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Quantitative and qualitative evaluation of IgG synthesis in 8,947 cerebrospinal fluid samples

Avaliação quantitativa e qualitativa da síntese de IgG em 8.947 amostras de líquido cefalorraquidiano

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ABSTRACT

Introduction: Increased intrathecal immunoglobulin class G (IgG) synthesis can be found in several neuroinflammatory diseases. **Objective:** The aim of this study was to analyze quantitative and qualitative methods of intrathecal immunoproduction evaluation. **Methods:** We retrospectively assessed data from cerebrospinal fluid (CSF) and serum samples sent to Senne Liquor Diagnóstico from 2001 to 2017. Cytological, biochemical, and immunological data were compared between cases with and without oligoclonal bands (OCBs). Comparisons between samples with OCBs (OCB+) and without them (OCB-) were carried out, and the ability to predict the presence of OCBs was assessed with ROC analysis. **Results:** We included 8,947 samples (2,599 OCB+ samples and 6,348 OCB- samples). CSF lymphocytes and monocytes were significantly associated with the presence of OCB ($p < 0.001$). All the inflammatory parameters were significantly associated with OCB, and the methods with the highest predictive ability for OCB were IgG index and Reiber diagram [area under the receiver operating characteristic (AUROC) curve = 0.881 and 0.863, respectively]. A small percentage of cases without OCB had high IgG index (9.56%) or Reiber diagram, showing increased IgG production (4.6%). **Conclusion:** We showed a strong association between OCB and other CSF inflammatory parameters. The presence of cases with increased quantitative CSF IgG synthesis without OCB suggests that both quantitative and qualitative methods should be performed in the evaluation of neuroinflammatory processes.

Key words: cerebrospinal fluid; oligoclonal bands; immunoglobulin G.

RESUMO

Introdução: *O aumento da síntese intratecal de imunoglobulina da classe G (IgG) pode ser encontrado em diferentes doenças neuroinflamatórias.* ***Objetivo:*** *Avaliar retrospectivamente diferentes métodos de avaliação da imunoprodução intratecal.* ***Métodos:*** *Dados de líquido cefalorraquidiano (LCR) e soro enviados para o Senne Liquor Diagnóstico entre 2001 e 2017 foram avaliados. Dados de citologia, bioquímica e imunologia foram comparados entre amostras com e sem bandas oligoclonais (BOCs). Comparações dos parâmetros do LCR entre amostras com (BOC+) e sem BOCs (BOC-) foram realizadas, e a habilidade de predizer a presença de BOCs foi avaliada por meio de curvas ROC.* ***Resultados:*** *Foram incluídas 8.947 amostras, sendo 2.599 BOC+ e 6.348 BOC-. Contagens de linfócitos e monócitos foram significativamente associadas à presença de BOC (*p *< 0,001). Todos os métodos de avaliação da IgG foram significativamente associados a BOC+; os com maior associação foram o índice de IgG e o Reiber [área sob a curva* receiver operating characteristic *(AUROC) = 0,881 e 0,863, respectivamente]. Entre os casos BOC-, o índice de IgG foi elevado em 9,56%, e o Reiber mostrou aumento de produção de IgG em 4,6%.* ***Conclusão:*** *Houve forte associação entre BOC e outros parâmetros neuroinflamatórios do LCR. A existência de casos BOC- com aumento da produção de IgG por métodos quantitativos sugere que tanto métodos qualitativos quanto quantitativos devem ser utilizados na avaliação dos processos neuroinflamatórios.*

Unitermos: *líquido cefalorraquidiano; bandas oligoclonais; índice de IgG.*

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RESUMEN

Introducción: *El incremento de la síntesis intratecal de inmunoglobulina G (IgG) puede ser encontrado en diferentes enfermedades neuroinflamatorias.* ***Objetivo:*** *Analizar retrospectivamente diferentes métodos de evaluación de la producción intratecal.* ***Métodos:*** *Se examinaron datos del líquido cefalorraquídeo (LCR) y suero enviados para el Senne Liquor Diagnóstico entre 2001 y 2017. Se compararon datos de citología, bioquímica e inmunología entre muestras con y sin bandas oligoclonales (BOC). Se hicieron comparaciones de los parámetros del LCR entre muestras con (BOC+) y sin BOC (BOC-), y la habilidad para predecir la presencia de BOCs fue evaluada mediante curvas ROC.* ***Resultados:*** *Se incluyeron 8.947 muestras, con 2.599 BOC+ y 6.348 BOC-. Recuentos de linfocitos y monocitos fueron significativamente asociados a la presencia de BOC (*$p < 0,001$*). Todos los métodos de evaluación de IgG fueron significativamente asociados a BOC+; aquellos con mayor asociación fueron el índice de IgG y el Reiber (área bajo la curva ROC = 0,881 y 0,863), respectivamente. Entre los casos BOC-, el índice de IgG fue alto en 9,56% y el Reiber demostró incremento de producción en 4,6%.* ***Conclusión:*** *Hubo fuerte asociación entre BOC y otros parámetros neuroinflamatorios del LCR. La existencia de casos BOC- con incremento de producción de IgG mediante métodos cuantitativos sugiere que tanto métodos cualitativos como cuantitativos deben ser usados en la evaluación de procesos neuroinflamatorios.*

Palabras clave: *líquido cefalorraquídeo; bandas oligoclonales; índice de IgG.*

INTRODUCTION

Increased synthesis of immunoglobulin class G (IgG) in the cerebrospinal fluid (CSF) is found in several disorders, including infectious and inflammatory neurological diseases. Quantitative and qualitative methods are used to assess IgG production in the central nervous system (CNS)[1, 2]. The most used quantitative methods are CSF IgG determination, IgG index[3], and Reiber diagram[4, 5]. Qualitative evaluation of intrathecal immunoproduction of IgG is carried out with oligoclonal band (OCB) detection by isoelectric focusing (IEF)[6].

OCB identification by IEF is considered the most sensitive method to detect intrathecal IgG synthesis[7]. There are five standardized interpretations: type 1 – normal; type 2 – OCB in CSF and not in serum; type 3 – OCB in CSF and serum, but with more bands in CSF than in serum; type 4 – with the same OCBs in serum and CSF; type 5 – with monoclonal bands in CSF and serum. The number of bands is not taken into account, but the correspondence of bands between serum and CSF, explaining why this method is qualitative and not quantitative[8]. Types 2 and 3 commonly indicate intrathecal synthesis of IgG, type 4 reveals a systemic inflammation in which serum antibodies cross the blood-brain-barrier (BBB), and type 5 is found in systemic paraproteinemias[3].

The correlation of OCB with other methods of evaluation of intrathecal synthesis of IgG, as well as with other CSF parameters, is not fully understood[9, 10]. The aim of this study was to assess the association of OCB with other methods of CSF IgG synthesis evaluation and other CSF inflammatory parameters.

METHODS

This was a retrospective study evaluating data of CSF and serum samples sent to Senne Liquor Diagnóstico between January 2001 and July 2017 for intrathecal IgG synthesis analysis. The data were obtained from Senne Liquor Diagnóstico databank.

CSF cell count and CSF biochemical data were recorded. Detection of IgG OCBs was performed with IEF in combination with immunoblotting[11]. Albumin and IgG were determined by nephelometry. Gamma globulin fraction was assessed with agarose gel electrophoresis[12]. IgG index and Reiber diagram were calculated as subsequently shown[3, 4]. Basically, IgG index is: [IgG (CSF) × albumin (serum)/IgG (serum) × albumin (CSF)] × 100. Reiber is found by plotting IgG and albumin quotients – Q IgG (CSF IgG/serum IgG) and Q albumin (CSF albumin/serum albumin) – into the Reiber diagram (Q IgG on the coordinate axis and Q albumin on the abscissa axis), thus identifying the area (quadrant). When the plot is located in area 4 (upper left quadrant) it indicates high Q IgG and low Q albumin, demonstrating intrathecal immunoproduction. OCB detection patterns with types 2 or 3 were classified as OCB+ and the remaining patterns were classified as OCB-.

Data normality was assessed with Kolmogorov-Smirnov test. We compared CSF data of OCB+ and OCB- samples. The *t* Student test was used for comparison of continuous data. Categorical data were compared with chi-square test. Variables with significant difference by univariate analysis were further assessed with binary logistic regression analysis. A sensitivity analysis was conducted

to compare the ability of other CSF variables to predict OCB+. Area under the receiver operating characteristic curve (AUROC) was calculated as a measure of predictive ability. Statistical analysis was performed using SPSS version 17.0 for Windows. The significance level was set at $p < 0.05$.

RESULTS

We included 8,947 samples (2,599 OCB+ samples and 6,348 OCB- samples). Cytological and biochemical data were compared between OCB+ and OCB- samples. CSF leukocyte count was 8.72 ± 36.2/mm^3 among OCB+ and 4.84 ± 79.4/mm^3 among OCB- samples ($p = 0.066$). The leukocyte subset comparisons between OBC+ and OCB- were: lymphocytes 3.84 ± 12.17/mm^3 vs. 1.55 ± 9.6/mm^3 ($p < 0.001$); monocytes 0.3 ± 1.9/mm^3 vs. 0.14 ± 1.7/mm^3 ($p < 0.001$); neutrophils 0.59 ± 18.7/mm^3 vs. 0.82 ± 53.8/mm^3 ($p = 0.834$). Mean total protein concentration in OCB+ was 35.5 ± 23.3 mg/dl and 37.5 ± 29.1 mg/dl in OCB- ($p = 0.018$). Mean lactate was 14.8 ± 4.1 mg/dl among OCB+ and 15.1 ± 4.6 mg/dl among OCB- ($p = 0.063$).

Gamma globulin fraction was significantly higher among OCB+ than in OCB- samples (6.1 ± 8.7 mg/dl vs. 4.1 ± 6.1 mg/dl, $p < 0.001$). CSF IgG concentration was higher among OCB+ than among OCB- samples (6.6 ± 32.7 mg/dl vs 3.8 ± 21.7 mg/dl, $p < 0.001$). Mean IgG index was significantly higher among OCB+ samples (0.74 ± 0.78 vs. 0.37 ± 0.34; $p < 0.001$). A small percentage of OCB- cases (9.56%) had IgG index above 0.7, which is the usually adopted cut-off for IgG index. IgG index was above 0.8 in 6.05% of OCB- cases. Most (71.5%) OCB+ cases and only 4.6% of OCB- cases had Reiber diagram in area 4, which is the area that indicates increased intrathecal immunoproduction ($p < 0.001$). All the parameters that were significantly different between OCB+ and OCB- by univariate analysis were also significantly different by multivariate analysis (**Table**).

The ROC curves are shown in the **Figure**. The highest AUROC was of IgG index (0.881), followed by Reiber (0.863), CSF IgG (0.842), gamma globulin (0.761), and lymphocytes (0.745). The other parameters had AUROC under 0.6.

DISCUSSION

In this study we showed a strong association between the detection of OCB and other CSF neuroinflammatory parameters, such as lymphocyte and monocyte count, gamma globulin, IgG, IgG index, and Reiber diagram. This finding is consistent with the mechanism of intrathecal production of IgG, which involves B-cell activation in the context of neuroinflammatory processes. This activation results in plasma cell activation within the CNS, thus explaining increased IgG production[13]. Quantitative and qualitative methods of intrathecal immunoproduction evaluation have different sensitivity and specificity levels to detect IgG synthesis. For instance, in patients with multiple sclerosis (MS), qualitative detection of increased IgG synthesis was shown to be a more sensitive method. While OCB detection using IEF occurs in 90%-95% of MS patients, quantitative IgG analyses are abnormal in nearly 75% of MS cases[14-16]. However, the frequency and the meaning of detecting quantitative increased IgG production

TABLE – CSF data compared between OCB+ and OCB- samples

	OCB+	OCB-	p^* (univariate analysis)	p^{**} (multivariate analysis)
Leukocytes (mm^3)	8.72 ± 36.2	4.84 ± 79.4	0.066	-
Lymphocytes (mm^3)	3.84 ± 12.17	1.55 ± 9.6	< 0.001	< 0.001
Monocytes (mm^3)	0.3 ± 1.9	0.14 ± 1.7	< 0.001	0.001
Neutrophils (mm^3)	0.59 ± 18.7	0.82 ± 53.8	0.834	-
Protein (mg/dl)	35.5 ± 23.3	37.5 ± 29.1	0.018	< 0.001
Lactate (mg/dl)	14.8 ± 4.1	15.1 ± 4.6	0.063	-
Gamma globulin fraction (mg/dl)	6.1 ± 8.7	4.1 ± 6.1	< 0.001	< 0.001
CSF IgG (mg/dl)	6.6 ± 32.7	3.8 ± 21.7	< 0.001	< 0.001
IgG index	0.74 ± 0.78	0.37 ± 0.34	< 0.001	< 0.001
Reiber area 4 (%)	71.5	4.6	< 0.001	< 0.001

*CSF: cerebrospinal fluid; OCB: oligoclonal bands; IgG: immunoglobulin class G; *univariate analysis with t test or chi-square test; **multivariate analysis with binary logistic regression.*

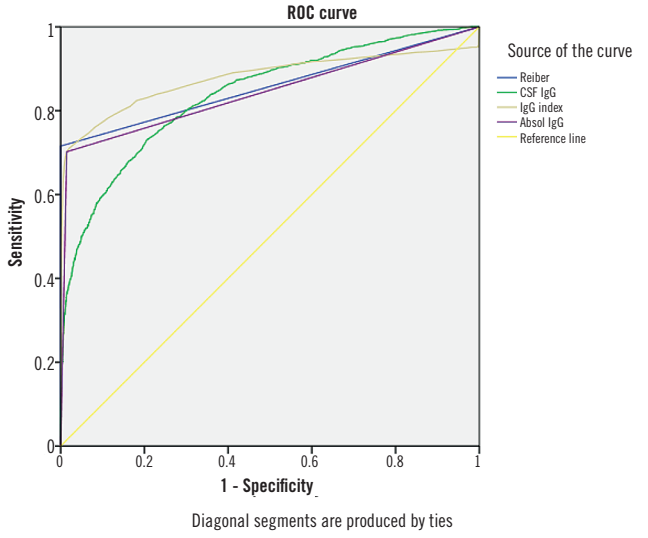

FIGURE – *ROC curves of IgG index, Reiber diagram, CSF IgG, and gamma globulin fraction ability to predict OCB+*

are not well established in cases in which OCBs are not found. In neuroinflammatory diseases other than MS, the sensitivity and specificity of quantitative and qualitative methods are still unknown.

In the present study we evaluated the performance of quantitative methods in relation to the detection of OCBs. IgG index was the test with the best ability to predict OCB+. Also, we found that 9.56% of the OCB- cases had IgG index above 0.7, which is the most frequently adopted cut-off. We found that 6.05% of the OCB- cases had IgG index above 0.8. Reiber was the second quantitative parameter with the best association with the presence of OCB. We found that 4.6% of the cases without OCB had Reiber diagram in area 4, which is the area suggestive of intrathecal synthesis of IgG. The positivity of quantitative methods in relation to OCB detection was evaluated in some previous studies. In one of them, researchers found that OCBs were always detected in cases with IgG index above 0.8[17]. A more recent study found IgG index above 0.8 in some OCB- cases[9]. There are two possibilities to explain discrepant results between OCB and quantitative methods. One is that quantitative methods in the absence of OCBs are truly indicators of intrathecal IgG synthesis, thus enhancing the chance of identifying a neuroinflammatory process. Another possible explanation is that altered IgG index and Reiber diagram in OCB- cases are false positive. In the present study we were not able to assess sensitivity and specificity, but these discrepant results suggest a cautious and individual interpretation of the results when OCB and quantitative methods lead to distinct results. In MS, OCB detection with IEF is the gold standard for IgG immunoproduction[13, 15]; however, it is not yet possible to entirely rule out that quantitative methods may disclose increased intrathecal immunoproduction in a few cases with no OCB[18]. In other neurological disorders, the relationship between quantitative and qualitative method results is still less well understood than in MS. It is therefore reasonable to recommend that both qualitative and quantitative analyses are individually interpreted when discrepant results occur.

Our study has limitations that deserve to be mentioned. We were not able to evaluate sensitivity and specificity of different methods to assess intrathecal IgG production, since this was a retrospective study from a CSF databank. Also, we could not evaluate the performance of these different methods in different diseases, as we had not access to clinical data on the patients. We were also not able to correlate CSF data with radiological data and disease activity. However, the large sample size included in our study, larger than those included in most previous studies in the area, strengthens the value of our findings. Future studies should address, in different neuroinflammatory diseases and in different stages of the diseases, the value of quantitatively and qualitatively assessing intrathecal IgG synthesis with different methods and parameters.

CONCLUSION

Our data suggest that both qualitative and quantitative tests should be carried out in the evaluation of intrathecal IgG production.

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