Utility of Cerebrospinal Fluid κ Free Light Chains

Authors: Nicholas Armfield, Nicola Williams. Neuroimmunology, The Walton Centre NHS Foundation Trust.
Nicholas.Armfield@nhs.net. Nicola.Williams90@nhs.net.

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that leads to demyelination, axonal damage and neuronal loss. The prevalence of MS is steadily increasing with around three million people affected worldwide in 2020, predominately women [1]. Oligoclonal bands (OCB) are the gold standard for the determination of intrathecal immunoglobulin G synthesis. OCBs were recently included in the McDonald criteria of 2017 to diagnose relapsing MS as a substitute for dissemination in time [2]. OCBs are also found across other neuroinflammatory conditions such as acute disseminated encephalomyelitis or neuromyelitis optica, mainly as a sign of demyelination. OCB testing is based on non-quantitative techniques and demands considerable methodological experience. Measurement of CSF immunoglobulin free light chains (FLC) have been described as a promising biomarker that might replace OCB detection [3]. The Siemens N Latex FLC kappa and Lambda assays are fully automated assays for the quantitative determination of FLC. The current study describes the aforementioned method in comparison to both classical OCB testing and clinical correlations.

Methods

Paired samples of serum and CSF (n=104) were sourced from the Liverpool Neurosciences Biobank at the Walton Centre (Ref. LNBW 21_05) and surplus diagnostic material. The cohorts were split in 2 ways; by OCB band type (T1 T2,3, T4 and T6) and diagnosis. To demonstrate, the cohort included 53 samples with ≥2intrathecal IgG OCBs and 47 patients with MS. All samples were analysed on receipt and stored at ≤-20°C. Methods included CSF leukocyte count via haemocytometer, OCBs via isoelectric focussing [4], serum albumin using a Roche Cobas c311 and CSF albumin, CSF and serum IgG and κFLC via nephelometry using a Siemens BNProSpec. Calculated values included use of a hyperbolic reference Reibergram [5], quotient ratios and index calculations using CSF and serum parameters. Mann Whitney scores (≤0.01 = significant) were employed as each cohort distributed askew. Box-whisker plots were developed to display the distributed data for each OCB type. Receiver operator curves (ROC) were used to described specificity and sensitivity in 2 ways; with or without disease or OCBs. Specificity and sensitivity studies helped derive reference ranges for quotient κFLC and κFLC index.

Results

Comparison against OCBs produced successful results. Intrathecal findings for QκFLC and κFLC index agreed with OCBs as all parameters gave significantly different results between T1, T4 and T2,3 OCB groups (p=≤0.01). Figure 1 displays the data distribution for the quantitative parameters measured in the OCB type cohorts in which those subjects with evidential intrathecal inflammation produced high ranges. Reibergram reference hyperbolic curves agreed with all 100% of positive OCBs, yet only 59% of negative OCBs. Utility of ROCs showed a κFLC index cut-off = ≥31 and a QκFLC cut-off = ≥0.20 agreed with 95% of positive OCBs and 89% negative OCBs (AUROC = 0.97, 0.99 respectively). Clinical correlation utilised ROCs against those with multiple sclerosis (figure 2) as well as demyelinating disease (n=63) and neuroinflammatory conditions (n=67) in which all specificity and AUROCs increased yet sensitivity decreased as conditions became more general.

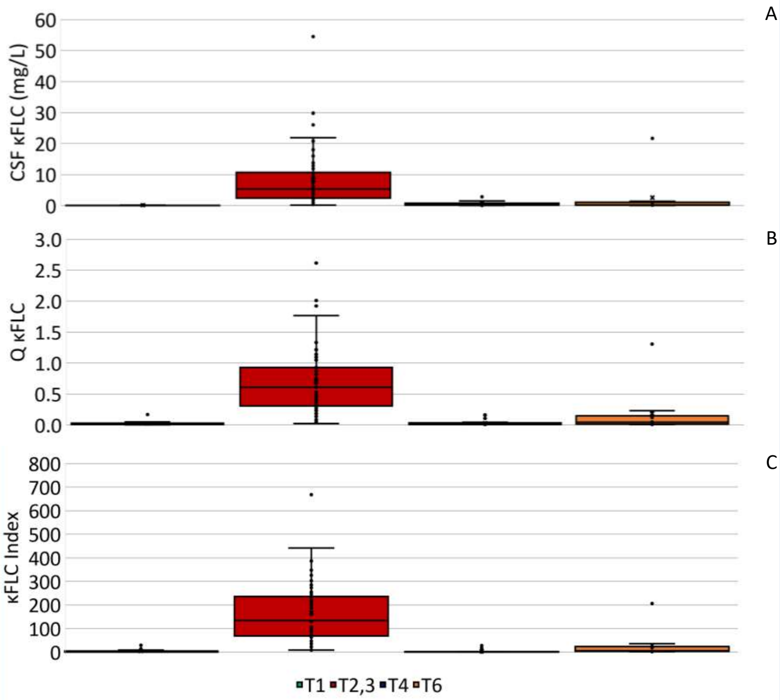
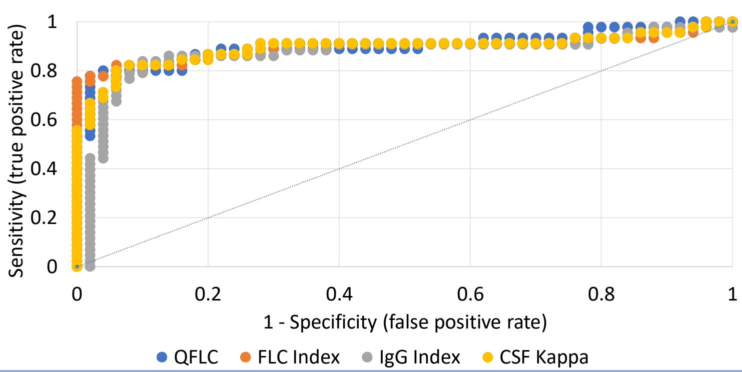


Figure 1. Box-whisker plots for CSF κFLC (mg/L) (A), Q κFLC (B), κFLC Index (C) in OCB type cohorts. Mann-Whitney scores between T2,3 group and all others in A, B and C p=<0.01.



(n=50)	OCB Type	FLC Index	QFLC	CSF Kappa (mg/L) [6]	CSF Index	Reibergram [5]
Cut-off	T2,3	≥31	≥0.2	≥0.1	≥0.7	Above ref. line
True Positive	47	41	41	50	42	50
% True Positive	94	82	82	100	84	100
% True Negative	87	84	84	44	86	56
AUROC	N/A	0.90	0.90	0.89	0.87	N/A

Figure 2. Receiver operator curve for quantitative measurements in neuroinflammatory parameters with associated detail for qualitative results in the Multiple Sclerosis cohort. (n=50).

Discussion

CSF κFLC is empirically demonstrated both in pure measurement and in quotient forms to correlate with an existing, valuable OCB method and final diagnosis. Figure 1 displays subjects with ≥2 intrathecal bands where each κFLC parameter is significantly elevated in comparison to all other OCB types (p=<0.01). Although factoring in serum analytes does not change significant differences between OCB type, it would be judicious to include such measurements when isolating inflammation to intrathecal origin. Therefore, κFLC Index is a developed calculation to propose for clinical practice. Utilising OCB correlations and the literature, 'cut-offs' for the κFLC parameters were developed to associate clinical diagnosis. ROC proved a justified comparative method. Varying to Gaetani et al who proposed a κFLC Index at 10.61 [7] , the proposed higher κFLC Index at ≥31 suited this population and produced an AUROC at 0.9, specificity at 84% and sensitivity at 82% in MS cohorts. This difference may be attributed to the population of the study where most referrals to a tertiary centre have confirmed illness without formal diagnosis. Succinctly, OCB type proved to be the better balance between sensitivity and specificity in all grouped clinical diagnoses 87%/94% (MS n=50) and 84%/100% (demyelinating disease n=63). Reibergrams demonstrated exceptional performance in true positive rates (100% in MS cohort) yet could not balance the negatives with low specificity (56% in MS cohort). This finding is in concordance with other works reporting very high sensitivities through MS [8]. Concerns surround the absence of λFLC in the CSF, where it is hypothesised epitopes targeted by nephelometric methods are monomer only [9], potentially missing a subset of demyelinating disease should CSF FLC be used in common practice. Future works will surround mass separations of FLC in CSF via electrophoresis of HPLC to investigate. Akin to mass separations, a case is made for the use of IEF of κ and λ FLC in paired CSF and serum samples for intrathecal inflammation detection. For now, these findings and associated concerns do not support a move to CSF FLC analysis. It is recommended OCBs remain in situ with a view to use CSF κ nephelopmetric methods as in supportive diagnosis or potentially prognosis.

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