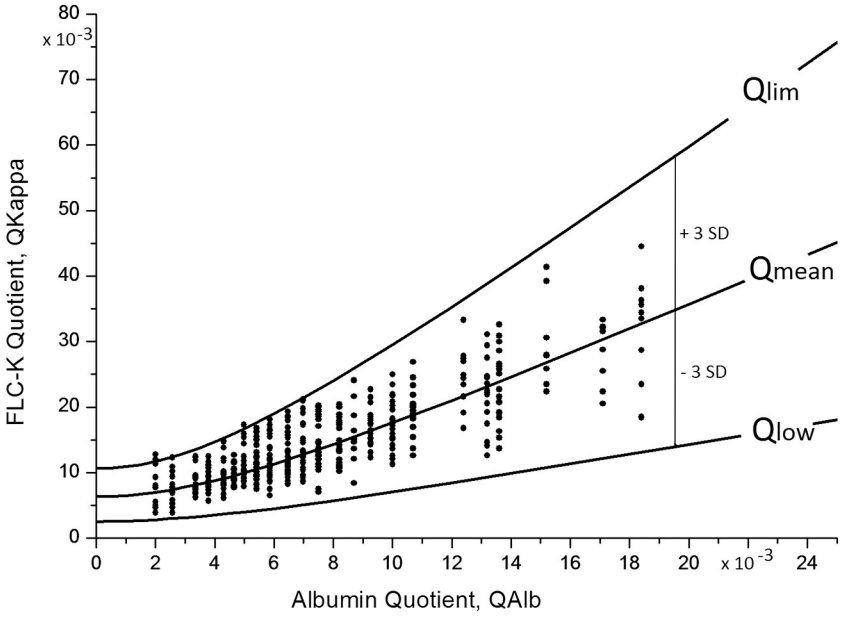

Fig. 2. Reference range of CSF/serum quotients, Q_{Kappa}, in a quotient diagram with linear axis. Qlim and Qlow are calculated from Qmean ± 3 CV (Table 3). Increasing standard deviations (SD) have a constant coefficient of variation, CV = 22.5% (Table 2). The reference range includes 99% of the controls. Data are accumulated from group 1a (Table 2), group 2a and 3a (Total N = 433).

The use of a double log plot for FLC-K analysis in Fig. 3, corresponding to the Reibergrams for IgG, IgA and IgM [1,6], has also the advantage to spread optically the range between $Q_{Alb} = 2 \times 10^{-3}$ and $Q_{Alb} = 20 \times 10^{-3}$ where the largest number of data from patients of diagnostic interest (e.g., with Multiple sclerosis) are localized but also provides a possible display of data up to values of QAlb $= 150 \times 10^{-3}$. The implemented Q_{mean} curve (dotted line) in Fig. 3 indicates the optical asymmetry in the double log diagrams. The square symbols represent the ungrouped data set from Fig. 2, expanded with values from patients with extremely increased QAlb (group 3 in Table 1). This shows the reliability of the theoretically founded extension of the hyperbolic reference range beyond the statistically treated data groups in Figs. 1 and 2.

The upper limit of the reference range, the diagnostically relevant discrimination line is.

$$Q_{kappa}\,(\mathrm{lim}) = 3.27\,[Q_{Alb}^{2} + 33]^{0.5} - 8.2\;(\times 10^{-3}) \qquad (3)$$

This function is valid in the QAlb range below 155×10^{-3} (Table 3). The diagnostic border line for QAlb values above 150×10^{-3} is given in Discussion and Table 3.

An extrapolation to QAlb below 1.6×10^{-3} is not justified. The ordinate interval (a-c) is influenced by the different molecular transfer mechanisms into the ventricular CSF [17], which are not restricted to diffusion, the precondition for a hyperbolic function.

3.3. Quantitation of intrathecal synthesis

With these functions according to Table 3 and the serum concentrations S_{Kappa}, we can calculate the locally, in brain synthesized FLC-K fraction in CSF. The quantity is expressed, like for the immunoglobulins [5,12], either as the locally synthesized absolute amount of FLC-K, K_{loc}, or the relative intrathecal fraction, K_{IF} (Fig. 3):

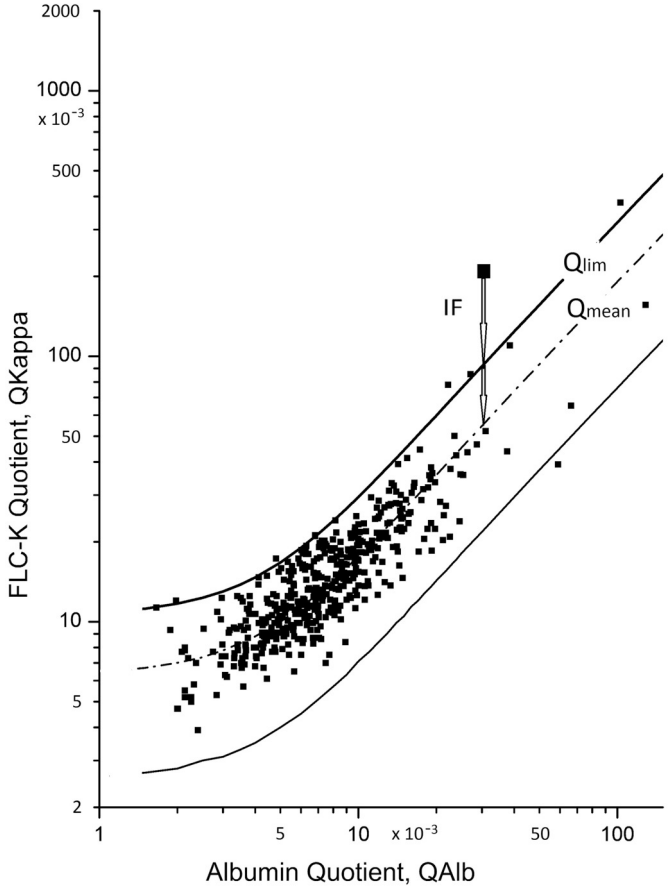

Fig. 3. FLC-K control data in a double logarithmic quotient diagram (Reibergram). The hyperbolic functions (Table 3) are shown up to QAlb $= 150 \times 10^{-3}$. The data from Fig. 2 are shown ungrouped and extended by additional data for QALb above 20×10^{-3} ($N = 30$, group 3b in Table 1, total $N = 463$). 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 (K_{IF} in %) or with reference to Qmean for statistical purposes, K_{IF}(mean), eventually calculated as quantitative value K_{loc}(mean) in mg/l.

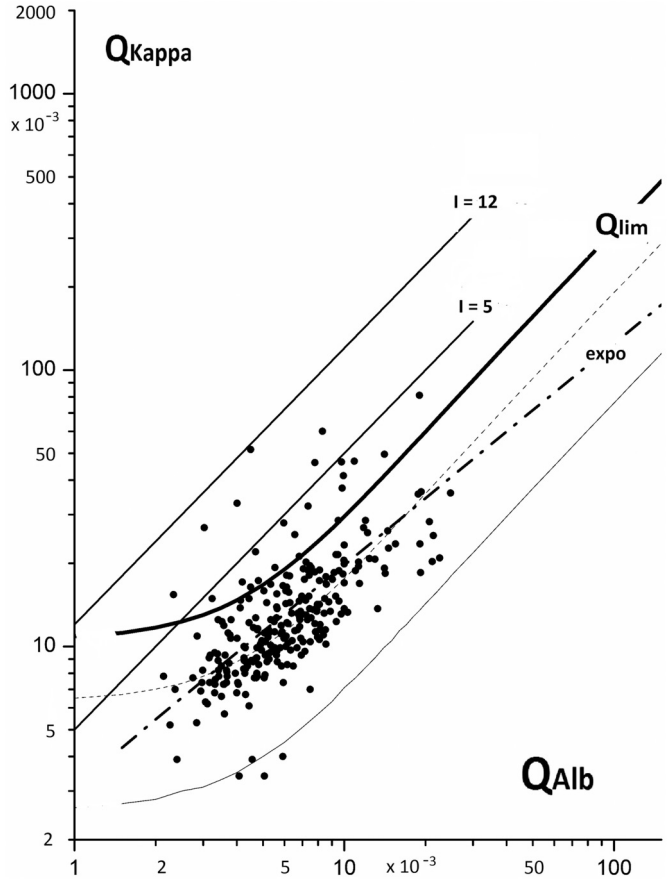

Fig. 4. Control group in the FLC-K Reibergram for comparison of different interpretation concepts. Routine laboratory data from biochemically defined controls without oligoclonal IgG, with normal IgG, IgA, IgM, normal cell count and normal lactate (N = 256 patients, group 1b). The bold line represents the hyperbolic Qlim curve (Table 3). 20/256 data pairs (8%) are outside the reference range, i.e., false negative interpretations of samples with negative oligoclonal IgG. Examples of the linear Index, I_{Kappa} = 5 (similar to [11,14]) and I_{Kappa} = 12 [7] show 15/20 or 20/20, respectively, false negative interpretations, compared to Qlim. The bold dash-dot line (expo) is the Qlim value of an exponential function [20] with 77/256 = 30% false positive interpretations of normal controls.

$$K_{loc} = [Q_{kappa}(total) - Q_{kappa}(lim)] \times S_{kappa}\ [mg/l] \quad (4)$$

$$K_{IF} = K_{loc}/CSF_{kappa} \times 100 \text{ or } (1 - Q_{lim}/Q_{kappa}) \times 100\ [\%] \quad (5)$$

With K_{loc} = 0 mg/l and K_{IF} = 0% at Qlim, the quantitative values for the intrathecal synthesis represent the calculable minimal amount of intrathecal FLC-K in CSF. In case of statistics for the comparison of patient groups (Fig. 5) it is more relevant to refer K_{loc} to Qmean (like IF in Fig. 3): K_{loc} (mean) = [Q_{kappa}(total) - Q_{kappa} (mean)] × S_{kappa} in mg/l. Examples are calculated in paragraph 3.6 for the data of the MS and CIS patients in Fig. 5.

3.4. Sensitivity of FLC-K analysis in different interpretation methods

Fig. 4 shows subsequently analyzed controls in the routine laboratory without any selection. With Q_{Kappa} above Q_{Kappa}(lim) in 20/256 or 8% of the patients (Fig. 4) the FLC-K analysis is more sensitive for an intrathecal immune response compared to other basic CSF parameters, in particular oligoclonal IgG, normal intrathecal IgG, IgA, IgM fractions, cell count and lactate.

Fig. 4 shows also two examples of reported thresholds values of the linear Index (I_{Kappa} = 5 and I_{Kappa} = 12). I_{Kappa} = 12 is including all OCB negative controls, i.e. there are no false positive interpretations, but compared to QKappa(lim), this Index I_{Kappa} = 12 creates 20/256 or 8% false negative interpretations. The cut-off value I_{Kappa} = 5 creates 14/256 = 6% false negative interpretations. This means, that the application of the hyperbolic reference line instead of the Index cut-off values would dramatically increase the sensitivity of FLC-K analysis reported in earlier studies [7,10–14].

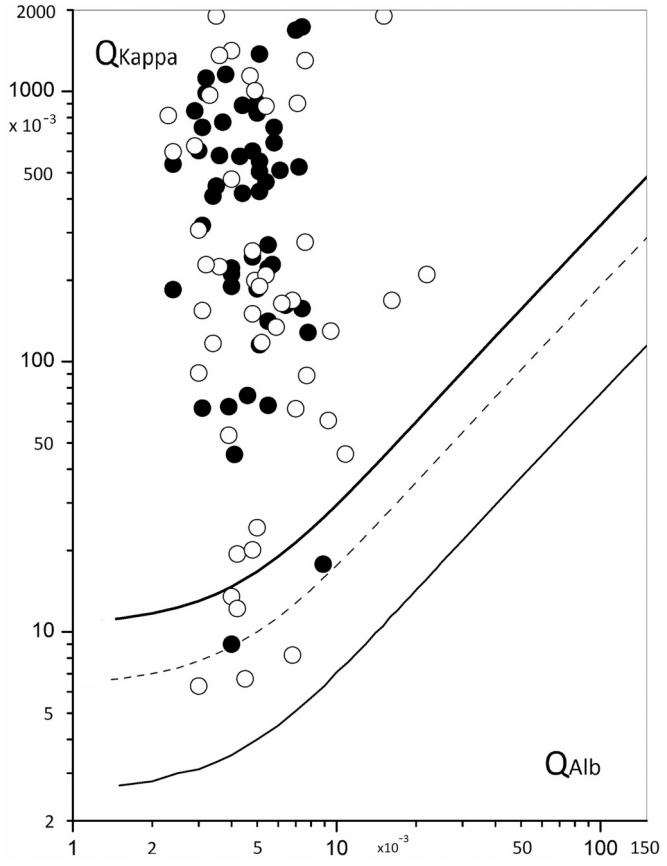

Fig. 5. MS and CIS patient data in the FLC-K Reibergram.
Filled circles are Multiple sclerosis patients (N = 45) and open circles represent Clinically isolated syndrome patients, later found as MS (N = 50). FLC-K analysis detects 93% of total 95 MS patients. For detailed comparison of quantitative intrathecal synthesis in the CIS and MS groups see text. A corresponding software for the evaluation of FLC-K in this quotient diagram is available as free download [27].

From the groups with molecular size dependent exponents of QAlb [14,20,21] the best fitting function Q_{Kappa}(Lim) = 31,276 QAlb $^{0.8001}$ [18] is shown as dash - dot line (expo) in Fig. 4. The negative bent border line creates 77/256 = 30%! false positive interpretations of normal OCB negative controls in Fig. 4. The percentage of false positive interpretations will be increasing for further increasing QAlb values.

The additional patients with extreme barrier dysfunctions, i.e., large QAlb values up to 128 × 10^{-3} in Fig. 3, show the superiority of the hyperbolic reference range over the other methods, which completely fail to fit this range of data.

3.5. Diagnostic sensitivity: multiple sclerosis

The total group of patients with a definite MS (N = 95, 94% OCB in CSF) is shown in Fig. 5 as filled and open circles. We find intrathecal FLC-K in 93% of the patients with reference to the hyperbolic border line, 90% with reference to I_{Kappa} = 5, or 85% with reference to I_{Kappa} = 12 and 98% with reference to the exponential border line.

These results mirror the methodological bias with a false low sensitivity for the Index and a false high sensitivity for the exponential border line. For a final judgement whether FLC-K can replace OCB in MS diagnosis remains to be investigated with larger patient groups.

3.6. Multiple sclerosis and clinically isolated syndrome in FLC-K diagrams

The data of the patient groups 4 (MS) and 5 (CIS) are shown in the FLC-K Reibergram in Fig. 5. At the time of first diagnostic CSF puncture the definite MS cases presented in 10% with false negative interpretations and in 2% of the CIS group with later confirmed definite MS with Q_{Kappa} below $Q_{Kappa}(lim)$. The quantitation of intrathecal synthesis changes this first impression. $K_{loc}(mean)$ with reference to $Q_{Kappa}(mean)$ is calculated for each patient and subsequent the means of the groups. The mean intrathecally synthesized amounts with $K_{loc}(mean) = 3.3$ mg/l for the MS group is lower than the mean $K_{loc}(mean) = 4.4$ mg/l for the CIS group. This result allows to conclude that there is no tendency for a weaker immune response in CIS to support a pathophysiological conversion from CIS to MS as stated by several researchers [22,23]. The CIS group had the lowest ($Q_{Kappa} = 6 \times 10^{-3}$) but also highest ($Q_{Kappa} = 2700 \times 10^{-3}$) quotient values. These extremely high Q_{Kappa} quotients mirror the higher relative intrathecal fractions, $K_{IF,}$ compared to the total IgG, $IgG_{IF,}$ with the Qmean related values $K_{IF}(mean) = 95\%$ for FLC-K but only $IgG_{IF}(mean) = 36\%$ for total IgG. This means that in average 95% of the CSF concentration of FLC-K is intrathecally synthesized compared to 36% of total IgG. This threefold larger relative intrathecal response of FLC-K compared to total IgG is the reason why the quantitative FLC-K analysis has a much better diagnostic sensitivity of 93% in the MS group compared to the correspondingly poor sensitivity of total IgG with about 70% [24].

4. Discussion

4.1. Reliability of the hyperbolic reference range

The method of grouped data analysis (Table 2) for a known curve function is superior to a regression analysis of the total data cloud in a scatter plot. From the data in the range of the lowest QAlb values in Fig. 1 (mean $QAlb = 2 \times 10^{-3}$, range $QAlb = 1.66 \times 10^{-3}$ to 2.3×10^{-3}) we learn that the slope of the curve Q_{Kappa} (mean) is approaching zero as expected for a hyperbolic curve. These data are not biased by the sensitivity of the method as only those values were included for which the serum values and corresponding CSF values were high enough.

The extrapolation of the curves to QAlb values larger than in the group of Table 2 and Fig. 1 is justified by the theory-based concept [15,17] and gains plausibility by higher QAlb values up to QAlb $= 128 \times 10^{-3}$ in Fig. 3. These concentration ranges, which are 10-fold the concentrations of the average quotients from MS patients, are barely considered in the many studies with other interpretation concepts [7,10–14,20–23]. But this would have been crucial to see the general reliability of the reference line in the individual model.

Table 4
Analytical sensitivity of FLC-K compared with oligoclonal IgG (group 2b in Table 1).

	Total	2 Bds	3 Bds	4–24 Bds
OCB +	116	7	8	101
FLC-K +	110	3	6	101

From the subsequent analysis of 320 patients in the routine laboratory with total 116 OCB positive CSF samples (OCB ≥ 2 Bds) we get the described frequencies of 2 to 24 bands. Of the CSF samples neg for OCB, 7/204 had a single band of which 3 had an intrathecal FLC-K synthesis. 26/204 cases without intrathecal IgG synthesis had ≥ 2 oligoclonal FLC-K bands in CSF [8].

4.2. Biophysically defined validity ranges of $Q_{Kappa}(lim)$

The hyperbolic reference range in CSF/serum quotient diagrams was discovered empirically [25] and later confirmed with a larger set of immunoglobulin data combined with the theoretical derivation of the hyperbolic function from the laws of diffusion [15]. The concept was applied to several different blood-derived proteins in CSF [16]. The free light chain type kappa (22.5 kDa) is the first molecule smaller than the reference albumin (64 kDa) to be investigated with this concept. The basic difference comes with the faster diffusion of FLC-K than albumin. The FLC-K concentration in CSF reaches the blood concentration ($Q_{Kappa} = 1$) before albumin reaches the equilibrium (QAlb $= 1$). Therefore, we need to describe eventually the complete Qlim curve, i.e. also for values of $Q_{Kappa} > 0.5$. For this purpose we have to remember that for the derivation of the hyperbolic function [15] from the diffusion equation we need a trigonometric series to get an approximation for the mathematical solution of this differential equation. Depending on the concentration range of the diffusing molecule there are two complementary trigonometric series. In the usual range of the low concentrations in CSF this is the error function complement, erfc [15,17]. At high concentrations, i.e., at $Q_{Kappa} > 0.5$ or 500×10^{-3} we have to use mathematically the alternative trigonometric function (error function, erf [15,17], i.e., we get an inverted hyperbolic function in the quotient diagram which, like a sigmoid curve, approaches to $Q_{Kappa} = 1$ at QAlb $\approx 308 \times 10^{-3}$. This is shown explicitely in [17].

According to Table 3 we reach $Q_{Kappa}(lim) = 0.5$ at a QAlb $= 155 \times 10^{-3}$, the limit for the validity of the hyperbolic function Qlim in Eq. (3).

For the rare cases with QAlb above 150×10^{-3} instead of the inverted hyperbolic function we can use simpler approximations. In the range QAlb = 150 to 300×10^{-3} we can use the asymptote $Q_{Kappa} = 3.27 \times$ QAlb as upper reference line and for QAlb above 300×10^{-3} any value of QKappa larger than 1 or 1000×10^{-3} must be due to an intrathecal synthesis of FLC-K. The inaccuracy by this approximation for the few rare cases is negligible compared to the analytical imprecision.

4.3. Clinical relevance of FLC-K analysis

4.3.1. FLC-K analysis compared to oligoclonal IgG

The theoretically expected symmetrical distribution of data in the reference range [15–17] is obviously fulfilled for FLC-K as shown in the distribution of data around Qmean in Fig. 2. This allows to conclude that the patients in Fig. 4 with Q_{Kappa} above $Q_{Kappa}(lim)$ have a probability of > 99.5% to have an intrathecal FLC-K synthesis in spite of absent oligoclonal IgG (OCB).

Any relevant comparison between the clinical relevance of OCB versus FLC-K -analysis must consider two different aspects: The quality of the OCB method and the particular disease (paragraph 4.3.2). In this study ($N = 388$ OCB negative samples)) we find $N = 31$cases (8%) with intrathecal FLC-K synthesis. The reference is a widespread used automated OCB method but we regarded two bands as positive oligoclonal IgG in contrast to the common 3 bands in routine diagnosis. Table 4 shows the relevance of this decision for the frequency of intrathecal FLC-K: 7/116 cases of our group 2b (Table 1) had only two bands which would be missed in the cut off with ≥ 3 bands. From these 7 cases with 2 bands only three cases had intrathecal FLC-K. But in the group of negative oligoclonal IgG ($N = 204$), which included 7 cases with one single OCB, we found with quantitative FLC-K analysis three patients and with qualitative FLC-K analysis 26 patients with intrathecal FLC-K.

So, any figure about false negative results of OCB, like our 8%, depends on several aspects of the quality and the interpretation of the OCB method.

The second comparison for the clinical relevance of FLC-K analysis is the diagnostic sensitivity for Multiple sclerosis. Our data for a small MS group and CIS group in Fig. 5 have been introduced for the

demonstration of the different results dependent on the interpretation concept with false low values for the Index (85%) and false high values for the exponential curve (98%) compared to the hyperbolic reference range (93%). A second motivation for this presentation of a CIS and MS group is the possibility to quantitate intrathecal synthesis for pathophysiological interpretations. For a reliable figure for the diagnostic sensitivity of FLC-K analysis we would need a larger cohort of MS patients and essentially an application for other diseases (s. 4.3.2).

4.3.2. Theoretical limits for quantitative FLC-K analysis

The analytical sensitivity of a quantitative method, with a statistically determined reference range, can't reach the analytical sensitivity of qualitative method like the oligoclonal IgG analysis in CSF [3] with a direct comparison in the individual patient.

1. The theoretical limits of diagnostic sensitivity for quantitative FLC-K analysis can be calculated from the coefficient of variation, CV: A healthy patient at the lower border of the reference range Q_{kappa}(low) (Fig. 2) may have to synthesize in brain additional 135% (6 × SD in Fig. 2) of its original FLC-K concentration in CSF to reach significantly Q_{Kappa}(lim). It would need still more to generate a detectable intrathecal synthesis of FLC-K with a value above Q_{Kappa}(lim). This is in contrast to the qualitative detection of OCB in IEF, which can detect under optimal conditions already 0.5% intrathecal IgG in the total CSF IgG [3].
2. In Multiple sclerosis patients this theoretical limitation is compensated by a relatively high intrathecal FLC-K fraction in CSF (K_{IF}(mean) = 95%, paragraph 3.6). But there are other diseases with a less prominent IgG class response, like neuroborreliosis [20,26] with dominant intrathecal IgM class response in a two to three class pattern or neurotuberculosis with a dominant intrathecal IgA class response in a one to two class pattern [1,6]. The investigations of the clinical sensitivity of the intrathecal FLC-K response have to consider the relation of OCB to FLC-K in different diseases. Of course, both, OCB as well as FLC-K analysis, may be less relevant with respect to the most specific and most sensitive parameter(s), like the specific borrelia antibody Index [26].

4.3.3. Comparison of different interpretation methods of FLC-K data in CSF

Among all published attempts to define a limit between normal and pathologically increased FLC-K concentration in CSF the absolute concentration of FLC-K is the worst choice [7,10–12]. First, normal CSF concentrations depend directly on the serum concentration which varies more than ± 100% (serum concentrations of FLC-K vary between 6 and 100 mg/l with a median of 12.9 mg/l). Second, this approach ignores the influence of the age on the blood CSF barrier function [3]. In group 1a we find 50% increase of QAlb between 30 and 70 years of age. Third, the largest increase of the blood-derived FLC-K concentrations in CSF comes with the up to 100-fold increasing concentration by blood-CSF barrier dysfunctions [1,3,6].

The most frequent concept uses a linear Index, $I_{kappa} = Q_{kappa}/QAlb$. Fig. 4 shows two examples of reported thresholds values of the linear Index ($I_{Kappa} = 5$ and $I_{Kappa} = 12$). Together with the data from MS analysis in Fig. 5 we can conclude that the Index related interpretations produce 8% false negative controls as well as 15% false negative MS interpretations. This means, that the sensitivity of FLC-K analysis with reference to the hyperbolic function is higher than reported in earlier Index studies [7,10–14].

Among the groups with nonlinear functions for the reference range those groups with molecular size dependent exponents of QAlb (e.g., $QAlb^{1.035}$ for IgG [18] and $QAlb^{0.6687}$ [14,21] or $QAlb^{0.8001}$ [20] for FLC-K) showed the worst empirical results with 30%! false positive interpretations of normal controls in Fig. 4. As a consequence of the obviously much to low border lines [14,21] the studies get a much to high FLC-K sensitivity (e.g.98% for the detection of an intrathecal immune response in MS in Fig. 5). In addition to the questionable empirical data fit the biophysical concept [18] is wrong. The molecular size dependence comes with the diffusion not with the CSF flow related change of QAlb. This approach is also not compatible with the physiological and pathophysiological data [15–17].

As a summary, these data from Fig. 4 show that compared to the hyperbolic reference range including 99% of the normal FLC-K controls, the Index values come along with underestimated sensitivity while the exponential approaches make as many as 30% of the controls to a false pathological case and may pretend to have a higher sensitivity of the FLC-K analysis.

Former publications that calculated sensitivity and specificity and compared with oligoclonal IgG in Multiple sclerosis or other diseases should reevaluated with our new approach. A free software for the evaluation and graphical presentation of FLC-K data in quotient diagrams is available [27].

4.3.4. Relevance for diagnosis and laboratory practice

The hyperbolic reference range in the FLC-K analysis avoids the false positive and false negative interpretations of the other methods reported and provides a reference range valid also for very high albumin quotients, i.e. in patients with the severest blood CSF barrier dysfunctions. With the possibility to calculate the intrathecally synthesized amounts of FLC-K the new reference functions provide also pathophysiological information.

For diagnosis of Multiple sclerosis the quantitative FLC-K analysis has a reasonably high sensitivity (93%), but could fail in other diseases with a less intense intrathecal immune response, where the theoretically founded advantage of the qualitative method over the quantitative method counts.

The quantitative FLC-K analysis has the advantage, compared to the sophisticated qualitative OCB method, that it can be easily performed in each laboratory with an equipment for protein analysis, considering the different qualities of the commercial assays (s. Methods). So, the laboratories without OCB analysis could gain a basic improvement in their analytical performance with FLC-K analysis. In the laboratory with OCB analysis the FLC-K detection could be complementary for cases with ambiguous interpretations presenting with 1–3 bands.

Our study, which is focused on the development of the reference range for FLC-K analysis, can't conclude whether FLC-K analysis could replace the more capricious OCB analysis. We would like to see a re-evaluation of the former studies with larger cohorts of MS cases with the new concept to avoid false positive and false negative interpretations.

Principally, like for IgG, also for the FLC-K analysis in CSF the qualitative detection of specific oligoclonal FLC-K bands after isoelectric focusing [8,28] could be a more sensitive method than the quantitative FLC-K analysis (s. legend to Table 4). But this would again create the problems with the capricious isoelectric focusing.

4.4. Free light chains and the blood-CSF barrier function

The molecular size dependent passage of molecules between blood and brain or CSF is mirrored by the mathematical function for the reference range in quotient diagrams [15,17], in particular by the slope of the asymptote s = a/b of the hyperbolic curve (Fig. 1). In the quotient diagrams with reference to QAlb the molecules larger than albumin have a slope for Qmean of $s < 1$ (IgG s = 0.65, IgA and CEA s = 0.47, IgM s = 0.33 [9]), but for molecules smaller than albumin we find the slope exceeding 1, e.g., s = 1.95 for FLC-K (Fig. 1 and Table 3). For the hyperbolic function of FLC lambda (FLC-L) we get a slope of about s = 1.2 (unpublished results of D. Zeman et al.). This means that FLC- K passes the barrier primarily as the smaller, monomeric molecule (22.5 kDa), compared to the primarily dimeric FLC-L (45 kDa) with a slope approaching to 1.0 like the value found e.g., for the transthyretin/retinol binding protein complex (54 + 21 kDa) with s = 1 [16].

As a consequence, the normal ratio of Kappa/Lambda free light

chain concentrations in CSF must be different from the normal relation in blood, due to the slower passage of the dimeric FLC-Lambda through the barrier and a subsequently lower concentration in CSF compared to the smaller monomeric molecule FLC-K. The ratio of monomeric to dimeric FLC-K reported from SDS PAGE electrophoresis with western blot detection [29] may not reflect the real relation that is present in physiological solutions, i.e., in blood and CSF.

4.5. Pathophysiology of MS and CIS

Several authors [21–23] investigated FLC-K analysis as a biomarker to prognose a conversion [22] of the Clinically isolated syndrome, CIS, into Multiple sclerosis, MS. The Qmean related statistical evaluation of the FLC-K intrathecal synthesis with Eq. (4) (free software available at www.albaum.it [27]) detects no differences in immune response between CIS and MS: There is no smaller mean amount of intrathecal FLC-K synthesis in CIS (K_{loc}(mean) = 4.4 mg/l) compared to MS (K_{loc}(mean) = 3.3 mg/l) which could support a conversion between CIS and MS. This idea of CIS as an early pathologically defined phase in the course of the disease may derive from insensitive methods or from the false interpretation of an analytical parameter. A study which declares intrathecal IgM synthesis to be a risk factor for conversion of CIS to MS [30], simply ignores that only 50% of the MS patients have an intrathecal IgM synthesis at all and that the isotype specific B cells immigrate in the individual MS patient as an arbitrary combination with locally different antibody specificities [31]. From our earlier investigations on juvenile MS [4] we also learned that already at the earliest time in the course of the disease we find the complete set of immune reactions, including the M-,R-,*Z*-antibody reaction [32]. There has never been shown a direct conversion e.g. from an acute disseminated encephalomyelitis, ADEM, without MRZ antibodies [4] to a chronic form, i.e., to MS with the typical polyspecific immune response [32].

5. Perspectives

The question in the editorial of D Zeman [33] about the relevance of oligoclonal IgG detection when FLC-K analysis is available cannot be answered as long as the nonlinear interpretation scheme described here has not been applied to larger patient cohorts with varying diseases. It is mandatory to use the concept of complete disease-related data patterns [1], as well as disease-specific parameters and to consider the context of the differential diagnostic questions to further establish the clinical relevance of FLC-K analysis. The fashionable search for the ONE, disease-specific marker molecule and the data evaluations with linear regression lines and linear reference ranges may fit the common preference for linear cause and consequence models but keeps missing the understanding of chronic diseases [34]. For this behavior we find a very simple and clear comment from the Austrian naturalist and artist Friedensreich Hundertwasser: “The straight line is a crime”.

References

[1] H. Reiber, Cerebrospinal fluid data compilation and knowledge-based interpretation of bacterial, viral, parasitic, oncological, chronic inflammatory and demyelinating diseases: diagnostic patterns not to be missed in neurology and psychiatry, Arq. Neuropsiquiatr. 74 (4) (2016) 337–350.

[2] B. Wildemann, P. Oschmann, H. Reiber (Ed.), Laboratory diagnosis in Neurology, Thieme Verlag, Stuttgart, 2010.

[3] H. Reiber, Knowledge-base for interpretation of cerebrospinal fluid data patterns - essentials in neurology and psychiatry, Arq. Neuropsiquiatr. 74 (6) (2016) 501–512.

[4] H. Reiber, M. Teut, D. Pohl, K.M. Rostasy, F. Hanefeld, Paediatric and adult multiple sclerosis: age related differences and time course of the neuroimmunological response in cerebrospinal fluid, Mult. Scler. 15 (2009) 1466–1480.

[5] M. Andersson, J. Alvarez-Cermeño, G. Bernardi, I. Cogato, P. Fredman, J. Frederiksen, et al., Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report, J. Neurol. Neurosurg. Psychiatry 57 (8) (1994) 897–902, https://doi.org/10.1136/jnnp.57.8.897.

[6] H. Reiber, J.B. Peter, Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs, J. Neurol. Sci. 184 (2) (2001) 101–122, https://doi.org/10.1016/S0022-510X(00)00501-3.

[7] F. Duranti, M. Pieri, D. Centonze, F. Buttari, S. Bernardini, M. Dessi, Determination of κFLC and κ index in cerebrospinal fluid: a valid alternative to assess intrathecal immunoglobulin synthesis, J. Neuroimmunol. 263 (1–2) (2013) 116–120.

[8] D. Zeman, P. Kušnierová, Z. Švagera, F. Všianský, M. Byrtusová, P. Hradílek, B. Kurková, O. Zapletalová, V. Bartoš, Assessment of intrathecal free light chain synthesis: comparison of different quantitative methods with the detection of oligoclonal free light chains by isoelectric focusing and affinity-mediated immunoblotting, PLoS ONE 11 (11) (2016; Nov 15), https://doi.org/10.1371/journal.pone.0166556.

[9] C. Fischer, B. Arneth, J. Koehler, J. Lotz, K.J. Lackner, Kappa free light chains in cerebrospinal fluid as markers of intrathecal immunoglobulin synthesis, Clin. Chem. 50 (2004) 1809–1813.

[10] M. Christiansen, M.C. Gjelstrup, M. Stilund, T. Christensen, T. Petersen, H. Jon Møller, et al., Clin. Chem. Lab. Med. 57 (2) (2018) 210–220, https://doi.org/10.1515/cclm-2018-0400.

[11] D. Zeman, P. Kušnierová, V. Bartoš, P. Hradílek, B. Kurková, O. Zapletalová, Quantitation of free light chains in the cerebrospinal fluid reliably predicts their intrathecal synthesis, Ann. Clin. Biochem. 53 (2016) 174–176.

[12] K.M. Gurtner, E. Shosha, S.C. Bryant, B.D. Andreguetto, D.L. Murray, S.J. Pittock, M.A.V. Willrich, CSF free light chain identification of demyelinating disease: comparison with oligoclonal banding and other CSF indexes, Clin. Chem. Lab. Med. 56 (2018) 1071–1080.

[13] M. Süße, M. Hannich, A. Petersmann, S. Zylla, M. Pietzner, M. Nauck, A. Dressler, Kappa free light chains in cerebrospinal fluid to identify patients with oligoclonal bands, Eur. J. Neurol. 25 (2018) 1134–1139.

[14] S. Presslauer, D. Milosavljevic, W. Huebl, S. Parriger, G. Schneider-Koch, T. Bruecke, Kappa free light chains: diagnostic and prognostic relevance in MS and CIS, PLoS ONE 9 (2) (2014) e89945, , https://doi.org/10.1371/journal.pone.0089945.

[15] H. Reiber, Flow rate of cerebrospinal fluid (CSF)- a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases, J. Neurol. Sci. 122 (1994) 189–203.

[16] H. Reiber, Proteins in cerebrospinal fluid and blood: barriers, CSF flow rate and source-related dynamics, Restor. Neurol. Neurosci. 21 (2003) 79–96.

[17] H. Reiber, Blood-brain and Blood-CSF barriers: physiology, pathophysiology and the common diffusion/flow interface, Fluids and Barrier Research (2019) submitted.

[18] M. Auer, H. Hegen, A. Zeileis, F. Deisenhammer, Quantitation of intrathecal immunoglobulin synthesis – a new empirical formula, Eur. J. Neurol. 23 (2016) 713–721.

[19] E. Mundwiler, L. Bernasconi, Detection of intrathecal immunoglobulin synthesis: A rapid and economical approach using a combination of kappa free light chain index and oligoclonal bands, Poster, Autoimmunity Conference, 2018 (Lisbon).

[20] H. Hegen, D. Milosavljevic, W. Huebl, A. Manowiecka, J. Walde, F. Deisenhammer, S. Presslauer, Cerebrospinal fluid free light chains as diagnostic markers in neuroborreliosis, Clin. Chem. Lab. Med. 56 (2018) 1383–1391.

[21] S. Presslauer, D. Milosavljevic, W. Huebl, F. Aboulenein-Djamshidian, W. Krugluger, F. Deisenhammer, M. Senel, H. Tumani, H. Hegen, Validation of kappa free light chains as a diagnostic biomarker in multiple sclerosis and clinically isolated syndrome: a multicenter study, Mult. Scler. 22 (2016) 502–510.

[22] L.M. Villar, M. Espiño, L. Costa-Frossard, A. Muriel, J. Jiménez, J.C. Álvarez-Cermeño, High levels of cerebrospinal fluid free kappa chains predict conversion to multiple sclerosis, Clin. Chim. Acta 413 (2012) 813–816.

[23] M. Senel, H. Tumani, F. Lauda, et al., Cerebrospinal fluid immunoglobulin kappa light chain in clinically isolated syndrome and multiple sclerosis, PLoS ONE 9 (2014) e88680.

[24] H. Reiber, Ungefehr St, Jacobi Chr, The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis, Mult. Scler. 4 (1998) 111–117.

[25] H. Reiber, K. Felgenhauer, Protein transfer at the blood-CSF barrier and the quantitation of the humoral immune response within the central nervous system, Clin. Chim. Acta 163 (1987) 319–328.

[26] H. Reiber, Ressel Chr, A. Spreer, Diagnosis of Neuroborreliosis-improved knowledge base for qualified antibody analysis and cerebrospinal fluid data pattern related interpretations, Neurol. Psychiatry Brain Res. 19 (2013) 159–169.

[27] W. Albaum, FLC- K Statistics and Graphic Program, Freeware www.albaum.it 7 (2019).

[28] S. Goffette, M. Schluep, H. Henry, T. Duprez, C. Sindic, Detection of oligoclonal free kappa chains in the absence of oligoclonal IgG in the CSF of patients with suspected multiple sclerosis, J. Neurol. Neurosurg. Psychiatry 75 (2004) 308–310.

[29] B. Kaplan, B.M. Aizenbud, S. Golderman, R. Yaskariev, B.A. Sela, Free light chain monomers in the diagnosis of multiple sclerosis, J. Neuroimmunol. 229 (2010) 263–271.

[30] A. Huss, A. Abdelhak, Halbgebauer St, B. Mayer, M. Senel, M. Otto, H. Tumani, Intrathecal immunoglobulin M production: a promising high-risk marker in clinically isolated syndrome patients, Anal. Neurol. 83 (2018) 1032–1036.

[31] H. Reiber, H. Kruse-Sauter, C. Quentin, Antibody patterns vary arbitrarily between cerebrospinal fluid and aqueous humor of the individual multiple sclerosis patient: specificity-independent pathological B cell function, J. Neuroimmunol. 278 (2015) 247–254.
[32] H. Reiber, Polyspecific antibodies without persisting antigen in multiple sclerosis, neurolupus and Guillain-Barré syndrome: immune network connectivity in chronic diseases, Arq. Neuropsiquiatr. 75 (8) (2017) 580–588, https://doi.org/10.1590/0004-282X20170081.
[33] D. Zeman, Free light chains in the cerebrospinal fluid. Do we still need oligoclonal IgG? (Editorial), Clin. Chem. Lab. Med. 56 (2018) 1011–1014.
[34] H. Reiber, Chronic diseases with delayed onset after vaccinations and infections: a complex systems approach to pathology and therapy, J. Arch. Mil. Med. 5 (3) (2017) e12285, , https://doi.org/10.5812/jamm.12285.