Evaluation of CSF kappa free light chains for the diagnosis of multiple sclerosis (MS): a comparison with oligoclonal bands (OCB) detection via isoelectric focusing (IEF) coupled with immunoblotting

Muhammad Abbas Abid,1 Sibtain Ahmed,2 Siraj Muneer,1 Samia Khan,1 Maria Helena Santos de Oliveira,2 Rizwana Kausar,1 Imran Siddiqui1

ABSTRACT
This study was done to evaluate the diagnostic accuracy of cerebrospinal fluid kappa free light chain (KFLC) for diagnosis of multiple sclerosis, against isoelectrofocusing (IEF) to detect oligoclonal bands (OCB) as gold standard. 64 cases were divided into positive and negative based on the OCB results. Diagnostic accuracy was calculated for the 1 mg/L cut-off. The 1 mg/L cut-off yielded a percent agreement of 86.1% and Cohen’s kappa value of 0.8. Youden’s index, yielded a cut-off of 0.92 mg/L as optimal (90.3% specificity and 90.9% sensitivity). The analytical time was 3 hours and 55 min for IEF and 25 min for KFLC. The cost of a single OCB test was PKR12 000 (US$68.17) compared with PKR4150 (US$23.58) for KFLC. KFLC proved to be an accurate, cheaper and time-saving alternative and can be performed prior to the contemporary testing.

INTRODUCTION
Multiple sclerosis (MS) is a demyelinating disorder of the central nervous system (CNS), which often leads to disability.1 It is the most common disabling neurological disease in young adults, between 20 and 40 years of age.2 The prevalence of MS in Pakistan is estimated to be 10 per 100 000 population.3 Intrathecal immunoglobulin synthesis is commonly found in MS.4 The diagnosis of MS is largely clinical and is supported by diagnostic tests including brain MRI, spinal fluid analysis and neurophysiological testing, with oligoclonal banding (OCB) via isoelectric focusing electrophoresis (IEF) being the gold standard. Establishing the fact that the immunoglobulins are of extensively cerebrospinal fluid (CSF) origin is imperative as systemic immunoglobulins can passively obliterate the blood–brain barrier and hence require to be distinguished from intrathecally incpected immunoglobulins suggestive of CNS pathology.5 However, IEF is a time-intensive and labour-intensive technique requiring subjective/visual interpretation of IgG bands from paired CSF and serum with an average time for analytical processing of over 4 hours.6 This further makes it susceptible to human errors and biases.7 Simultaneous samples of both the CSF and serum are needed, making it inconvenient for the patient as well. There is no standard definition of OCB positivity can be characterised by anything from 1 to 4 unique CSF bands, which significantly affects diagnostic accuracy of the assay (figure 1).8 Kappa free light chain (KFLC) in CSF can provide a quantitative value for diagnosis and can reduce the labour requirements as well as the rater-dependent biases of the traditional diagnostic method. In the clinical laboratory setting, CSF KFLC has a noteworthy logistic advantage as it is an automated quantitative measure, which has further eliminated the need for paired serum analysis.9 Furthermore, it will reduce the turn-around time of results reporting aiding swift patient-centred decision making by clinicians.10 CSF KFLC has a broader utility in cases with a clinically isolated syndrome, which is a clinical episode where patient reports symptoms (eg, optic neuritis) characteristic of inflammation and demyelination of the CNS and warrants evaluation by a neurologist.11 This study aims to validate and determine the diagnostic accuracy of CSF KFLC taking different interpretation approaches OCB via IFE as the gold standard. To the best of our knowledge, and based on a thorough literature review, this will be the first study from Pakistan to evaluate KFLC for MS.12 In this study, we proposed replacing OCB IFE testing with a more objective, less costly assay, such as measurement of CSF KFLC by nephelometry. Replacing OCB IFE testing would be advantageous for low and high complexity laboratories.

METHODOLOGY
The study was carried out at the Section of Chemical Pathology, Department of Pathology & Laboratory Medicine, Aga Khan University (AKU), Karachi, Pakistan.

OCB via IFE is routinely performed at the lab for patients with suspicion of MS or clinically isolated syndrome. Paired patient CSF and serum specimens are collected. The OCBs are detected by IFE on agarose gel using Interlab Diagnostics CSF IFE kit and manual immunoblotting steps to transfer the proteins on transfer membranes. Simultaneous analysis is also conducted on serum samples. The pattern of OCB in CSF is reviewed by two pathologists and their findings are noted as positive or negative for presence of CSF OCB shown in figure 1A,B.
OCB, the 2017 McDonald criterion of two unique bands was used as the primary definition of a positive test result.12

Sixty-four samples were received during June to August 2021 for OCBs via isoelectrofocusing (IEF), requested by physicians as part of clinical care. The aim of this sample selection was to reflect the diversity in the real clinical setting rather than having a focused group. Samples were stratified into two groups: positive (n=33), negative (n=31), as reported in their final OCB results available in the Integrated Laboratory Information Management System.

CSF samples were used for KFLC analysis after the retention policy limits have been saturated and the samples are ready to be discarded (3 days after the day of reporting results). A 0.5 mL CSF sample was analysed for KFLC analysis by nephelometry on Beckman Immage-800 analyzer using kits from Freelite (The Binding Site, Birmingham, UK) according to the manufacturer’s instructions. As CSF protein concentrations are smaller in CSF than serum, alternative dilutions and standard curve concentrations were utilised to validate the alternative matrix type for the measurement of KFLC in CSF. Precision estimates were carried out with measurements of 3 levels of calibrator-spiked artificial CSF: low (<0.5 mg/L) below the analytical cut-off limit medium (0.5–1 mg/L), near the analytical cut-off limit and high (>1 mg/L), above the analytical cut-off limit. Five precision measurements were taken within one analytical run and within-run imprecision was <7% Coefficient of variation (CV) across the levels.

Demographic characteristics of age and sex were compared between the groups according to OCB criteria, using Mann-Whitney’s U test and Fisher’s exact test, respectively. As most of the patients were outside referrals, chart reviews were available for nine cases. KFLC was compared using Mann-Whitney’s U test as well. Using a KFLC cut-off of 1 mg/L to assign patients as positive, the McNemar test was performed to identify significant change in categorisation compared with the OCB criteria. Percent agreement between KFLC (calculated for the 1 mg/L cut-off) and OCB IEF, and Cohen’s Kappa was calculated. Further to identify an optimal cut-off Youden’s index was determined. All statistical analyses were performed using R software (The R Project for Statistical Computing, Vienna, Austria).

**RESULTS**
Out of the 64 samples that were included in the study, 34% were male (n=22). The mean age was 28.6 years (range=2–68 years). Considering the OCB criteria for MS diagnosis, there were no significant differences in age or sex between positive and negative patients. The demographic details are listed in table 1.

A significant difference in KFLC was identified, as positive patients showed significantly higher KFLC compared with negative patients (Median and IQR 5.12 (3.15–5.12) vs 0.31 (0.20, 0.51), p<0.001). The McNemar test did not find significant change in categorisation using either OCB criteria or Kappa >1 mg/L to diagnose patients. A per cent agreement of 89.1% and Cohen’s kappa value of 0.8 between OCB IEF and KFLC was determined (table 2). Using Youden’s index, a slightly lower cut-off of 0.92 mg/L was considered optimal, providing the same specificity as the previous cut-off, but higher sensitivity at 90.9%.

Brain MRI findings were available for the 9 cases with positive OCB results. The MRI results were suggestive of MS. The CSF KFLC results were above the cut-off in all the cases with a concordance of 100%. This further supported the diagnostic utility of KFLC.

The cost of a single OCB test using paired samples is PKR was 7650 (US$41.35), while that of a single KFLC test was PKR4150 (US$22.43). This is a 45.75% decrease in cost of a single test between the two methods. The details of cost difference between the two tests is listed in table 3. The time for the performance of OCB via IFE was 3 hours and 55 min whereas FKLC is available in the Integrated Laboratory Information Management System.

**Table 1 Demographic details**

| Variable | Overall | OCB IEF |
|----------|---------|---------|
|          | Negative | Positive | P value |
| Age (range) | 27.5 (2–68) | 31 (9–68) | 33 (2–52) | 0.213* |
| Sex       |          |         |         |       |
| Male      | 22       | 11 (50%) | 11 (50%) | 1† |
| Female    | 42       | 20 (47.6%) | 22 (52.4%) | |
| KFLC      | 0.99 (0.34–5.12) | 0.31 (0.20–0.51) | 5.12 (3.16–5.12) | <0.001† |

Values are median (IQR) or n (%).

*Mann-Whitney test.
†Fisher’s exact test.
IEF, isoelectrofocusing; KFLC, kappa free light chain; OCB, oligoclonal bands.

**Table 2 KFLC test performance using 1 mg/L as the cut-off**

| KFLC | n | Negative | Positive | P value (McNemar test) |
|------|---|----------|----------|-----------------------|
|      |   |          |          |                       |
| Negative | 31 | 28 (87.5%) | 4 (12.5%) | 1                     |
| Positive  | 33 | 3 (9.4%)  | 29 (90.6%) |                       |
| Agreement (%) | 89.1 |        |         |                       |
| Cohen’s k  | 0.8 |          |          |                       |

IEF, isoelectrofocusing; KFLC, kappa free light chain.
The breakdown of time difference between the two tests is shown in Figure 2.

**DISCUSSION**

MS is one of the most common causes of neurological disability in young adults, second to trauma. The need for prompt and accurate diagnosis of MS is imperative because evidence suggests that early treatment proves to be beneficial in delaying disease progression. OCB coupled with IgG-specific immunoblotting (IgG-IEF) identifies immunoglobulins specific to the CNS. It is part of the diagnostic criteria for MS. However, OCB is a labour-intensive and time-intensive technique that includes manual identification of IgG bands to report negative or positive results. The results of our study show that KFLC, when considered positive at a concentration >1.0 mg/L, has a substantial agreement based Cohen’s kappa. Moreover, the differences between this test and the OCB analysis in relation to diagnostic accuracy are not statistically significant ($p=0.20$) as evident by the comparable performance between the two diagnostic tests with per cent agreement of 89.1%.

The use of KFLC for the diagnosis of MS has been well studied and validated in other regions of the world. Saadeh et al reported a sensitivity and specificity of 68.2% and 86.1% in the retrospective group while 78.6% and 87.1% in the prospective cohort, respectively. In another study, Gurtner et al reported an 86% sensitivity and 77% specificity of KFLC in diagnosis of demyelinating disease. They reported an optimal cut-off of 0.06 mg/dL (6 mg/L). The difference in reported optimal cut-off could be due to inherent differences due to different geographic location, and therefore, it is important to develop population-specific cut-off for KFLC. The results of KFLC may have implications beyond the diagnosis. Rudick et al reported that KFLC in the upper quartile implied rapid progression of disease. Similarly, Rinker et al reported that KFLC of >1.53 µg/mL predicted enhanced disease progression.

Although the literature on the utility of KFLC is present, to our knowledge, no study from the South-Asian region reported the use of KFLC for diagnosing the demyelinating disorder in this population. Hence, making it a first-of-its-kind study from the region.

Apart from similar diagnostic potential, KFLC has several other benefits over the traditional OCB via IEF. KFLC does not require a paired serum sample, making it relatively easier, not only for the patients, but also the laboratories by speeding up workflow. The turnaround time for KFLC test performance in the lab (25 min) is significantly lesser than that for OCB via IEF (3 hours and 55 min). This not only leads to lesser labour and time requirements of the performing technologists, but also results in quicker result reporting and accelerate the process for the prescribing physicians (Figure 2). Third, the cost of KFLC is significantly lesser than that for OCB via IEF. At our setting, the cost of a single KFLC is PKR4150 (US$22.43) against PKR7650

---

**Table 3** Breakdown of cost differential between KFLC and OCB via IEF

| Methods                                      | Cost for material (single patient) | Cost for personnel (single patient) | Total cost (single patient) | Cost per 64 patients (as per study results) | Difference in cost (compared with exclusive IFE) |
|----------------------------------------------|------------------------------------|-------------------------------------|----------------------------|--------------------------------------------|-----------------------------------------------|
| Exclusive IFE                               | PKR7419 (US$40.1) PKR231 (US$1.3)  | PKR7650 (US$41.4) PKR4809 600 (US$2,646.5) | PKR8035 (US$42.8) PKR5064 200 (US$2,731.1) | PKR529 750 (US$2,864) | −45.8%                                           |
| Exclusive KFLC                              | PKR4093 (US$ 22.1) PKR57 (US$0.4)  | PKR4150 (US$22.4) PKR265 600 (US$1,435.7) | PKR269 900 (US$1,438.1) PKR140 700 (US$7,142.4) | PKR265 600 (US$1,435.7) | +54.2%                                           |
| OCB and KFLC for all patients               | PKR11 800 (US$63.8)                 | PKR755 200 (US$4,082.2)              | PKR830 000 (US$4,514.4) | PKR529 750 (US$2,864) PKR265 600 (US$1,435.7) | −30.8%                                           |
| KFLC for all patients and IEF for positives* | PKR529 750 (US$2,864)               | PKR265 600 (US$1,435.7)              | PKR830 000 (US$4,514.4) | PKR529 750 (US$2,864) | −30.8%                                           |

*Using an average positive rate of 15%.

IEF, isoelectrofocusing; KFLC, kappa free light chain; OCB, oligoclonal bands; PKR, Pakistan Rupee.

---

**Figure 2** Breakdown of the time and steps involved in analysis of KFLC (top) and IEF (bottom). CSF, cerebrospinal fluid; IEF, Isoelectric Focusing Electrophoresis; KFLC, kappa free light chain.
Our study was limited by sample size and a lack of clinical information for all subjects as most of the patients were outside referrals. As OCB IEF can be positive in other demyelinating disorders in addition to MS, moreover, owing to lack of healthy controls and true positive cohort the sensitivity and specificity can be suboptimal. A cost analysis of total savings when KFLC is performed prior to OCB was not performed. However, this was a first study from Pakistan reporting highly favourable results for KFLC demonstrating the utility of this test in this population. Further larger and multi-centre studies are required to assess the overall diagnostic and monetary impact of incorporating KFLC in routine practice.

Provided the similar diagnostic potential along with the additional benefits and convenience, KFLC should be advocated as an initial test in undiagnosed patients. This testing is particularly valuable in patients presenting with a clinically isolated syndrome, reporting symptoms such as optic neuritis, characteristic of inflammation and demyelination of CNS. In instances where neurologist has a high suspicion of MS, but the diagnosis is not yet confirmed. It can also be recommended in cases where the imaging findings are inconclusive.

CONCLUSION
Our study demonstrated that KFLC can serve as a cheaper and more convenient alternative to OCB via IEF, without compromising on diagnostic accuracy. Moreover, it simplifies testing being a standardised quantitative test, less error prone and significantly reduced cost and turnaround time. Based on our study incorporation of this test as an initial screen for the evaluation of MS in resource limited set ups like Pakistan, as it will not only be cost effective but would prove beneficial in both clinical and research settings.

Handling editor Tahir S Pillay.
Twitter Sibtain Ahmed @sibtain_7
Contributors SA conceived the study; MAA, SK, SM and RK performed the experiments and collected the data; MHSdO performed the statistical analysis; MAA and SA wrote the original draft; IS assisted with writing the manuscript and critically reviewed the final draft. All authors reviewed and approved the final version of the manuscript.
Funding This study was supported by the Aga Khan University Student and Trainee Initiated Research (STIR) program (MC “88”).
Competing interests None declared.
Patient consent for publication Not applicable.
Ethics approval The study was approved by the institutional ethical review committee of the Aga Khan University (ERC# 2021-6714-19104).

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID ID
Sibtain Ahmed http://orcid.org/0000-0003-0316-2622

REFERENCES
1. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018;17:162–73.
2. Shah Z, Wasay M, Chaudhry BZ, et al. Multiple sclerosis in Pakistan: current status and future perspective. *J Neurol Sci* 2020;418:117066.
3. Wasay M, Ali S, Khatri IA, et al. Multiple sclerosis in Pakistan. *Mult Scler* 2007;13:668–9.
4. Bonnau M. Intrathecal IgG synthesis: a resistant and valuable target for future multiple sclerosis treatments. *Mult Scler Int* 2015;2015:1–15.
5. Wolinsky JS, Group PRS, PROMiSe Study Group. The diagnosis of primary progressive multiple sclerosis. *J Neurol Sci* 2003;206:145–52.
6. Keren DF. Procedures for the evaluation of monoclonal immunoglobulins. *Arch Pathol Lab Med* 1999;123:126–32.
7. Mayo Clinic Laboratories. Immunoglobulin kappa free light chain, spinal fluid. Available: https://www.mayocliniclabs.com/test-catalog/Clinical-and-Interpretive/65372 [Accessed 11 Apr 2022].
8. Link H, Huang Y-M. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. *J Neuroimmunol* 2006;180:17–28.
9. Schwenkenbecher P, Koenen FF, Wurster U, et al. Reiber’s diagram for kappa free light chains: the new standard for assessing intrathecal synthesis? *Diagnotics* 2019;9. doi:10.3390/diagnostics9040194. [Epub ahead of print: 16 11 2019].
10. Crespi I, Sulas MG, Mora R, et al. Combined use of kappa free light chain index and isoelectrofocusing of cerebro-spinal fluid in diagnosing multiple sclerosis: performances and costs. *Clin Lab* 2017;63:551–9.
11. Presslauer S, Milosavljevic D, Huebl W, et al. Validation of kappa free light chains as a diagnostic biomarker in multiple sclerosis and clinically isolated syndrome: a multicenter study. *Mult Scler* 2016;22:502–10.
12. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292–302.
13. Saadeh R, Pittock S, Bryant S. CSF kappa free light chains as a potential quantitative alternative to oligoclonal bands in multiple sclerosis. *Neurology* Apr 2019:92.
14. Dobson R, Giovannoni G. Multiple sclerosis - a review. *Eur J Neurol* 2019;26:27–40.
15. Kavalunas A, Manouchehrinia A, Stawiarz L, et al. Importance of early treatment initiation in the clinical course of multiple sclerosis. *Mult Scler* 2017;23:1233–40.
16. Gurtner KM, Shoxha E, Bryant SC, et al. CSF free light chain identification of demyelinating disease: comparison with oligoclonal banding and other CSF indexes. *Clin Chem Lab Med* 2018;56:1071–80.
17. Rudick RA, Medendorp VN, Namey M, et al. Multiple sclerosis progression in a natural history study: predictive value of cerebrospinal fluid free kappa light chains. *Mult Scler* 1995;1:150–5.
18. Rinker JR, Trinka K, Cross AH. Elevated CSF free kappa light chains correlate with disability progression in multiple sclerosis. *Neurology* 2006;67:1288–90.
19. Awad A, Hemmer B, Hartung H-P, et al. Analyses of cerebrospinal fluid in the diagnosis and monitoring of multiple sclerosis. *J Neuroimmunol* 2010;219:1–7.