

KAPPA FREE LIGHT CHAINS IN CEREBROSPINAL FLUID OF PATIENTS WITH IDENTIFIED OLIGOCLONAL IMMUNOGLOBULIN G

Marina Vasilj^{1,3}, Miro Klarić^{2,3}, Nada Vrkić⁴, Ivanka Mikulić^{1,3}, Marijana Marković Boras^{1,3},
Nevenka Jelić-Knezović³ & Violeta Šoljić³

¹Department of Laboratory Medicine, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

²Department of Psychiatry, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

³Faculty of Health Studies, University of Mostar, Mostar, Bosnia and Herzegovina

⁴Clinical Institution of Chemistry, Clinical Hospital Center „Sestre milosrdnice“, Zagreb, Croatia

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SUMMARY

Background: Production of kappa free light chains (KFLC) represents a part of humoral immune response, along with the synthesis of intrathecal immunoglobulins. Increased concentrations of immunoglobulin G light chains, kappa and lambda chains, were identified through research of numerous diseases of central nervous system. The qualitative method of isoelectric focusing (IEF) followed by immunofixation currently represents the accepted standard in identifying oligoclonal bands (OCB), but establishing a sensitive immunonephelometric method for quantification of kappa free light chains (KFLC) in cerebrospinal fluid (CSF) has paved a way for new diagnostic possibilities. Andersson classified the pattern types of OCB, ranging from type 1 to type 5, wherein types 2 and 3 indicate intrathecal synthesis. Our aim was to determine KFLC in CSF of patients with clinically isolated syndrome (CIS) who had presented with type 2 and type 3 OCB, to determine if there is a difference in concentrations between those two groups and to establish a borderline value of KFLC which would enable differential diagnostics.

Subjects and methods: 70 patients, who underwent lumbar puncture for CSF analysis and had their blood sampled through the cubital vein, participated in the study. Patients were classified according to Andersson as type 2 or type 3, which besides adulthood, represented the inclusion criteria. The average age of patients classified as type 2 was 36 years, and those classified as type 3 was 39 years, where it is evident that there was not a statistically significant difference ($p=0.0685$). We used a qualitative electrophoretic technique of IEF with agarose gel followed by immunofixation, and a quantitative immunonephelometric method. All results were interpreted on a level of statistic significance of $p<0.05$.

Results: CSF KFLC concentrations in type 3 were statistically and significantly elevated with regard to type 2 (Mann-Whitney test, $p=0.0430$). The median for KFLC in type 2 was 0.9 mg/L, while the median for KFLC in type 3 was 2.71 mg/L, and the detection limit for both types was 0.18 mg/L. We used a statistical ROC curve to determine that KFLC concentration can be used for differential diagnostics, meaning it can discriminate type 2 from type 3 with clinical sensitivity of 61% and clinical specificity of 71% ($AUC=0.641$) ($p=0.037$).

Conclusion: Despite the obtained statistically significant differences in concentrations of KFLC between types of OCBs and ROC analysis results, determination of KFLC by a nephelometric method, insufficiently strong clinical sensitivity and specificity does not justify abandonment of IEF method followed by immunofixation.

Key words: kappa free light chains - immunoglobulin G - intrathecal synthesis - isoelectric focusing - oligoclonal bands

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INTRODUCTION

The synthesis of intrathecal immunoglobulins is usually present during inflammation of central nervous system (CNS) (DeCarli et al. 1987). Considering that the blood-brain barrier (BBB) greatly prevents immunoglobulins from entering the bloodstream, they start depositing in the CNS (Compston & Coles 2008). Plasma cells, which are present in the intrathecal space, mainly produce immunoglobulin G (IgG) (Montalban et al. 2010, Freedman et al. 2005). Production of immunoglobulins is a part of humoral immune response, along with the production of FLC.

FLCs show as kappa isotypes (KFLC) and lambda isotypes (LFLC). Elevated levels of light chains, especially kappa chains, were found through researching

diseases of CNS and through analysis of CSF, which also changed their CSF/serum ratio (Rudick et al. 1989, Krakauer et al. 1998, Goffette et al. 2004, Kaplan et al. 2010). Kappa quotient (Q KFLC) represents the KFLC CSF/KFLC serum ratio. In evaluation of patients with documented first episode that was defined as clinically isolated syndrome (CIS), and with the suspected diagnosis of multiple sclerosis (MS), the detection of IgG intrathecal synthesis, which can be proven by a laboratory analysis of CSF, is of great importance (Link 1991).

The test which represents the accepted standard and a diagnostic criteria for MS is identification of two or more oligoclonal IgG zones with the qualitative method of isoelectric focusing followed by immunofixation. Oligoclonal zones are present in approximately 85%

CIS patients and they are an independent risk factor for MS conversion. Considering that this method is qualitative and technically very complicated, sometimes even an experienced analyst has troubles identifying OCB because of their low resolution and weak visualization. A need for a quantitative specific immunochemistry method in controlled environment, such as nephelometry, has arisen because of the absence of standardization and quality control (Freedman et al. 2010, te Velthuis et al. 2010). Although the qualitative detection of intrathecal IgG synthesis through IEF followed by immunofixation is generally regarded as a highly reproducible method, this reproducibility still has not been thoroughly researched on a higher number of patients (Olsson et al. 1984, Keir et al. 1990).

According to Andersson, there are five types of OCB IgG which are classified as: type 1 – normal CSF; type 2 – OCB present in CSF only, which represents intrathecal synthesis; type 3 – many OCBs present in CSF, some of which can be found in serum, which also represents intrathecal synthesis; type 4 – identical OCBs in CSF and serum, which do not demonstrate intrathecal synthesis; type 5 – strongly expressed monoclonal bands in CSF and serum, which also do not demonstrate intrathecal synthesis (Andersson et al. 1994).

The aim of this study was to compare the qualitative and quantitative methods and the possibility of quantitative immunonephelometric method to prove intrathecal synthesis. Furthermore, the aim is to explore the possibility of differentiation between type 2 and type 3 according to Andersson, which are already discriminated by the number of OCBs through IEF followed by immunofixation.

SUBJECTS AND METHODS

Patients

The study was conducted on 70 samples of CSF and serum at the Clinical Institute for Medical Chemistry of the Clinical Hospital Center „Sestre milosrdnice“. Patients with the diagnosis of CIS underwent lumbar puncture in the Neurology Department and had their blood sampled simultaneously in the morning. Patients' evaluation was ordained by a specialist neurologist, which included demonstration of oligoclonal immunoglobulins as one of important differential-diagnostic procedures which can indicate a localized immunological reaction in the central nervous system (CNS). The remaining CSF and serum samples of patients who were classified as type 2 or type 3 were used for a quantitative immunonephelometric determination of KFLC in CSF and serum.

The inclusion criteria were patients' adulthood and classification into type 2 or type 3 of oligoclonal IgGs. Patients whose CSF was hemorrhagic and who were classified as type 1, type 4 or type 5, were excluded from the study.

The included patients were separated into two groups (type 2 and type 3). Out of total 70 patients, 31 (11 males, 20 females) were classified as type 2, while the other 39 patients were classified as type 3 (10 males, 29 females). The average age of patients classified as type 2 was 36 years (18-54), and those classified as type 3 was 39 years (22-76).

The study was approved by the Ethics Committee of Clinical Hospital Center „Sestre milosrdnice“ and all patients gave their written consent for using the surplus of CSF and serum. The study was conducted in accordance with the Helsinki Declaration.

Methods

OCB detection

Immunoglobulins were separated by electrophoretic technique of IEF on agarose gel in pH gradient of 6-10, fixated (immunoprecipitated) with an antibody for IgG and visualised with chromogen. Patient's serum and CSF were applied to agarose gels (gel, chromogen and Hydrasis instrument for IEF, Sebia, France) in order to compare and detect OCBs, which, if there are at least two in CSF, indicate intrathecal synthesis.

Determination of kappa free light chains

Concentrations of KFLC in serum and CSF were determined with an immunonephelometric method on an automated analyzer BN II (Siemens, Germany). The reagent contains specific monoclonal antibodies (N Latex FLC assay, Siemens, Marburg, Germany).

Statistical analysis

Statistical analysis was conducted using MedCalc software (MedCalc Statistical Software version 14.8.1, Mariakerke, Belgium, <http://www.medcalc.org>; 2014). For normality testing, the Kolmogorov-Smirnov test was used. Non-parametric Mann-Whitney test was used to compare two independent groups. We tried to determine the borderline value for KFLC which provides the best sensitivity and specificity in comparing OCBs in type 2 and type 3. All results were interpreted at the level of statistical significance $p < 0.05$.

RESULTS

Seventy patients classified as type 2 (n=31) and type 3 (n=39) participated in this study. Concentrations of KFLC in CSF were significantly higher in type 3 than those in type 2, which is shown in Table 1. Concentrations of KFLC in serum in type 2 and type 3, along with Q KFLC, are also presented in Table 1. Values in Table 1 are expressed as medians with interquartile ranges. We used the 25-75 percentile as interquartile range.

Table 1. Demographic and biochemical characteristics of patients

Type of IgG according to Andersson	Number (M/F)	Age (years)	CSF FLC (mg/L)	Serum FLC (mg/L)	Q FLC***
Type 2	31 (11/20)	36 (18-54)	0.90* (0.617-2.532)**	10.10* (9.090-13.925)**	0.1178* (0.0504-0.2449)**
Tip 3	39 (10/29)	39 (22-76)	2.71* (0.810-6.123)**	12.10* (10.200-16.325)**	0.2009* (0.0689-0.4849)**

KFLC – kappa free light chains; Q FLCt – FLC_{CSF}/FLC_{serum} ratio; *Values expressed as median; distribution does not follow Gaussian distribution; **Values expressed as interquartile range; ***kappa light chain quotient; N – number (male/female); CSF FLC – cerebrospinal fluid free light chains; Serum FLC – serum free light chains; Q FLC – free light chains quotient

Figure 1. shows the comparison of CSF KFLC between type 2 and type 3 where we established that concentration of CSF KFLC was significantly higher in type 3 ($p=0.0430$). Median with its belonging interquartile ranges for KFLC in type 2 was 0.90 mg/L (0.61-2.53), whereas median for KFLC in type 3 was 2.71 mg/L (0.810-6.123). Lower detection limit for both types was 0.18 mg/L. Nine patients had values lower than the lower detection limit. Out of these 9 patients, 3 of them (9.6%) were classified as type 3, whereas other 6 patients (15.3%) were classified as type 2.

We also determined Q KFLC values from a mathematical relation between CSF KFLC and serum KFLC. Median for Q KFLC was 0.1178 (0.0504-0.2449) for type 2, and 0.2009 (0.0689-0.4849) for type 3. Q KFLC between type 2 and type 3 reached borderline significance ($p=0.06$).

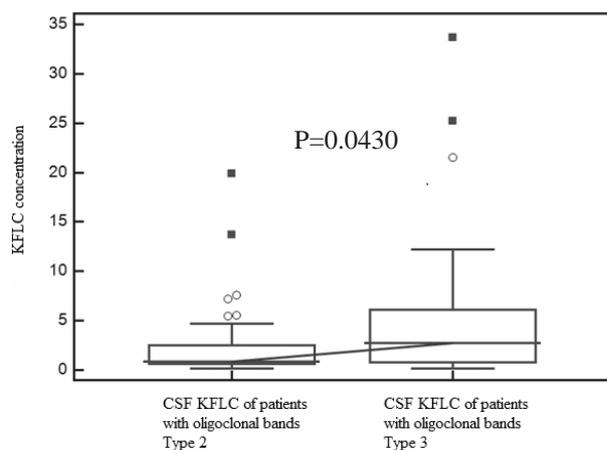


Figure 1. Comparison of CSF KFLC concentrations between oligoclonal IgG type 2 and type 3

Serum KFLC concentrations of type 3 are higher than type 2, but without statistical significance ($p=0.069$). The same results were found for Q KFLC comparison – higher values of type 3 did not show statistical significance ($p=0.068$).

Figure 2. shows the ROC (Receiver Operating Characteristics) analysis. By using ROC analysis, we tried to determine the borderline value for KFLC which gives the best sensitivity and specificity in discriminating type 2 and type 3. With the borderline value of 1.89 mg/L, the diagnostic sensitivity was 61.5%, whereas the diagnostic specificity was 71.0%. The ROC analysis for

serum concentration of KFLC and for Q KFLC showed worse results (AUC=0.627 and 0.628; sensitivity =66% and 46%; specificity =67% and 83%) with $p=0.0671$ and 0.0619.

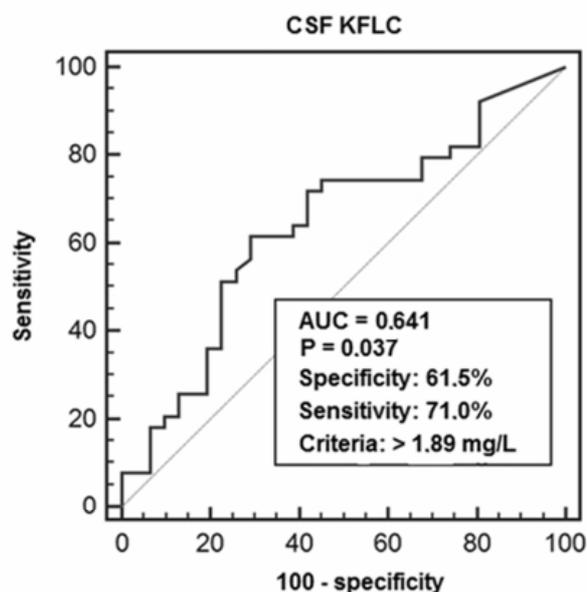


Figure 2. ROC analysis of kappa free light chains in CSF for possibility of differentiation between oligoclonal IgG type 2 and type 3

DISCUSSION

OCB immunoglobulins are synthesized in plasma blasts and plasma cells in CSF or CNS, and their determination represents an international recommendation for detection of intrathecal inflammation and synthesis of oligoclonal immunoglobulins which originate from two or more B lymphocyte clones (Awad et al. 2010, Passerini et al. 2016). The activity of intrathecal IgG synthesis from plasma cells can be qualitatively shown through IEF followed by immunofixation, which is a very demanding and very often equivocal method, especially if the OCBs are weakly expressed or presented in CSF only (Franciotta & Lolli 2007, Andlovic et al. 2012, Link & Huang 2006). IgG FLCs, which are synthesized through B lymphocytes, consist of two heavy chains and two light chains, which exist as kappa and lambda isotypes (Kaplan et al. 2011, van der Heijden et al. 2006). KFLCs have already been reported

as a surrogate marker of intrathecal immunoglobulin synthesis, but in a differential diagnostics of multiple sclerosis they need to be complemented with clinical observations, anamnestic data, imaging, biochemical, cytological and microbiological analyses. Besides these, the MRZ reaction (antigen-specific immunoglobulin G to Measles, Rubella and Varicella Zoster Virus) improves the specificity of oligoclonal IgG findings. The quantitative determination of immunoglobulins is still not being used in these diagnostics (Passerini et al. 2016). In contrast to IEF followed by immunofixation, determination of KFLC is a simple, quantitative method which can be performed by using ELISA or nephelometry (Senel et al. 2014).

In this study, we report KFLC measuring as a possible method for detection of intrathecal synthesis which allows incorporation of KFLC analysis in routine laboratory diagnostic algorithms (Presslauer et al. 2014). Comparing CSF KFLC values in two evaluated groups, somewhat higher KFLC concentrations were found in type 3 than in type 2. We have to emphasize that during this study we used laboratory data only and excluded all radiological data. For the complete overview of significance and clinical value of KFLC quantification, it is necessary to correlate these with the findings of MRI and clinical presentation in the future.

It is well known that other authors found significantly elevated Q KFLC values with present IgG OCBs, with Q KFLC being elevated in patients classified as type 2 and type 3 which represent intrathecal IgG synthesis in regard to type 1 which excludes MS (Senel et al. 2014, Rudick et al. 1989). However, in this study we tried to distinguish type 2 from type 3 based on the laboratory data of KFLC and its quotient. Determination of CSF KFLC was statistically significant ($p < 0.05$), whereas comparing Q KFLC between type 2 and type 3 reached borderline significance only ($p = 0.06$). ROC analysis of KFLC in type 2 and type 3 indicated a diagnostic sensitivity of 61.5% and diagnostic specificity of 71.0%. The area under the curve was 0.641.

CONCLUSION

Although we obtained a statistically significant difference in KFLC determination in this study, which indicates that a nephelometric method could be used to distinguish two types in Andersson's classification, its low sensitivity and specificity does not confirm the commonality of findings, so further using of technically more demanding method of IEF followed by immunofixation is still recommended. Additional determination of KFLC with a nephelometric method would be useful for patients with unclear results of IEF followed by immunofixation and with low resolution of KFLC, therefore this method should further be assessed in multicentered prospective studies on a larger number of patients classified according to Andersson as type 2 or type 3.

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Contribution of individual authors:

Marina Vasilj: design study, analyses of data results, statistical analyses, literature searches;

Miro Klarić: design study, analyses of data results, statistical analyses, literature searches;

Nada Vrkić: design study, analyses of data results, statistical analyses, literature searches;

Ivanka Mikulić: analyses of data results, literature searches;

Marijana Marković Boras: analyses of data results, literature searches;

Nevenka Jelić-Knezović: analyses of data results, literature searches;

Violeta Šoljić: analyses of data results, literature searches.

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Correspondence:

Marina Vasilj, Master of Chemistry and Biology
Department of Laboratory Medicine, University Clinical Hospital Mostar
Bijeli Brijeg bb, 88000 Mostar, Bosnia and Herzegovina
E-mail: vasiljmarina87@gmail.com