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Association of cerebrospinal fluid kappa free light chains with the intrathecal polyspecific antiviral immune response in multiple sclerosis

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ABSTRACT

The polyspecific B-lymphocyte response to neurotropic viruses such as measles (M), rubella (R) and varicella zoster (Z), known as MRZ reaction, is to-date the most specific neurochemical marker for multiple sclerosis (MS). The aim of this study was to investigate a possible association of immunoglobulin (Ig) kappa (κ-) and lambda (λ-) free light chains (FLC) with the presence of the MRZ reaction in multiple sclerosis.

Immunoglobulin κ- and λ-FLC, MRZ reaction, oligoclonal IgG bands (OCB), and cerebrospinal fluid (CSF) routine parameters were measured in 65 MS patients.

OCB were detected in 97% of MS patients, intrathecal IgG synthesis according to Reiber was detectable in 57%, an elevated IgG index (> 0.7) in 66% and the MRZR was positive in 45%. All investigated κ-values (CSF κFLC, CSF-serum ratio of κFLCs (QκFLC), and κFLC index (κFLC/QAlbumin)) were significantly higher in patients with positive MRZ reaction as compared to MRZ negative MS patients. In contrast, λ-values showed no significant differences.

Additionally to the putative diagnostic sensitivity and prognostic value of κFLC, the association of κFLC with a highly specific neurochemical marker for MS – the MRZ reaction, especially the determination of κFLCs is an informative tool to assess the B-cell response and determine its extent in MS patients.

1. Introduction

Detection of intrathecal B-lymphocyte activation is one of the most important tools to establish the diagnosis of Multiple Sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system (CNS). The diagnostic criteria of MS evolved over the past decades and underwent the latest revisions in 2005 [1], 2010 [2] and recently in 2017 [3]. One main adjustment of the last revision is the inclusion of cerebrospinal fluid (CSF) specific oligoclonal IgG bands (OCB) as an addition to dissemination in space detected by MRI to fulfil the diagnostic criteria of MS [3]. A positive OCB finding indicates chronic inflammation in the CNS and can be detected in approximately 95% of MS patients [4–6]. However, OCB are not specific for MS but are also frequently detected in other autoimmune/inflammatory CNS diseases [7,8]. The determination of OCB requires technical expertise to lead to reliable results [9,10]. In recent years, immunoglobulin kappa (κ) and lambda (λ) free light chains (FLC), both produced by terminal B lymphocytes, have been discussed as quantitative parameters for intrathecal IgG synthesis with similar diagnostic precision for MS as OCB [11–15]. Furthermore, they could serve as a prognostic marker in patients with a first clinical event suggestive of MS [16–18].

As for OCB, κ- and λ-FLC show high sensitivity for MS but are elevated in other inflammatory CNS disorders as well [14]. To evaluate whether κ- and λ-FLC have an added diagnostic value over OCB for MS, its diagnostic specificity needs to be studied. Up to now the most specific neurochemical marker for MS is the so-called MRZ reaction (MRZR), which in more detail is the detection of intrathecally produced IgG antibodies against measles (M), rubella (R) and varicella zoster (Z), whereof at least two out of the three have to be positive [19,20].

As a new aspect in contrast to previous studies which investigated the diagnostic sensitivity of FLC compared to OCB [15,18,21–23], we aimed to investigate a possible association of CSF immunoglobulin κ- and λ-FLC with the presence of the MRZ reaction in multiple sclerosis.

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2. Methods

2.1. Patients

This study included 65 MS patients who were seen at the Department of Neurology of the University of Ulm and fulfilled the revised McDonald criteria 2017 [3]. CSF and serum samples were obtained in parallel for diagnostic purposes and were handled accordingly to the consensus protocol for CSF analysis [24].

2.2. Determination of CSF and serum parameters

CSF leukocyte count (cells/μl) was determined using the Fuchs Rosenthal Counting Chamber. CSF total protein (g/l), CSF lactate (mmol/l), the albumin CSF-serum concentration ratio (QAlb), CSF and serum immunoglobulin G, A and M levels were obtained as previously described [25,26].

Intrathecal IgG synthesis was assessed quantitatively by calculating the IgG index (QIgG/QAlb) and according to IgG synthesis by Reiber [27].

OCB were detected by isoelectric focusing (IEF) on polyacrylamide gels followed by immunoblotting using an IgG-specific antibody staining. Paired CSF and serum samples adjusted for IgG concentrations were applied in the same assay. A more detailed protocol is given in a previous work [28].

MRZR (Measles, Rubella, Zoster antibodies) was determined as previously described [29] using an enzyme-linked immunosorbent assay (ELISA) according to the instructions as supplied by the manufacturer (Genzyme Virotech, Rüsselsheim, Germany). Quantitative expression of the intrathecal immune response was based on the calculation of the CSF/serum quotients (Q) of specific antiviral IgG antibodies, and the intrathecal synthesis of antibodies was detected by calculation of the corresponding antibody indices (AI). AI values ≥ 1.5 were considered to be indicative of intrathecal IgG synthesis against the respective antigen, and MRZR was considered positive if two or more AI values were ≥ 1.5.

Immunoglobulin kappa (κ) and lambda (λ) free light chains (FLC) were measured by nephelometry (Siemens N Latex FLC kappa and lambda assays on Siemens BN ProSpec®) according to the instructions supplied by the manufacturer. We calculated the CSF-serum ratio of κFLC (QκFLC) and λFLC (QλFLC) and determined the κFLC index (QκFLC/QAlb) and λFLC index (QλFLC/QAlb) by correcting for QAlb.

2.3. Statistical analysis

All statistical analyses were performed using Graph Pad Prism 6 (Graph Pad Software Inc., La Jolla, CA, USA). Data sets were tested for normality using D'Agostino & Pearson normality test and accordingly parametric or non-parametric statistical tests were used; i.e. unpaired *t*-test or Mann-Whitney *U* test for the comparison of two groups and one-way ANOVA with Tukey's correction for multiple comparison or Kruskal-Wallis test with Dunn's correction for multiple comparisons for the comparison of three or more groups. A p-value $< .05$ was considered as significant.

2.4. Ethics statement

Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki, and the study was approved by the ethics committee of the University of Ulm (No. 10/20).

3. Results

3.1. Subject description

Demographic data, main CSF characteristics, and both qualitative (OCB) and quantitative (IgG index, intrathecal IgG synthesis, κ- and λ-FLC) parameters of intrathecal B-cell response are shown in Table 1.

Table 1
Patients' characteristics. Numbers show quantity or median, brackets give percentage (%) or interquartile range (25–75%).

	All patients $n = 65$
Age (years)	32 (25–47)
Female (n)	49 (75%)
CSF leukocyte count [/mm^3]	4 [2 − 11]
QAlb [$\times 10^{-3}$]	5.0 [4.0–6.4]
Lactate CSF [mmol/l]	1.6 [1.5–1.8]
QIgG [$\times 10^{-3}$]	4.2 [3.0–6.3]
IgG index	0.79 [0.64–1.10]
Presence of intrathecal IgG synthesis according to Reiber (n)	37 (57%)
Presence of CSF specific OCB (n)	63 (97%)
MRZR positive (n)	29 (45%)
κFLC CSF [mg/l]	2.96 (1.00–7.34)
κFLC serum [mg/l]	10.10 (8.84–12.20)
QκFLC [$\times 10^{-3}$]	348.8 (120.3–679.5)
λFLC CSF [mg/l]	0.67 (0.29–1.66)
λFLC serum [mg/l]	12.90 (10.85–16.35)
QλFLC [$\times 10^{-3}$]	60.34 (27.1–109.6)

OCB were detected in 97% of MS patients, intrathecal IgG synthesis according to Reiber was detectable in 57%, an elevated IgG index (> 0.7) in 66% and the MRZR was positive in 45%.

In MRZR positive MS patients, 100% showed positive OCB, 86% showed elevated IgG index (> 0.7) and 72% showed intrathecal IgG synthesis according to Reiber.

3.2. Comparison of MRZR with CSF immunoglobulin κ- and λ-FLC

MS patients with positive MRZR showed significantly higher QκFLC levels as compared with MRZR negative MS patients ($p < .01$, Fig. 1). In contrast, QλFLC levels showed no significant difference (Fig. 1).

This difference was even more striking for the comparison of κFLC- and λFLC indices (QκFLC/QAlb and QλFLC/QAlb) between MRZR positive and negative patients (Fig. 2, $p = .0009$ and $p = .984$, respectively).

In more detail, all investigated κ-values (CSF κFLC, QκFLCs, and κFLC index) were significantly higher in patients with at least one positive AI (≥ 1.5) compared to patients with no positive AI (Fig. 3). In contrast, no significant differences were seen for this comparison for λ-values (CSF λFLC, QλFLC, and λFLC index) (Fig. 3).

4. Discussion

Chronic inflammation of the CNS is a major hallmark of MS [30] and can be demonstrated by the detection of an intrathecal IgG production. Here, the detection of oligoclonal IgG bands is the gold standard for an intrathecal IgG production, which can be found in approximately 95% of MS patients [4–6]. Furthermore, κ- and λFLC are discussed as putative markers for the detection of intrathecal IgG production, while in particular κFLC performs almost as well as OCB in terms of diagnostic sensitivity [13,18]. The main advantages of FLC determination are the less laborious and complex processing and the quantitative results obtained in contrast to OCB as a qualitative parameter. To our best knowledge in all conducted studies analyzing FLC, their performance concerning diagnostic sensitivity or prognostic value was compared to OCB and other quantitative parameters of intrathecal IgG synthesis [13,16,18,31–34]. However, the to-date most specific neurochemical marker for MS is the so-called MRZ reaction with a diagnostic specificity of up to 97% [19,35,36], whereas the sensitivity is lower compared to OCB or FLC with approximately 60% [19]. Here, we aimed to investigate a possible association of CSF κ- and λFLC with the presence of the MRZ reaction in multiple sclerosis.

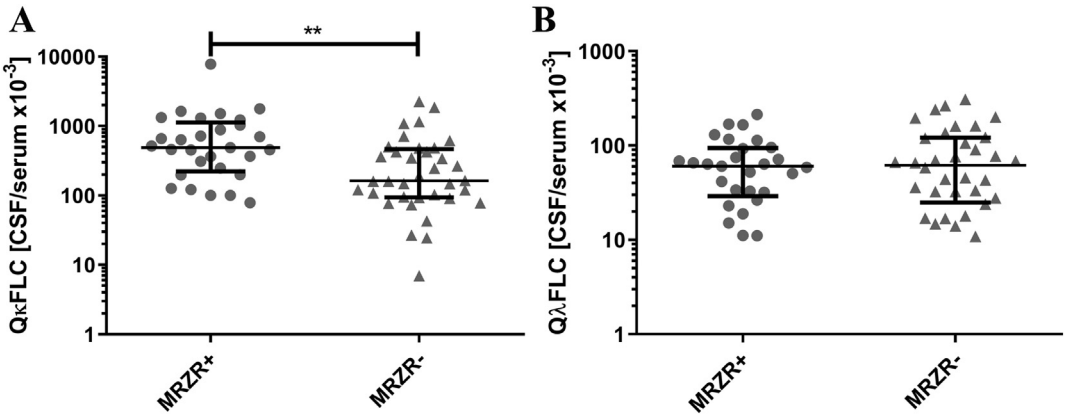

Fig. 1. Comparison of κFLC and λFLC CSF/serum quotient for a positive (2/3 AI of measles (M), rubella (R) and varicella zoster (Z) ≥ 1.5) or negative MRZ reaction. Lines show median and IQR 25–75%. ** = $p < .01$. FLC = free light chain, CSF = cerebrospinal fluid.

In our study population of 65 MS patients, we could observe higher values for all κFLC parameters (CSF κFLC, QκFLC, and κFLC index) for patients with at least one out of three positive AI of MRZ. This finding was even more distinct if the complete MRZR was positive (i.e. 2/3 AI ≥1.5) compared to a negative MRZR (< 2/3 AI ≥1.5). In contrast to κFLC, there were no significant differences for λFLC parameters neither for CSF λFLC, QλFLC nor for λFLC index for patients with a positive MRZR.

Whereas OCB in the CNS are produced by clonally expanded B cells [37–40], which are present in meningeal follicles [41] and the brain parenchyma [42], the MRZR has been suggested to reflect a non-specific bystander activation of B cells or, more recently, to be the result of nonsense activity of immortalized B cell clones and probably not directly involved in the pathogenic process [19,29]. Even though the origin of FLCs is distinct from that of the MRZR, at least κFLC production is associated with the MRZR and therefore both display activation of an intrathecal immune response in MS.

As so far mostly the diagnostic sensitivity of FLC was investigated in large cohorts [15,23], further studies are needed to show the role of FLC in the pathogenesis of MS, possible targeted structures and their predictive value for disease progression, as it was done extensively for OCBs [37,43,44].

5. Conclusion

We conclude that additionally to the putative diagnostic sensitivity and prognostic value of κFLC, the association of κFLC with a highly specific neurochemical marker for MS – the MRZ reaction, especially the determination of κFLCs is an informative and particularly quantitative tool to assess B-cell response in MS patients. The utility of κFLC analysis as a marker for the differentiation of a persistent/chronic B-cell activation and a serological scar should be systematically investigated with regard to its applicability as a therapeutic B-cell activity marker.

Contributors

MS, AH and HT were involved in the conception and design of the study. Data were acquired by AH, FMY, FB, TF, JL, MO, MS and HT. AH, FMY, HT and MS were involved in the statistical methods and analysis. The first draft of the manuscript was designed by AH and MS, followed by a critical revision of all authors. The final version for submission was approved by all authors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

AH has nothing to declare.
FMJ has nothing to declare.
FB has nothing to declare.
TF has nothing to declare.
JL has received honoraria for speaking and travel grants from Bayer, TEVA, CHDI and the Movement Disorders Society.

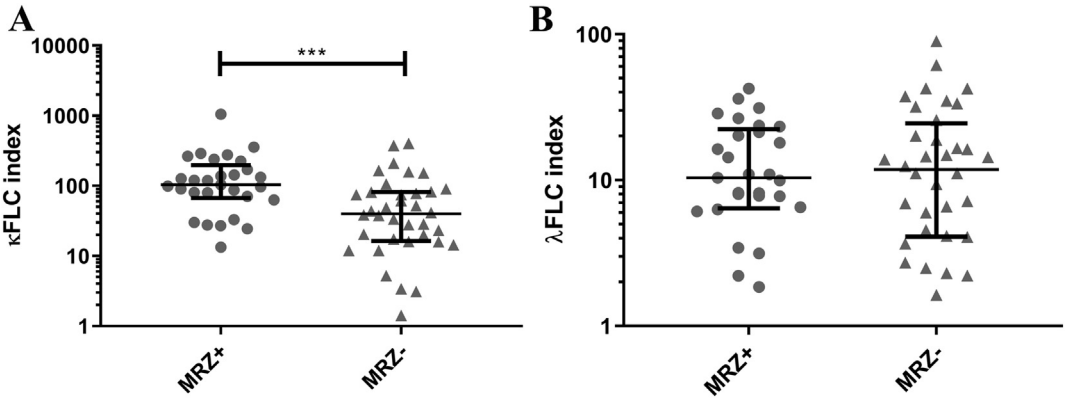

Fig. 2. Comparison of κFLC- and λFLC-indices for a positive (2/3 AI of measles (M), rubella (R) and varicella zoster (Z) ≥ 1.5) or negative MRZ reaction. Lines show median and IQR 25–75%. *** = $p < .001$. AI = antibody specificity index, FLC = free light chain.

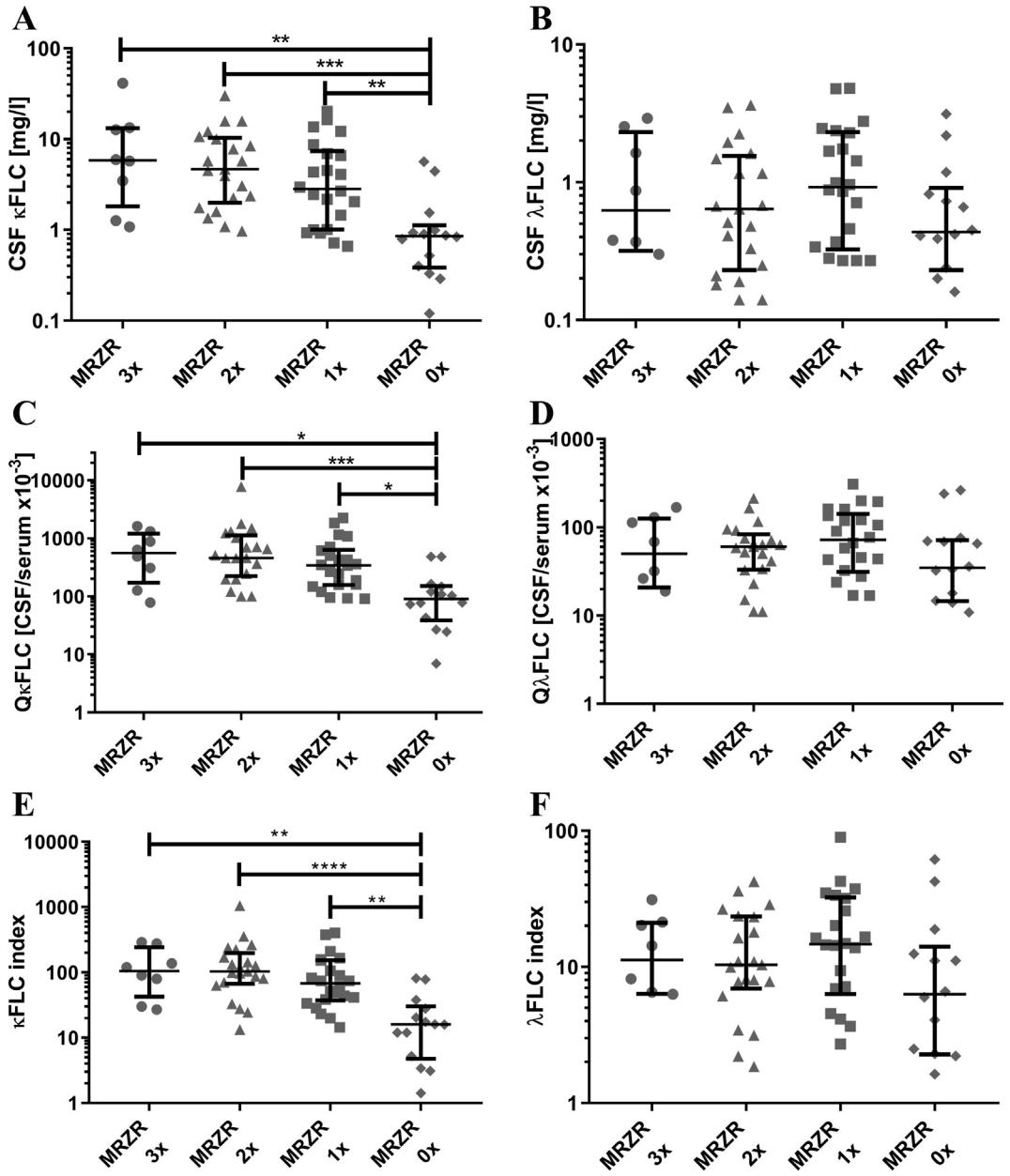

Fig. 3. κ-values (CSF κFLC, QκFLCs, and κFLC index) and λ-values (CSF λFLC, QλFLC, and λFLC index) compared to the MRZR with 0×, 1×, 2× and 3× positive AI (≥ 1.5). Lines show median and IQR 25–75%. * = $p < .05$, ** = $p < .01$, *** = $p < .001$, **** = $p < .0001$. AI = antibody specificity index, CSF = cerebrospinal fluid, FLC = free light chain, MRZR = MRZ reaction.

MO has nothing to declare.

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