Kappa Free Light Chains in Cerebrospinal Fluid as Markers of Intrathecal Immunoglobulin Synthesis

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Background: Intrathecal immunoglobulin synthesis is observed in several inflammatory disorders of the central nervous system, but its detection by current laboratory tests is either tedious or relatively insensitive. We assessed the diagnostic accuracy of an assay for κ free light chains (κFLC) in cerebrospinal fluid (CSF) and serum, and compared it with traditional tests for intrathecal immunoglobulin synthesis.

Methods: κFLCs were measured by nephelometry in CSF/serum pairs from 112 patients. Samples were excluded if artificial blood contamination of CSF (n = 12) or monoclonal bands in both CSF and serum (n = 5) were present. The remaining sample pairs were grouped according to the presence (n = 71) or absence (n = 24) of oligoclonal bands. Data were analyzed as κFLC concentrations in CSF, as κFLC CSF/serum ratios, and by use of the quotient diagram described previously for immunoglobulins.

Results: Both κFLC concentrations in CSF and the κFLC CSF/serum ratio identified patients with oligoclonal bands with high specificity and sensitivity. The areas under the ROC curves were 0.991 (95% confidence interval, 0.944 – 0.998) and 0.978 (0.924 – 0.996), respectively. Exclusion of patients with impaired blood–CSF barrier function further improved diagnostic accuracy. To account for patients with impaired blood–CSF barrier function, data were also analyzed in a quotient diagram. Only two patients without detectable oligoclonal bands would have been misclassified by this approach.

Conclusions: Our data indicate that the nephelometric assay for κFLCs in CSF reliably detects intrathecal immunoglobulin synthesis. This automated and quantitative method could simplify the diagnostic procedure for CSF analysis in the routine laboratory.

Intrathecal immunoglobulin synthesis is commonly observed in inflammatory disorders of the central nervous system (CNS)3 of either infectious or autoimmune origin (1), and has been shown to be of high diagnostic value. Because small amounts of immunoglobulins typically enter the cerebrospinal fluid (CSF) by passive transfer across the blood–CSF barrier, differentiation of the origin of immunoglobulins in CSF is needed before intrathecal immunoglobulin synthesis can be diagnosed (2). This can be achieved either by calculation of the CSF/serum ratios of immunoglobulins compared with the CSF/serum ratio of albumin, which is not synthesized intrathecally. For both approaches, numerous protocols have been devised. In general, CSF/serum ratios and quotient diagrams are not sufficiently sensitive to reliably differentiate intrathecal immunoglobulin synthesis from passive transfer across the blood–CSF barrier, and the analysis of OCBs is time-consuming, not quantitative, and subject to investigator bias (4).

In theory, the determination of free light chains (FLCs) might be a sensitive alternative to the above-mentioned approaches. Immunoglobulin light chains are typically secreted together with intact immunoglobulins by plasma cells. Although the half-life of κFLCs in serum is very short (2–4 h) because of rapid renal elimination, this clearance pathway is not available from CSF; therefore, one can assume that the half-life of κFLCs in CSF is comparable to that of other proteins. Thus, even small amounts of intrathecal immunoglobulin synthesis with concomitant κFLC secretion will disproportionally increase the CSF concentration of κFLCs, making them a

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3 Nonstandard abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; OCB, oligoclonal band; FLC, free light chain; and MS, multiple sclerosis.
potentially sensitive marker of intrathecal immunoglobulin synthesis. Attempts to determine FLCs in CSF have been made previously (5, 6). The methods described to date for the detection or quantification of FLCs are laborious and time-consuming; however, therefore, they have never be incorporated into routine CSF analysis. Recently, an automated nephelometric assay for the detection of monoclonal gammopathy based on specific polyclonal antibodies against epitopes of FLCs that are hidden in intact immunoglobulins was introduced (7). We applied this test to CSF/serum pairs and evaluated its diagnostic accuracy in the detection of intrathecal immunoglobulin synthesis in comparison with the determination of OCBs, which is considered to be the most sensitive procedure and was taken as the reference standard for our study.

**Patients and Methods**

**Patients**

CSF/serum pairs from 112 patients who had been routinely investigated for intrathecal immunoglobulin synthesis were analyzed retrospectively for κFLCs. All patients had undergone an elective spinal puncture in the Mainz University Hospital between August 2002 and January 2004; a request for OCBs had been submitted, and sufficient surplus CSF and serum were available for additional tests. Patients were divided into two groups. Group 1 (n = 24) had no detectable intrathecal fraction and no OCBs; group 2 (n = 71) had positive OCBs. Forty-nine patients in group 2 had a diagnosis of multiple sclerosis (MS) according to the McDonald criteria (8) (group 2A), whereas 22 patients had other neurologic diseases (group 2B). CSF samples from 12 patients were excluded because of artificial blood contamination. Of these patients, nine have been assigned to group 1 (no OCBs; no detectable intrathecal fraction) and three to group 2 (positive OCBs). Another five patients were excluded because they had monoclonal bands in both the CSF and serum. The characteristics of the patients are shown in Table 1. The anonymous use of surplus patient material is approved by the local ethics committee, and informed consent was obtained from the patients.

**Methods**

Immunoglobulin and albumin concentrations were measured by nephelometry (BN II; Dade Behring) in fresh CSF and serum samples. Intrathecal production of immunoglobulin was assessed according to a quotient diagram described previously (2).

OCBs were determined by immunofixation-peroxidase (Hydragel 6 CSF; Sebia) on a semiautomated agarose electrophoresis system (Sebia Hydrasys LC). This method performs comparably to other analytical methods such as immunofixation electrophoresis, high-resolution agarose gel electrophoresis (9), and isoelectric focusing (10). The system can detect OCBs in a concentration range of 30–125 μg/L. Analysis was performed by laboratory technicians, and the results were read by an experienced staff physician (B.A. or J.L.) and given as positive or negative. After analysis of immunoglobulin concentrations and OCBs, samples were aliquoted and stored at −20 °C until further use.

Nephelometric measurement of κFLCs was performed with the Freelite™ Human Kappa Free Kit (The Binding Site Ltd.). Briefly, a monospecific antibody against κFLCs was coated on polystyrene latex. Antibodies were generated from sheep immunized with FLCs, and subsequent antisera were adsorbed extensively against intact immunoglobulins until they were specific for FLCs (7). FLCs in CSF and serum samples were measured according to the manufacturer’s protocol on the BN II automated analyzer. According to the manufacturer, the lower detection limit for κFLCs is 0.06 mg/L. The intraassay CV (10 replicate determinations of a serum-based control) was 4.0% at 0.54 mg/L. The interassay CV (10 determinations on separate days of the same control material) was 14% at 0.55 mg/L. Calibrators and controls were provided by the manufacturer and consisted of stabilized human sera containing polyclonal κFLCs; calibrators and controls were diluted to the appropriate concentrations for serum and CSF determinations.

Statistical analysis of diagnostic accuracy, including confidence intervals, was done with MedCalc 4.31.010.

**Results**

**Characteristics of Patient Groups**

The demographic and clinical characteristics of the 95 patients who were reassessed for κFLCs are shown in Table 1. Although the groups were not specifically matched, there were no major differences in age and sex distribution between the groups. The median albumin ratio was higher in group 2B (7.1 × 10^-3) than in group 1 (5.3 × 10^-3) and group 2A (4.8 × 10^-3), consistent with impaired blood–CSF barrier function as expected for this group, which included patients with infectious etiologies of intrathecal inflammation. The higher relationship between IgA/IgM and combined positivity in quotient diagrams in group 2B reflects the heterogeneity of neurologic diagnoses in this group.

**Detection of κFLCs**

In 33% of group 1 patients, the κFLC concentration in CSF was at or below the lower detection limit of 0.06 mg/L. The median CSF concentration of κFLCs in group 1 was 0.09 mg/L (range, 0.06–0.47 mg/L). Four patients had κFLC concentrations in CSF >0.3 mg/L: a 74-year-old man with rectal carcinoma metastatic to the brain (0.47 mg/L); a 44-year-old man with cerebral infarction (0.41 mg/L); and two women, 66 and 41 years of age, respectively, with carcinomatous meningitis in the course of breast cancer (0.38 and 0.31 mg/L, respectively). These patients also had relatively high κFLC CSF/serum ratios, ranging from 40 to 83 × 10^-3, and mild to moderate impairment of the blood–CSF barrier with albumin ratios...
ranging from 7.9 to $18.6 \times 10^{-3}$. CSF/serum ratios were calculated for all patients in group 1 and ranged from 5 to $83 \times 10^{-3}$, but it should be mentioned that these ratios are an overestimation for the cases with $\kappa$FLCs in CSF below the lower detection limit (see above).

$\kappa$FLCs were detectable in groups 2A and 2B, with medians of 2.7 mg/L (range, 0.29–27.1 mg/L) and 3.6 mg/L (range, 0.43–28.3 mg/L), respectively. The upper outlier in group 2B was a patient with tuberculous meningitis and strongly impaired blood–CSF barrier function ($Q_{alb} = 32.5 \times 10^{-3}$) and a $\kappa$FLC concentration of 28.3 mg/L. The CSF/serum ratio for $\kappa$FLCs was 1.14 for this patient. In contrast, $Q_{alb}$ was within the reference interval for all three upper outliers of group 2A. All three had $\kappa$FLC CSF/serum ratios $\geq 1.2$. Three patients in group 2A and one patient in group 2B had $\kappa$FLC concentrations in CSF $<0.5$ mg/L, which is above the highest value in group 1 (Fig. 1). When patients with impaired blood–CSF barrier ($Q_{alb} > 7 \times 10^{-3}$) were removed from the analysis, there was no overlap between $\kappa$FLC concentrations in groups 1 and 2. This indicates that determination of $\kappa$FLC concentrations in CSF provides information similar to that provided by measurement of OCBs provided that patients with impaired blood–CSF barrier function are excluded.

ROC analysis was performed for $\kappa$FLC concentration in CSF and for $\kappa$FLC CSF/serum quotients. The presence of OCBs was taken as the reference for intrathecal immunoglobulin synthesis. The areas under the curves were 0.991 (95% confidence interval, 0.944–0.998) and 0.978 (0.924–0.996), respectively (Fig. 2). As expected, removal of patients with disturbance of the blood–CSF barrier, as indicated by $Q_{alb} > 7 \times 10^{-3}$, from the analysis increased the area under the ROC curve.

### Table 1. Characteristics of the patient groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 24)</th>
<th>Group 2 (n = 71)</th>
<th>Group 2A (n = 49)</th>
<th>Group 2B (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M), n</td>
<td>16/8</td>
<td>34/15</td>
<td>12/10</td>
<td></td>
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<tr>
<td>Mean (minimum–maximum) age, years</td>
<td>46 (17–82)</td>
<td>38 (13–60)</td>
<td>45 (21–77)</td>
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<tr>
<td>Cerebrovascular disease/bleeding, n</td>
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<td>0</td>
<td>0</td>
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<td>Meningitis, encephalitis, n</td>
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<td>0</td>
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<td>Carcinomatous meningitis/cerebral metastasis, n</td>
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<td>0</td>
<td>0</td>
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<td>Guillain–Barré syndrome, n</td>
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<td>0</td>
<td>4</td>
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<tr>
<td>MS, n</td>
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<td>49</td>
<td>0</td>
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<tr>
<td>OND (not specified), n</td>
<td>14</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range) $Q_{alb}$</td>
<td>5.3 (4.3–7.9)</td>
<td>4.8 (3.4–7.0)</td>
<td>7.1 (4.8–10.0)</td>
<td></td>
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<td>Detectable intrathecal fraction</td>
<td></td>
<td></td>
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<tr>
<td>Positive for IgG only, n</td>
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<td>10</td>
<td></td>
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<tr>
<td>Positive for IgG and/or other immunoglobulins, n</td>
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<tr>
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<td>24</td>
<td>10 (20%)</td>
<td>2 (9%)</td>
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<tr>
<td>OCBs, n</td>
<td>0</td>
<td>49</td>
<td>22</td>
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</tbody>
</table>

" Retrobulbar neuritis/encephalomyelitis with positive OCBs.  
OND, other neurologic diseases.

CSF/serum ratios of $\kappa$FLCs and albumin
Because neither of the above-mentioned criteria takes into account blood–CSF barrier function, CSF/serum ratios of $\kappa$FLCs were plotted against the respective ratios of albumin as is typically done for immunoglobulins. Again, a clear separation of patients with and without intrathecal immunoglobulin synthesis could be achieved with little overlap between group 1 and groups 2A or 2B (Fig. 3).

The hyperbolic function commonly used for the immunoglobulin quotient diagrams:

$$Q_{FLC} = \frac{a}{b \sqrt{(Q_{alb})^2 + b^2}} - c$$
was applied to fit a curve to the kFLC data. To obtain an optimal fit, the following parameters were set empirically: $a/b = 6.5; b^2 \times 10^6 = 5; c \times 10^3 = 1$. With these parameters, two patients from group 1 were classified as having intrathecal kFLC production. One patient from group 1 had Borrelia infection, but no Borrelia-specific antibodies could be detected in CSF. A diagnosis of CNS involvement was therefore discarded. The second patient from group 1 had cerebral infarction with mild impairment of blood–CSF barrier function ($Q_{alb} = 7.88 \times 10^{-3}$). Neither patient had positive OCBs or evidence of intrathecal immunoglobulin synthesis according to analysis of quotient diagrams. Both patients had CSF leukocyte (<5/μL) and erythrocyte (negative) counts within the appropriate reference intervals.

**Discussion**

The use of kFLCs to detect intrathecal immunoglobulin synthesis was reported previously by other groups (5, 11, 12), but the test has not been incorporated into diagnostic use. The determination of free immunoglobulin light chains has been technically difficult in the past and not feasible in routine clinical diagnostics. Here we show that a novel, automated assay for the detection of kFLCs is suitable for routine detection of intrathecal immunoglobulin synthesis.

We reanalyzed 112 CSF/serum pairs from patients who underwent analysis of OCBs in CSF during their diagnostic workup. We assumed that the measurement of OCBs represents the most sensitive procedure to detect intrathecal immunoglobulin synthesis. Thus, any alternative procedure must be evaluated against this “gold standard”. We excluded 17 CSF/serum pairs because of artificial blood contamination or the presence of monoclonal bands. Blood contamination of CSF is known to bias quotient diagrams in favor of intrathecal synthesis. Inclusion of these patients in the study would not have changed the results appreciably (data not shown); all patients would have been classified properly. The number of patients with monoclonal bands was too small to draw conclusions about the diagnostic value of kFLC concentrations in CSF in this group.

The absolute concentration of kFLCs in CSF as well as the CSF/serum ratio of kFLCs identified intrathecal immunoglobulin synthesis with high sensitivity and specificity. Because group 1 did not include patients with severe impairment of blood–CSF barrier function, who might present with increased total kFLC concentrations or CSF/serum ratios, the high diagnostic sensitivity and specificity are limited to patients with normal or almost-normal blood–CSF barrier function.

The use of a quotient diagram plotting CSF/serum ratios of kFLCs vs albumin again provided good separation of patients with and without intrathecal immuno-
globulin synthesis. The separating line between the patient groups was empirical, but the data show that there was a clear separation into two major populations. Thus, with larger numbers of patients with and without intrathecal immunoglobulin synthesis, it should be possible to generate an improved calculation that can be used for differentiation.

Two patients were misclassified according to this procedure (see the Results). One of these suffered from a *Borrelia* infection presumably without intrathecal immunoglobulin synthesis. Although in this case no evidence of CNS involvement was found by conventional methods, it cannot be excluded with absolute certainty that the κFLC result is in fact correct and that the other methods missed intrathecal immunoglobulin synthesis. Only for the sake of clarity did we presume that the presence of OCBs is 100% sensitive for the detection of intrathecal immunoglobulin synthesis. However, this is not undisputed.

The predominance of κFLCs in intrathecal inflammation is widely accepted in the literature (6, 12–14), but the diagnostic use of κFLC quantitation is not yet clear. One group noted that the presence of FLC banding in CSF was highly correlated with the concentration of FLCs in CSF (15). Furthermore, the use of κFLC quantitation to monitor the time course of MS has been documented in the Avonex study (16). These investigators found a positive correlation between κFLC, quantified by ELISA (17), and cerebral lesion volume after 2 years. A previous report from the same group pointed to the predictive value of κFLC concentrations in CSF for the progression of MS, and they even proposed κFLCs as a target for intervention in therapeutic trials (18). The automated quantification of κFLCs in CSF that we describe here could facilitate the use of this marker in the routine laboratory.

In summary, we describe here a novel approach to quantitatively detect intrathecal immunoglobulin synthesis by measurement of κFLCs. This assay is rapid and reliable, reducing tedious manual procedures and uncertainty in result interpretation to a minimum. In addition, it may be useful to monitor the progression of inflammatory neurologic diseases, e.g., MS. One major advantage, in our opinion, is the ready availability of the assay for use on a routine nephelometric analyzer. Thus, daily assays would be easily integrated into laboratory processes. Furthermore, the turnaround time is ~1.5–2 h for this assay, compared with at least 4–5 h for OCB determinations. In addition, the analysis of κFLCs is more sensitive than the determination of intrathecal immunoglobulin synthesis by quotidian diagrams for intact immunoglobulins. The sensitivity of the κFLC assay is similar to that for OCB determinations. Different from OCBs, the κFLC assay is quantitative and provides a means to follow changes in intrathecal immunoglobulin synthesis. Clearly, experience with larger patient numbers is needed to come to a final evaluation of this method for κFLCs, but our data indicate that nephelometric determination of κFLCs in CSF and serum may be a useful diagnostic procedure for detection and potentially for monitoring of intrathecal immunoglobulin synthesis.

The reagents for the determination of κFLCs (Freelite™ Human Kappa Free Kit) were kindly provided by The Binding Site GmbH (Schwetzingen, Germany).

**References**

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