Kari M. Gurtner, Eslam Shosha, Sandra C. Bryant, Bruna D. Andreguetto, David L. Murray, Sean J. Pittock and Maria Alice V. Willrich*

CSF free light chain identification of demyelinating disease: comparison with oligoclonal banding and other CSF indexes

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Abstract

Background: Cerebrospinal fluid (CSF) used in immunoglobulin gamma (IgG) index testing and oligoclonal bands (OCBs) are common laboratory tests used in the diagnosis of multiple sclerosis. The measurement of CSF free light chains (FLC) could pose as an alternative to the labor-intensive isoelectric-focusing (IEF) gels used for OCBs.
Methods: A total of 325 residual paired CSF and serum specimens were obtained after physician-ordered OCB IEF testing. CSF kappa (cKFLC) and lambda FLC (cLFLC), albumin and total IgG were measured. Calculations were performed based on combinations of analytes: CSF sum of kappa and lambda ([cKFLC + cLFLC]), kappa-index (K-index) ([cKFLC/sKFLC]/[CSF albumin/serum albumin]), kappa intrathecal fraction ($KFLC_{IF}$) {([cKFLC/sKFLC]–[0.9358 × CSF albumin/serum albumin]^[0.6687 × sKFLC]/cKFLC)} and IgG-index ([CSF IgG/CSF albumin]/[serum IgG/serum albumin]).
Results: Patients were categorized as: demyelination (n = 67), autoimmunity (n = 53), non-inflammatory (n = 50), inflammation (n = 38), degeneration (n = 28), peripheral neuropathy (n = 24), infection (n = 13), cancer (n = 11), neuromyelitis optica (n = 10) and others (n = 31). cKFLC measurement used alone at a cutoff of 0.0611 mg/dL showed >90% agreement to OCBs, similar or better performance than all other calculations, reducing the number of analytes and variables. When cases of demyelinating disease were reviewed, cKFLC measurements showed 86% clinical sensitivity/77% specificity.
Conclusions: cKFLC alone demonstrates comparable performance to OCBs along with increased sensitivity for demyelinating diseases. Replacing OCB with cKFLC would alleviate the need for serum and CSF IgG and albumin and calculated conversions. cKFLC can overcome challenges associated with performance, interpretation, and cost of traditional OCBs, reducing costs and maintaining sensitivity and specificity supporting MS diagnosis.

Keywords: cerebrospinal fluid; free light chains; multiple sclerosis; nephelometry; oligoclonal banding.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease mainly characterized by demyelination and axonal loss. A formal diagnosis of MS is based on clinical and radiological dissemination in space (DIS) and time (DIT), with an increasing role of MRI examinations as established in the 2010 revision of the McDonald diagnostic criteria [1].

Cerebrospinal fluid (CSF) analysis, in particular detection of intrathecal immunoglobulin gamma (IgG) oligoclonal bands (OCBs), is no longer a required criterion for a diagnosis of the more common relapsing–remitting form of MS (RRMS). It does, however, continue to be a major criterion in the primary-progressive form of MS (PPMS) [2]. When OCBs are absent, clinicians are cautious of making a diagnosis of MS, compelling an alternative diagnosis to be considered. Although serologic requirements for CSF have lessened in terms of MS diagnosis, CSF analysis has prognostic implications in patients with clinically isolated syndrome (CIS). When intrathecal OCBs were detected, CIS patients were twice as likely to convert to MS than OCB-negative individuals, and they did so in a shorter time period [3]. Furthermore, having an absence of brain lesions and presence of OCBs had a higher conversion rate to MS (60%) versus similar patients without OCBs (21%).

***Corresponding author: Maria Alice V. Willrich,** PhD, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN, 55905, USA, Phone: +507-284-2511, E-mail: willrich.mariaalice@mayo.edu
Kari M. Gurtner and David L. Murray: Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
Eslam Shosha and Sean J. Pittock: Department of Neurology, Mayo Clinic, Rochester, MN, USA
Sandra C. Bryant: Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA
Bruna D. Andreguetto: University of Campinas, Sao Paulo, Brazil

When evaluating CSF in radiologically isolated syndrome (RIS), it was discovered that 82% of patients exhibited OCBs [4]. These observations play an important role in the significance of each demyelinating episode and their subsequent diagnosis.

In addition to MS, several other neurologic diseases generate CSF-specific OCBs as a humoral immune response [5–8]. Furthermore, OCBs can also be found in up to 8% of healthy subjects [9]. Although these conditions produce CSF-specific OCBs, analytical testing is ordered to assist the clinical guidelines that define MS disease diagnosis [8, 10–13].

Currently, due to its high sensitivity, isoelectric focusing (IEF) coupled with IgG-specific immunoblotting is the preferred technique in detecting OCBs. This manual technique requires paired CSF and serum specimens to be run in parallel, with a subjective visual interpretation. The multistep method is labor intensive and costly, with an average time for analytical processing of over 3 h. There is no standard definition of OCB amounts required for a clinically positive result. Despite the FDA approval of IEF testing, package inserts suggest establishing an individual laboratory reference interval within its own population. With differing approaches by varied institutions, positivity can be characterized by anything from 1 to 4 unique CSF bands [7], which significantly affects sensitivity and specificity of the assay.

In conjunction with OCBs, IgG-index is commonly offered as a diagnostic gauge of relative CSF IgG amounts compared to IgG present in serum. Increased IgG production in the central nervous system is reflected as an increase in IgG-index values and considered to be an indicator of inflammatory disease.

Over the past few decades, numerous studies have established immunoglobulin free light chain (FLC) presence in CSF as a beneficial biomarker used to correlate MS diagnosis, using either ELISA or nephelometry [6, 14–17]. Light chains are produced by plasma cells in molar excess to heavy chains [18, 19]. The excess amount of FLC is rapidly secreted and then cleared by the kidneys as FLC have a shorter serum half-life (hours) than intact immunoglobulins (days) [20]. Quantitative FLC assays use antisera directed against epitopes that are exposed only when the light chains are free (unbound to heavy chain) in solution. Nephelometric measurement of CSF kappa FLC (cKFLC) and CSF lambda FLC (cLFLC) demonstrated that concentration of cKFLC alone provides a higher degree of sensitivity and specificity in MS diagnosis as compared to traditional OCB detection [6]. To expand this comparison, prediction of intrathecal IgG FLC synthesis employing various formulas, including Reiber's formula for blood-CSF barrier function [21], as a representation of the humoral immune response produced similar improvements on sensitivity and specificity for MS diagnosis [22–25].

In this study, we proposed replacing OCB IEF testing with a more objective, less costly assay, such as measurement of CSF FLC by nephelometry. Replacing OCB IEF testing would be advantageous for low and high complexity laboratories. Both FLC presence and various published calculations/indexes that suggest representation of humoral immune response were calculated in patients having neurological disorders and physician-ordered OCB IEF testing.

Materials and methods

Clinical specimens

Paired CSF and serum samples were characterized between two cohorts. Cohort 1 included residual waste specimens obtained after physician-ordered OCB IEF clinical testing (Institutional Review Board [IRB] protocol 15-000480), n = 278. The second cohort comprised patients clinically characterized with autoimmune and/or inflammatory neurological disorders, n = 47 (IRB protocol 08-007810). Testing was performed on frozen specimens without prior knowledge of clinical diagnosis. Combining the two sources of specimens, a collection of 325 specimens was used for measurement of FLC. Chart review of all 325 patients' medical history was performed by two neurologists blinded to any laboratory test results. Chart review included demographics and diagnosis of each condition at the time of testing. The definition of MS was based on the revised McDonald criteria [1] or according to MAGNIMS 2016 [26].

Additionally, 130 de-identified, residual CSF samples were used for method validation of the CSF matrix, along with commercially available human CSF from healthy donors (Lee Biosciences, Maryland Heights, MO, USA). Specimens were pooled, spiked or diluted with human serum, calibrators or artificial CSF (Tocris, Minneapolis, MN) to obtain measurements across the assay measuring range.

Oligoclonal banding-isoelectric focusing testing

Paired patient CSF and serum specimens were tested according to manufacturer instructions along with the laboratory's standard operating procedure for the IgG-IEF gel assay (SPIFE IgG IEF Kit Cat. no. 3389) using the Helena SPIFE 3000 platform (Helena, Beaumont, TX, USA). Briefly, the IgG-IEF gel separates CSF and serum proteins according to isoelectric point (pI). Proteins on the agarose gel are transferred to a nitrocellulose membrane, which is then immunofixed to observe IgG-specific bands. Because IgG migrates into discrete regions as opposed to one distinct band, qualitative visual interpretation of band patterns is performed by comparing unique oligoclonal band presence in a patient's CSF that is not found in its corresponding serum. Unique CSF bands

are determined by subtracting the number of serum bands from the number of CSF bands, when those bands are exactly aligned on the gel. Serum bands are not subtracted from the CSF bands if they are not exactly aligned. Our institution has validated a specific classification of four unique CSF bands to be considered positive [7]. Banding interpretation is performed by three or more reviewing technologists.

FLC by nephelometry

Paired CSF and serum nephelometric measurements were generated using a Dade Behring BNII nephelometric system (Siemens, Marburg, Germany). Results are determined by the instrument, based on the proportion of light scatter from an antigen/antibody interaction. Both CSF and serum FLC measurements used Freelite® Human Kappa and Freelite® Human Lambda reagent kits (The Binding Site, Birmingham, UK). Serum measurement was performed per manufacturer instructions [27]. The measurement of FLC in CSF required validation of this alternative matrix type, and because CSF protein concentrations are smaller in CSF than serum, alternative dilutions and standard curve concentrations were used.

Method validation for CSF KFLC

cKFLC measurement was validated for within-run and within-laboratory imprecision, carryover, analyte stability, analytical sensitivity and analytical specificity. Briefly, precision estimates were carried out with measurements of 3 levels of calibrator-spiked artificial CSF: low (<0.05 mg/dL) below the analytical cutoff limit medium (0.05–0.10 mg/dL), near the analytical cutoff limit and high (>0.10 mg/dL), above the analytical cutoff limit. Twenty precision measurements were taken within one analytical run (within-run imprecision) in addition to 20 different analytical measurements over the course of 20 days (within-laboratory imprecision). Carryover was addressed to allow for continuous patient testing among other assay methodologies operating on the same instrument platform. Routinely, the BNII cuvettes are washed by the instrument and reused for various reactions throughout the day, including serum samples tested for FLC and serum monoclonal proteins. Briefly, to assess carryover using a random access instrument, 60 CSF specimens were loaded onto new, unused cuvettes. Following completion, 60 pooled residual monoclonal serum specimens (serum kappa FLC [sKFLC] 65 mg/dL) were then measured using those same cuvettes. Finally, the initial 60 individual CSF specimens were measured for cKFLC a second time, immediately following the monoclonal sKFLC specimens. cKFLC results were categorized as undetectable (<0.0060 mg/dL), negative (0.0060–0.0875 mg/dL) or positive (>0.0875 mg/dL) for comparison. Percent difference from original results was calculated. Error limit (EL) was established as acceptable if within 3 SD of the initial cKFLC measurement. Analyte stability was evaluated at five different time points over a 28-day period at ambient, refrigerated (4–8 °C) and frozen (–20 °C) temperatures, in addition to three freeze-thaw cycles. Analytical sensitivity studies included determination of limit of quantitation (LOQ) and analytical measuring range. Analytical specificity investigated potential interferences of hemolysis and bilirubin in CSF, as well as monoclonal proteins present in serum.

Other nephelometric measurements

Albumin (Siemens) and total IgG quantitation (Siemens) was performed using standard operating procedures employing nephelometry (Dade Behring BNII, Siemens) and used to generate a selection of calculations under consideration.

Calculations

IgG-index was calculated based on values obtained from both CSF and serum IgG and albumin:

$$\text{IgG-index} = \frac{(\text{CSF IgG} / \text{CSF Albumin})}{(\text{Serum IgG} / \text{Serum Albumin})}$$

The absolute measurements of both cKFLC and cLFLC were summed to create a total (ΣFLC):

$$\Sigma\text{FLC} = \text{cKFLC} + \text{cLFLC}$$

Additionally, previously published calculations were compared. Kappa-index (K-index), defined as a linear formula intended to represent intrathecal inflammation [24], is described below.

$$\text{K-index} = \frac{(\text{cKFLC} / \text{sKFLC})}{(\text{CSF Albumin} / \text{Serum Albumin})}$$

Kappa intrathecal fraction, or kappa synthesis ($KFLC_{IF}$) [22], combines the KFLC quotient (Q_{KFLC}) to the albumin quotient (Q_{Alb}) [25]. The formula takes into account the change in intrathecally synthesized KFLC concentration ($KFLC_{Loc}$), utilizing the Q_{KFLC} and the Q_{Alb}-dependent upper normal limit ($KFLC_{Lim}$) and displaying the final relative intrathecal fraction as a percentage. This has been reported as a non-linear calculation with comparable sensitivity and specificity as OCBs in the diagnosis of MS, but superior sensitivity when compared to OCBs in the diagnosis of CIS [22].

$$Q_{KFLC} = \frac{\text{cKFLC}}{\text{sKFLC}}$$

$$Q_{Alb} = \frac{\text{CSF Albumin}}{\text{Serum Albumin}}$$

$$KFLC_{Lim} = 0.9358 \times QAlb^{0.6687}$$

$$KFLC_{Loc} = (Q_{KFLC} - KFLC_{Lim}) \times \text{sKFLC}$$

$$KFLC_{IF} = (KFLC_{LOC} / \text{cKFLC}) \times 100.$$

Establishing medical decision points for CSF FLC analysis

FLC positivity, along with clinical sensitivity and specificity of varied cutoffs, was determined using patient clinical diagnosis of demyelination as true positives. From this, a medical decision point was created for cKFLC, cLFLC, ΣFLC, etc., through receiver operating characteristic (ROC) curve analysis. For OCBs, the clinical practice uses four or more bands as the critical value to define disease. For all other measures, the optimal point balancing sensitivity and specificity was calculated using the ROC curve point that minimizes the

distance from perfect sensitivity and specificity. In addition, other points on the ROC curve were investigated for some measures based on maximizing cKFLC and OCB agreement and on common clinical usage. Score confidence intervals were calculated for sensitivity, specificity and overall agreement. Wald confidence intervals are calculated for the AUC. R version 3.2.3 'ROC' function was used for calculating optimal cut points and SAS version 9.4 was used for all other analyses.

Results

Patient demographics

Following neurologist chart review, patients were divided into 10 clinical diagnoses categories (Table 1). The demyelinating disease category included 67 patients with definite MS (PPMS, RRMS or tumefactive MS, TMS) (n=62), CIS (n=3) and RIS (n=2). The remaining consisted of 258 patients with other neurological diseases, in which the diagnosis of MS was excluded. This group included autoimmune/paraneoplastic diseases (n=53) such as N-methyl-D-aspartate receptor autoimmune encephalitis, Parry-Romberg syndrome, stiff person syndrome and limbic encephalitis. Non-inflammatory disorders (n=50) included epilepsy, vascular disease, psychiatric disorders, vitamin deficiencies, headaches and genetic disorders. Inflammatory diseases (n=38) within the cohort ranged from systemic lupus erythematosus, sarcoidosis, myelitis, vasculitis, connective tissue disease and Sjögren's syndrome. Degenerative conditions (n=28) included multiple system atrophy, Lewy-body dementia, Alzheimer's disease, normal pressure hydrocephalus, progressive supranuclear cerebral palsy and mild cognitive impairment. Peripheral nervous system disorders (n=24) were comprised of neuropathies and myopathies. Infections (n=13) included human immunodeficiency virus, progressive multifocal leukoencephalopathy, borreliosis, varicella, and Creutzfeldt–Jakob disease. Cancers (n=11) included lymphoma, glioma and adenocarcinoma. Finally, aquaporin-4 channelopathies/neuromyelitis optica (n=8) and myelin oligodendrocyte glycoprotein (n=2) diagnoses were grouped separately. The remaining diagnoses were miscellaneous (others category, n=31), including spastic paraparesis, walking difficulties and fibromyalgia.

Table 1: Cohort demographics: age, gender and clinical conditions.

Demographics	Results (n=325)
Gender, F, n (%)	174 (54)
Age, years (median and range)	54, 6 months–94 years
Diagnosis	
Demyelinating disease	67
Autoimmune/paraneoplastic	53
Non-inflammatory	50
Inflammatory	38
Others	31
Degenerative	28
Peripheral	24
Infection	13
Cancer	11
NMO/MOG	10

NMO, neuromyelitis optica; MOG, myelin oligodendrocyte glycoprotein.

Validation of the CSF KFLC nephelometric assay

CSF was validated as a matrix for the cKFLC nephelometric assay, which is FDA-approved for serum specimens. Within-run imprecision was <7% CV across the three levels tested. Within-laboratory imprecision was <10% (Supplemental Figure s1). Instrument carryover due to the use of shared cuvettes was not significant, with cKFLC %CV under the established EL for all concentrations tested (Supplemental Table s2).

cKFLC was stable at ambient temperature for 1 day (5% mean difference from baseline), refrigerated 2–8 °C up to 4 days (3%) or frozen at –20 °C for up to 28 days (–5%), and it withstood up to a maximum of two –20 °C freeze/thaw cycles (–3%). The LOQ was established as the concentration with <20% imprecision (measured at 1:1). The lowest reportable value for cKFLC was defined <0.0083 mg/dL [<0.083 mg/L]. The upper LOQ study determined specimens will be reported up to 3.55 mg/dL (measured at 1:100); any specimens above the reportable range will be reported as >3.55 mg/dL (Supplemental Table s3).

Analytical specificity studies showed that hemoglobin spiked between 5 and 100 mg/dL (conversion factor to SI units [mmol/L] is 0.000626) did not significantly impact results (CV <13% from original measurements, n=10 residual CSF samples), nor did bilirubin between 6.25 and 100 mg/dL (conversion factor to SI units [µmol/L] is 17.1) (CV<8%, n=10 residual CSF samples). Therefore, icteric or hemolyzed samples may be accepted for analysis. Additionally, samples will be visually inspected and centrifuged prior to testing as a precautionary measure. Finally, the presence of monoclonal proteins was evaluated by reviewing all serum samples with skewed serum kappa/lambda FLC ratios (outside the established reference interval of 0.26–1.65). In the cohort of 325 subjects

analyzed for OCB IEF, there were 29 patients (9%) with serum FLC ratios outside of the reference interval (Supplemental Table s4). However, out of those, only 5 (17%) had a serum kappa/lambda FLC ratio above 3.0, suggesting a kappa monoclonal gammopathy. No cases of elevated serum FLC ratios had related elevated cKFLC values without cause, in which case either a supporting diagnosis with demyelinating disease or a corresponding positive OCB result was observed with this patient group. In addition, there was no correlation between elevated sKFLC and the presence of oligoclonal bands (Figure 1A), or when classifying the cKFLC into positive and negatives (Figure 1B). Likewise, there was no significant quantitative correlation between sKFLC and cKFLC results (Figure 1C, Spearman's correlation coefficient r = 0.172, 95% CI 0.061–0.279). Spearman's correlation was also applied to subgroups of OCB-positive samples (n = 113, r = 0.206, 95% CI 0.017–0.381) and OCB-negative samples (n = 211, r = 0.429, 95% CI 0.309–0.536). The correlation seems to be higher in OCB-negative samples but still very modest (r < 0.500) because one result would not be predictive of the other, giving the finding limited clinical significance.

Establishing a cutoff for positive CSF KFLC and calculations

Initially, OCB, CSF FLC measurements, IgG-index and novel formulas involving KFLC, LFLC, etc., were compared for their utility of diagnosing demyelinating disease (Table 2). Using demyelinating disease as true positives (n = 67 unique subjects) and every other condition as true negatives, the AUC of the ROC curve was calculated for each measurement. Using a cutoff of four unique CSF bands, OCBs showed a sensitivity of 86.6% and specificity of 78%, with a diagnostic odds ratio (OR) of 23.32. Changing the cutoff of OCB unique bands to 2 or 3 increases sensitivity while decreasing specificity, as expected. When a cutoff of 0.0529 mg/dL was used, cKFLC had a sensitivity of 92.5% and specificity of 71.7%, with diagnostic OR of 31.25, without the need for a paired serum sample. A higher cutoff of 0.0875 mg/dL improves the balance between sensitivity and specificity. The combination of OCB and IgG-index as a panel has no improvement on sensitivity and specificity over OCB alone; the same is true if IgG-index was added to a cKFLC panel.

Figure 2 shows the performance of each measure in every group of conditions. Several conditions used in the control group have positive OCB results and, therefore, also had positive results for cKFLC and many of the calculations. An alternative means of comparison

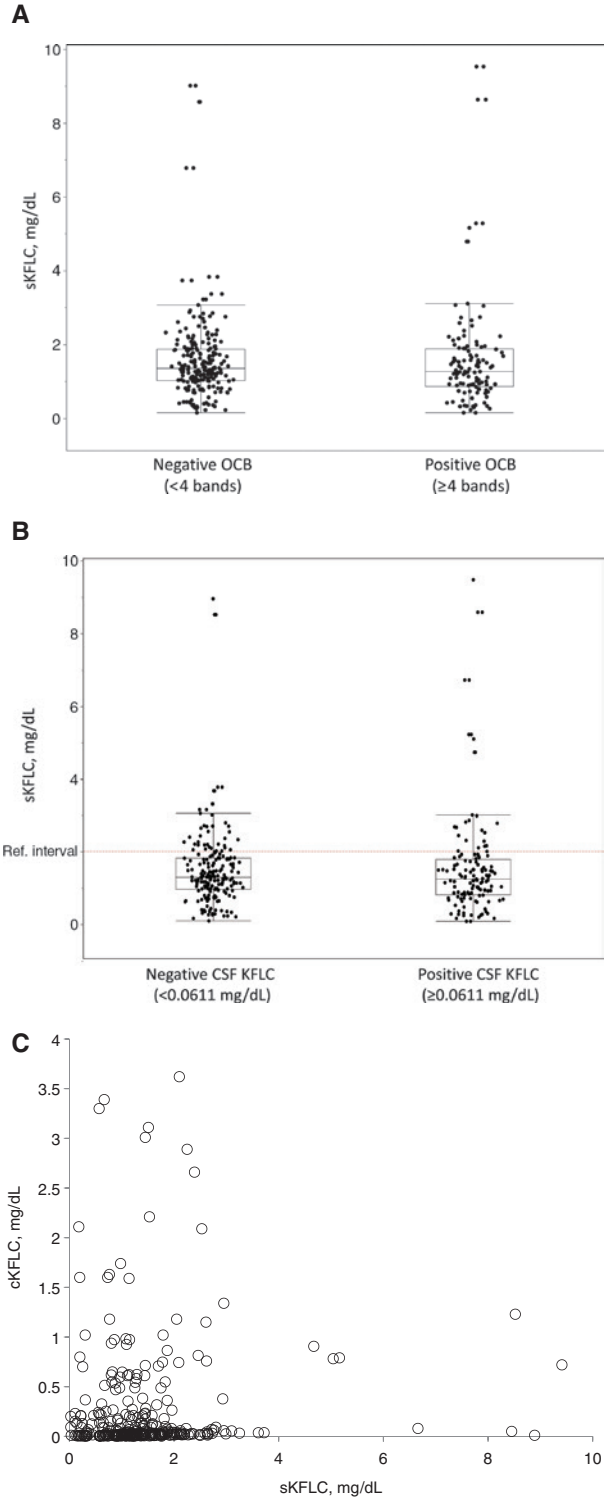

Figure 1: Comparison of serum kappa free light chain (sKFLC) concentrations with (A) presence of oligoclonal bands (*p = 0.3581), (B) presence of elevated CSF kappa free light chains (cKFLC) above cutoff (*p = 0.4250) and (C) cKFLC concentrations ($^{\#}r_s = 0.177$).

*Obtained from Wilcoxon non-parametric test (#) Spearman correlation coefficient. Conversion factor for mg/dL to mg/L, multiply by 10.

Table 2: Diagnostic utility of OCB and free light chain (FLC) measures for diagnosing demyelinating diseases.

Variable (positive test)	n	AUC	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Agreement (95% CI)	LR+	LR−	Diagnostic OR
OCB (≥4 bands)	325	0.854 (0.807, 0.900)	86.6 (76.4, 92.8)	78.3 (72.9, 82.9)	50.9	95.7	80.0 (75.3, 84.0)	3.99	0.17	23.32
OCB (≥3 bands)	325	0.854 (0.807, 0.900)	88.1 (78.2, 93.9)	76.7 (71.2, 81.4)	49.6	96.1	79.1 (74.3, 83.1)	3.78	0.16	24.37
OCB (≥2 bands)	325	0.854 (0.807, 0.900)	94 (85.6, 97.6)	72.5 (66.8, 77.6)	47	97.9	76.9 (72.0, 81.2)	3.42	0.08	41.30
cKFLC (≥0.0529 mg/dL)	325	0.840 (0.793, 0.886)	92.5 (83.6, 96.7)	71.7 (65.9, 76.8)	45.9	97.4	76.0 (71.1, 80.3)	3.27	0.10	31.25
cKFLC (≥0.0875 mg/dL)	325	0.840 (0.793, 0.886)	85.1 (74.7, 91.7)	77.1 (71.6, 81.8)	49.1	95.2	78.8 (74.0, 82.9)	3.72	0.19	19.23
cLFLC (≥0.0304 mg/dL)	320	0.746 (0.686, 0.806)	71.2 (59.4, 80.7)	72.4 (66.6, 77.5)	40.2	90.6	72.2 (67.0, 76.8)	2.58	0.40	6.49
ΣFLC (≥0.1067 mg/dL)	320	0.826 (0.778, 0.873)	87.9 (77.9, 93.7)	72.8 (67.0, 77.9)	45.7	95.9	75.9 (71.0, 80.3)	3.23	0.17	19.44
K-index (≥10.463)	320	0.864 (0.825, 0.902)	86.6 (76.4, 92.8)	76.3 (70.7, 81.1)	49.2	95.5	78.4 (73.6, 82.6)	3.65	0.18	20.81
$KFLC_{IF}$ (≥0%)	320	0.826 (0.780, 0.872)	95.5 (87.6, 98.5)	68.0 (62.0, 73.4)	44.1	98.3	73.8 (68.7, 78.3)	2.98	0.07	45.10
$KFLC_{IF}$ (≥29.91%)	320	0.826 (0.780, 0.872)	89.6 (80.0, 94.9)	74.7 (69.0, 79.7)	48.4	96.4	77.8 (72.9, 82.0)	3.54	0.14	25.44
IgG-index (≥0.611)	319	0.731 (0.656, 0.805)	74.6 (63.0, 83.5)	69.4 (63.5, 74.8)	39.4	91.1	70.5 (65.3, 75.3)	2.44	0.37	6.66
IgG-index (≥0.85)	319	0.731 (0.656, 0.805)	38.8 (28.0, 50.8)	87.7 (83.1, 91.2)	45.6	84.4	77.4 (72.5, 81.7)	3.15	0.70	4.52
IgG-index (≥0.85) or OCB (≥4 bands)	320	0.816 (0.768, 0.865)	86.6 (76.4, 92.8)	76.7 (71.1, 81.5)	49.6	95.6	78.8 (73.9, 82.9)	3.72	0.17	21.27
IgG-index (≥0.85) or cKFLC (≥0.0875 mg/dL)	319	0.802 (0.752, 0.853)	85.1 (74.7, 91.7)	75.4 (69.7, 80.3)	47.9	95.0	77.4 (72.5, 81.7)	3.46	0.20	17.51
IgG-index (≥0.85) or K-index (≥10.463)	318	0.815 (0.767, 0.862)	88.1 (78.2, 93.9)	74.9 (69.2, 79.9)	48.4	95.9	77.7 (72.8, 81.9)	3.51	0.16	22.09
IgG-index (≥0.85) or $KFLC_{IF}$ (≥29.91%)	318	0.822 (0.778, 0.866)	91.0 (81.8, 95.8)	73.3 (67.5, 78.4)	47.7	96.8	77.0 (72.1, 81.3)	3.41	0.12	27.76

OCB, oligoclonal band; cKFLC, CSF kappa FLC; cLFLC, CSF lambda FLC; ΣFLC, sum of both kappa and lambda FLC; K-index, kappa-index; $KFLC_{IF}$, relative intrathecal kappa FLC fraction – Measurements reported in mg/dL [10 mg/L].

was to use a stricter control group as true negatives. In a second comparison of diagnostic utility of all measures, only conditions expected to be negative for OCBs were used for comparison. These included cancer, degenerative, non-inflammatory, others and peripheral categories. This comparison, shown in Table 3, not surprisingly showed an improvement in AUC for all calculations. In this case, cKFLC AUC was the greatest (0.914), and a cutoff of 0.0611 mg/dL yielded 92.5% sensitivity and 86.1% specificity for diagnosis of demyelinating diseases with a diagnostic OR of 76.40; the highest of the entire group of measures.

Overall, each FLC measurement or calculation/index demonstrated ≥90% agreement with OCBs, with the exception of cLFLC and IgG-index, which only demonstrated 86% and 80% agreement, respectively, using the stricter control group. When employing the cutoff of 0.0611 mg/dL, cKFLC measurement used alone showed 93% agreement to OCBs, reducing the number of analytes measured and variables associated with calculations.

cKFLC measurement as a replacement for OCBs would allow the laboratory to decrease technologist bench time from 3+ h to 20 min/specimen, creating an automated setup with reduced turnaround time, subsequently reducing the overall testing-related costs by 75%, significantly simplifying the workflow of the laboratory (Figure 3).

Discussion

The cohort of samples studied included consecutively collected samples in the laboratory, at any stage of disease, along with chart review performed after sample collection and testing. The prevalence of demyelinating disease in the studied cohort was 21%. However, not all subjects' samples were obtained at time of diagnosis; some of the patients might have been undergoing immunosuppressant therapy. This diversity reflects clinical practice and was impossible to avoid.

cKFLC was fully validated for CSF, a matrix traditionally containing 100-fold less protein than serum. cLFLC validation was not pursued because the diagnostic performance was considerably inferior to cKFLC. The validation for cKFLC showed acceptable precision, accuracy, linearity and reportable range, making the assay suitable for CSF testing. Additionally, it was shown the assay performs well on the BNII instrument, with undetectable carryover, a limitation that could have prevented implementation when instruments for analysis of serum

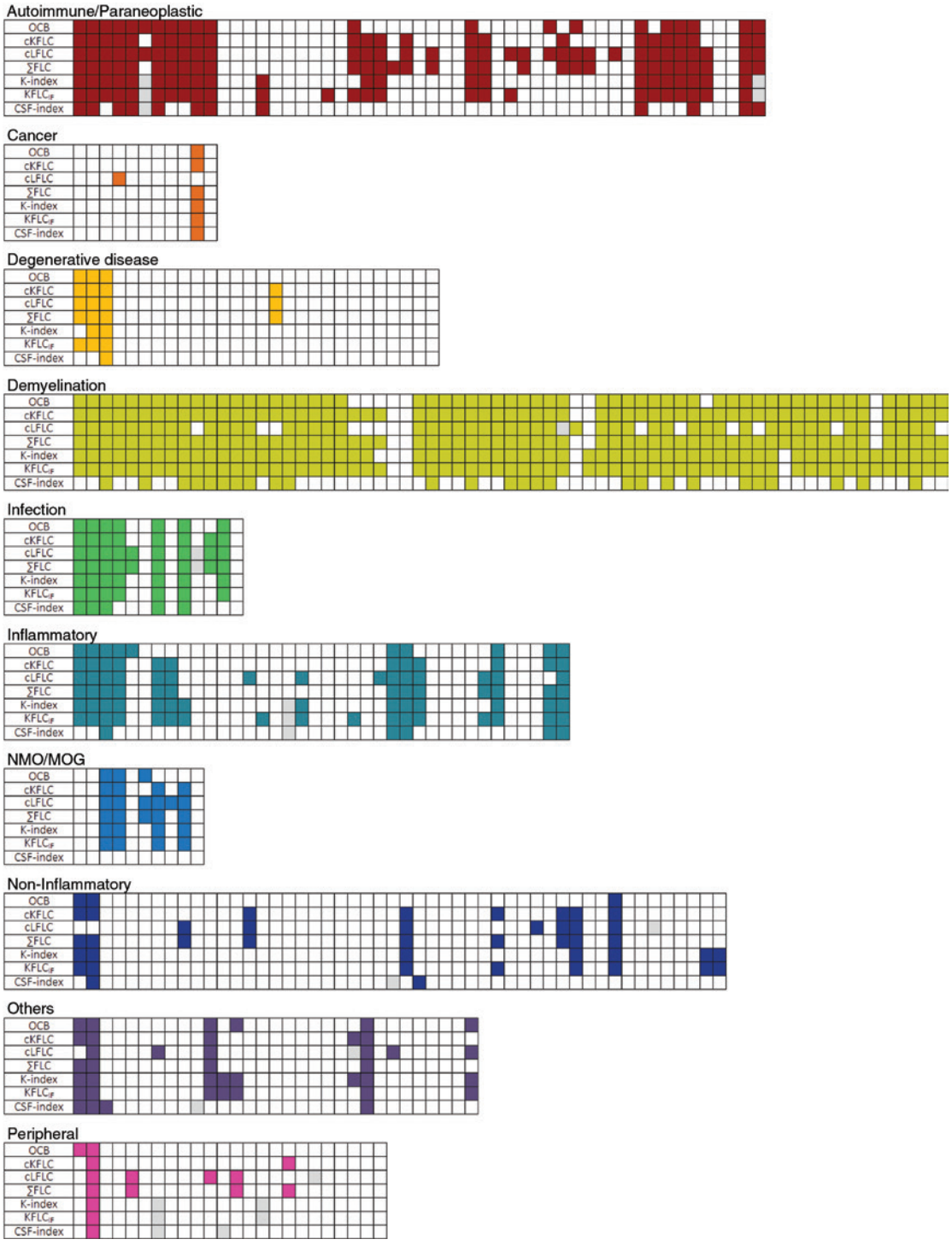

Figure 2: Visual representation of positive results obtained by each CSF measurement or calculation per disease state.
Each square represents a subject. Colored squares represent a positive result for the individual test or calculation: oligoclonal band (OCB), CSF kappa FLC (cKFLC), CSF lambda FLC (cLFLC), sum of both kappa and lambda FLC (ΣFLC), kappa-index (K-index), relative intrathecal kappa FLC fraction (KFLC$_{IF}$) and CSF-index. Squares in gray represent samples not tested.

proteins were shared with the CSF tests. Furthermore, during development, a common question was how to address the presence of monoclonal proteins in serum. Could the CSF elevated cKFLC be a reflex of leakage of sKFLC? The experiments provided here demonstrated there was no difference in sKFLC concentrations between

Table 3: Diagnostic utility of OCB and free light chain (FLC) measures for diagnosing demyelinating diseases.[a]

Variable (positive test)	n	AUC	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Agreement (95% CI)	LR+	LR−	Diagnostic OR
OCB (≥4 bands)	211	0.905 (0.859, 0.950)	86.6 (76.4, 92.8)	89.6 (83.5, 93.6)	79.5	93.5	88.6 (83.6, 92.2)	8.33	0.15	55.68
OCB (≥3 bands)	211	0.905 (0.859, 0.950)	88.1 (78.2, 93.9)	88.2 (81.9, 92.5)	77.6	94.1	88.2 (83.1, 91.8)	7.47	0.13	55.34
OCB (≥2 bands)	211	0.905 (0.859, 0.950)	94.0 (85.6, 97.6)	84.0 (77.1, 89.1)	73.3	96.8	87.2 (82.0, 91.1)	5.87	0.07	82.25
cKFLC (≥0.0529 mg/dL)	211	0.914 (0.870, 0.957)	92.5 (83.6, 96.7)	85.4 (78.7, 90.2)	74.7	96.1	87.7 (82.6, 91.5)	6.34	0.09	72.14
cKFLC (≥0.0611 mg/dL)	211	0.914 (0.870, 0.957)	92.5 (83.6, 96.7)	86.1 (79.5, 90.8)	75.6	96.1	88.2 (83.1, 91.8)	6.65	0.09	76.4
cKFLC (≥0.0875 mg/dL)	211	0.914 (0.870, 0.957)	85.1 (74.7, 91.7)	89.6 (83.5, 93.6)	79.2	92.8	88.2 (83.1, 91.8)	8.18	0.17	49.21
cLFLC (≥0.0244 mg/dL)	207	0.861 (0.806, 0.915)	75.8 (64.2, 84.5)	84.4 (77.5, 89.5)	69.4	88.1	81.6 (75.8, 86.3)	4.86	0.29	16.95
ΣFLC (≥0.0763 mg/dL)	207	0.912 (0.869, 0.955)	92.4 (83.4, 96.7)	84.4 (77.5, 89.5)	73.5	96.0	87.0 (81.7, 90.9)	5.92	0.09	65.78
K-index (≥8.868)	209	0.928 (0.893, 0.964)	88.1 (78.2, 93.9)	88.7 (82.4, 92.9)	78.7	94.0	88.5 (83.5, 92.2)	7.80	0.13	58.11
$KFLC_{IF}$ (≥0%)	209	0.913 (0.872, 0.955)	95.5 (87.6, 98.5)	81.7 (74.5, 87.2)	71.1	97.5	86.1 (80.8, 90.2)	5.22	0.06	94.75
$KFLC_{IF}$ (≥33.99%)	209	0.913 (0.872, 0.955)	88.1 (78.2, 93.9)	88.7 (82.4, 92.9)	78.7	94.0	88.5 (83.5, 92.2)	7.80	0.13	58.11
IgG-index (≥0.611)	207	0.785 (0.709, 0.861)	74.6 (63.0, 83.5)	80.0 (72.6, 85.8)	64.1	86.8	78.3 (72.2, 83.3)	3.73	0.32	11.75
IgG-index (≥0.85)	207	0.785 (0.709, 0.861)	38.8 (28.0, 50.8)	93.6 (88.3, 96.6)	74.3	76.2	75.8 (69.6, 81.2)	6.06	0.65	9.27
IgG-Index (≥0.85) or OCB (≥4 bands)	207	0.872 (0.823, 0.921)	86.6 (76.4, 92.8)	87.9 (81.5, 92.3)	77.3	93.2	87.4 (82.2, 91.3)	7.16	0.15	46.95
IgG-Index (≥0.85) or cKFLC (≥0.0611 mg/dL)	207	0.884 (0.840, 0.928)	92.5 (83.6, 96.7)	84.3 (77.4, 89.4)	73.8	95.9	87.0 (81.7, 90.9)	5.89	0.09	66.22
IgG-Index (≥0.85) or K-index (≥8.868)	206	0.883 (0.837, 0.929)	89.6 (80.0, 94.9)	87.1 (80.5, 91.7)	76.9	94.5	87.9 (82.7, 91.6)	6.95	0.12	58.17
IgG-Index (≥0.85) or $KFLC_{IF}$ (≥33.99%)	206	0.883 (0.837, 0.929)	89.6 (80.0, 94.9)	87.1 (80.5, 91.7)	76.9	94.5	87.9 (82.7, 91.6)	6.95	0.12	58.17

OCB, oligoclonal banding; cKFLC, CSF kappa FLC; cLFLC, CSF lambda FLC; ΣFLC, sum of both kappa and lambda FLC; K-index, kappa-index; $KFLC_{IF}$, relative intrathecal kappa FLC fraction – Measurements reported in mg/dL [10 mg/L]. [a]Controls for this table include 'Cancer', 'Degenerative', 'Non-Inflammatory', 'Others' and 'Peripheral' disease categories.

patients with negative or positive OCBs, nor was there a linear correlation between serum and CSF kappa FLC. In addition, the population tested for OCB did not present concomitantly with complaints related to monoclonal gammopathies. In the 325 records reviewed, there was no registry of a malignant monoclonal gammopathy such as multiple myeloma (serum kappa/lambda FLC ratio >10) or amyloidosis [28]. For the 29 patients with serum FLC ratios outside the reference interval of 0.26–1.65, 24 had FLC ratios between 1.66 and 3.0, an equivocal range usually associated with polyclonal gammaglobulinemia and poor renal function [29]. Five patients had serum FLC ratios greater than 3.0 (but below 10.0) and could be thought to have premalignant conditions such as monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma, although information supporting such diagnoses was not found during chart review. However, out of these five subjects, only one patient had demyelinating disease, with a positive OCB and positive cKFLC. One additional subject with a diagnosis of an inflammatory condition had a negative OCB and a positive cKFLC (0.0854 mg/dL). However, his sKFLC was within reference intervals (0.6610 mg/dL), and his sLFLC was suppressed at 0.2110 mg/dL, skewing the ratio to 3.13. Therefore, the elevated cKFLC result could be due to intrathecal synthesis and in this patient could have been further investigated.

The sample size of this study may be a limitation, although the method is now fully validated and the cKFLC marker shows potential as a replacement for OCBs. The confidence intervals on sensitivity, specificity and agreement with MS diagnosis indicate that extremely large sample sizes would be needed to determine testing benefit (over 1000 samples, data not shown). Rather, incorporating increased efficiency and decreased cost is a key factor in favor of changing the analyte for diagnosing MS.

Over the course of 14 months, our clinical laboratory performed over 75,000 tests to aid in the diagnosis of demyelinating diseases, which included CSF and serum albumin, CSF and serum IgG, and OCB. As shown, the cKFLC measurement alone had superior sensitivity as both K-index and $KFLC_{IF}$ to OCB when diagnosing MS patients. Similar in sensitivity, the ∑FLC requires both cKFLC and cLFLC measurements, further adding to the cost. Data analysis shows cKFLC alone performs equivalently to each formula calculation and OCB, deeming it the most cost effective solution for replacement of OCB. cKFLC alone not only saves the clinical laboratory in cost each year, but it also significantly reduces the pressure on the technologist involved with meticulously reading an

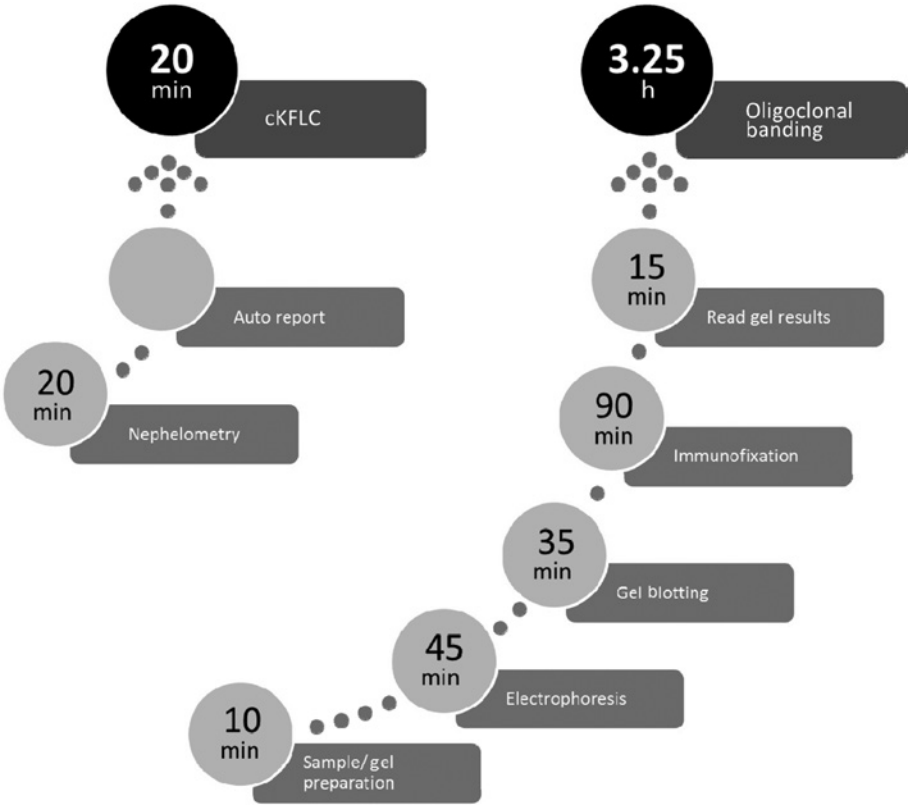

Figure 3: Visual representation of CSF kappa free light chains (cKFLC) vs. oligoclonal banding analytical time to result completion.

OCB pattern and overcomes the challenge of lack of harmonization of number of positive OCBs in the field.

Conclusions

The performance of cKFLC provides an equivalent performance as OCB and demonstrates increased sensitivity for demyelinating diseases. Replacing OCB and IgG-index with cKFLC would alleviate the need for serum IgG and albumin, CSF IgG and albumin, and calculated conversions. This would allow the laboratory to cut spending by reducing technologist time and eliminating expensive IEF kits. Additionally, cKFLC would replace subjective interpretation with quantitative values, overcoming the challenges associated with the performance and interpretation of OCB testing. It is true, however, that this change would be revolutionary. For it to be accepted, laboratorians should work closely with clinicians, and cKFLC should first be incorporated in consensus guidelines as an alternative to OCB.

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