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Short Communication

Analytical performances of SPAPLUS® turbidimeter for the quantification of albumin and IgG in serum and cerebrospinal fluid

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ABSTRACT

Objectives: The intrathecal production of IgG is part of the diagnosis criteria for Multiple Sclerosis. Its assessment requires both quantitative (quantification of IgG and albumin in serum and cerebrospinal fluid (CSF)) and qualitative assays (isoelectric focusing). We have evaluated the analytical performances of the SPAPLUS® immunoturbidimeter (The Binding Site®) for the quantification of IgG and albumin in serum and CSF.

Design and methods: Within-day and between-day precision, linearity and carry-over were assessed. Results obtained with SPAPLUS® were compared to those obtained with the nephelometer IMMAGE® 800, including albumin quotient and CSF IgG index. Isoelectric focusing was performed and considered as the gold standard for assessment of intrathecal production of IgG.

Results: The within-day and the between-day precisions were obtained at two concentration levels and were below the recommendations of the manufacturer and of the French Society of Clinical Biology. Our evaluation confirmed the linearity of the assays and the absence of contamination. An agreement above 94% was observed between the results obtained with SPAPLUS® and those obtained with IMMAGE® 800. The use of the new reference material DA470k did not significantly modify IgG and albumin values. The confrontation of CSF IgG index and isoelectric focusing results led to a sensitivity of 79% and a specificity of 97% of CSF IgG index quantified on SPAPLUS® for the presence of oligoclonal bands at IEF. The sensitivity of intrathecal IgG calculated with the Reiber's hyperbolic formula was 47.4% and specificity was 97% for the presence of oligoclonal bands at IEF. Automatic rerun managed by the device for concentrations outside the measuring range was satisfactory.

Conclusion: The SPAPLUS® immunoturbidimeter displays good analytical performances for the parameters evaluated in this work.

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1. Introduction

The intrathecal production of IgG is a useful parameter in case of Multiple Sclerosis (MS) suspicion to confirm the inflammatory nature of the disease and/or to exclude alternative diagnosis [1]. The intrathecal production of IgG is part of the diagnosis criteria for primary progressive Multiple Sclerosis which is characterized by a chronic progression from diagnosis without identified relapse [1,2]. It is therefore particularly indicated after one year of disease progression in association with the evidence of dissemination in space [1]. The evaluation of IgG production in cerebrospinal fluid (CSF) requires both quantitative and qualitative assays. The quantitative tests consist of quantifying IgG and albumin in serum and CSF. This allows the calculation of the albumin quotient (albumin CSF/albumin serum) which reflects the state of the blood–brain barrier, and that of CSF IgG index ([IgG CSF/albumin CSF]/[IgG serum/albumin serum]) which reflects the abnormal intrathecal production of IgG. However, the sensitivity of the CSF IgG index is insufficient to affirm the intrathecal production of IgG [3,4], therefore the isoelectric focusing (IEF) of IgG in serum and CSF is required.

Recent improvement of immunoturbidimetry led to consider this technique as a validated alternative to immunonephelometry, which is the reference method for the determination of specific proteins [5]. We have evaluated the analytical performances of SPAPLUS® immunoturbidimeter (The Binding Site) for the quantification of IgG and albumin in serum and CSF. This evaluation was performed according to CLSI (Clinical and Laboratory Standards Institute) protocols with minor modifications (between-day precision was assessed on 15 operating days and linearity was not assessed in duplicate).

2. Methods and results

2.1. Reagents

For each parameter, reagent kits include a specific antiserum, a reaction buffer, six calibrators and two levels of internal quality control.

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Calibrators consist of mixtures of human sera. IgG and albumin assays are calibrated with the International DA470k reference material.

2.2. Evaluation of precision

Albumin (Vialebex®, LFB, Courtaboeuf, France) and therapeutic immunoglobulins (TEGELINE®, LFB) were diluted at various concentrations in PBS (Gibco Life Technologies, Saint Aubin, France) to obtain samples in sufficient quantity, with concentrations corresponding to a normal serum, a normal CSF and a pathological CSF (i.e. with high albumin quotient and high CSF IgG index). These three samples were assayed 30 times the same day in the same series. The within-run precision in serum gave satisfactory results (Table 1) with CV less or equal to 3% which met the acceptability criteria of the manufacturer and of the French Society of Clinical Biology (SFBC) [6]. The CV obtained in CSF were <4% for IgG and albumin, leading to CV < 5.8% for albumin quotient and CSF IgG index.

For each parameter, the between-day precision was evaluated on two levels of internal quality controls provided in the kits. They were quantified twice a day during 14 to 15 consecutive working days. CV were below 5% for all the parameters studied, including calculated indexes (Table 1) and are in accordance with the SFBC's and manufacturer's recommendations.

2.3. Evaluation of linearity

Linearity was assessed in CSF obtained from two patients having IgG or albumin concentration close to the upper limit of the measuring range of SPAPLUS®. Because no sera were available with such characteristics, we used a mixture of albumin and therapeutic immunoglobulins for the assessment of linearity in serum. Six to seven diluted points covering the measuring range given by the supplier were quantified on SPAPLUS®. The linear regressions between measured concentrations and theoretical values had slopes near 1 and intercept close to zero, except for albumin in CSF. Excellent correlation coefficients (>0.995) were obtained (Supplemental Table 1).

2.4. Evaluation of carry-over

The carry-over was evaluated by measuring three times a sample with a high level for a parameter (H1, H2, H3) and then a sample with a low level for this same parameter (B1, B2, B3). Carry-over was calculated as follows: (B1 − B3) ∗ 100 / (average H − B3). Alternating samples with high concentrations with low concentration samples did not reveal any contamination (Supplemental Table 2).

2.5. Agreement with results from IMMAGE® 800 nephelometer

Fifty-three coupled samples of sera and CSF were obtained from 53 patients suspected of Multiple Sclerosis, of neurodegenerative disease, or of other inflammatory diseases or infections of the central nervous system. These samples were residues of routine analysis performed at our laboratory. IgG and albumin were quantified the same day on SPAPLUS® and on nephelometer IMMAGE® 800 (the device routinely used in our laboratory, Beckman Coulter, Villepinte, France). The comparison was performed on ten operating days. On IMMAGE, the IgG and albumin assays are calibrated with the international reference material CRM470.

The slopes of linear regressions were between 0.92 and 0.97 and the correlation coefficients were above 0.87 for the four analytes measured (Fig. 1A). With Bland and Altman analysis, IgG and albumin biases were below 0.3 g/L in serum and <2 mg/L in CSF (Fig. 1B). The agreement between the two assays was greater than 94% for the four measured parameters and >90% for the two calculated indexes. It is interesting to note that the use of the new reference material DA470k did not significantly modify IgG and albumin values.

Agreement was also evaluated for classification as normal or elevated albumin quotient (>6.5) and CSF IgG index (>0.65) [7] (Supplemental Table 3). Agreements between SPAPLUS® and IMMAGE® 800 for albumin quotient and CSF IgG index were 92% and 89%, respectively (see details below).

2.6. Agreement with isoelectric focusing results

Isoelectric focusing followed by IgG immunoblotting [8] was used as the reference technique for the assessment of intrathecal IgG production [3]. Six patients had normal CSF IgG index with results from SPAPLUS® while having elevated CSF IgG index with results from IMMAGE® 800. Among those, two patients had no oligoclonal bands (OCB) as determined by IEF (they had uveitis and unreported diagnosis, respectively), and four had OCB at IEF. All four had CSF IgG index obtained from SPAPLUS® very close to the threshold (values from 0.61 to 0.63 for a threshold at 0.65). Two of them suffered from Multiple Sclerosis.

Table 1
Evaluation of within-run and between-day precision.
For each parameter, the table shows the number of determinations, the mean of obtained values, the standard deviation and the CV, as well as the acceptability criteria from the French Society of Clinical Biology (SFBC) [6] and from the manufacturer (TBS = The Binding Site). IQC: internal quality control. If not stated, the evaluation was performed on a patient sample.

Precision	Samples	Parameter	N dosages	Mean	Standard deviation	CV (%)	Acceptability criteria	
							SFBC	TBS
Within-run	Serum	IgG (g/L)	30	6.5	0.14	2.08	4.5	5
		Albumin (g/L)	30	32.45	0.99	3.05	3.8	6
	Normal CSF	IgG (mg/L)	30	16.39	0.64	3.89		5
		Albumin (mg/L)	30	190.18	6.37	3.35		5
		Albumin quotient	30	5.87	0.27	4.68		
		CSF IgG index	30	0.43	0.02	5.77		
	Pathological CSF	IgG (mg/L)	30	68.88	1.85	2.69		5
		Albumin (mg/L)	30	380.41	8.15	2.14		5
		Albumin quotient	30	11.73	0.46	3.95		
		CSF IgG index	30	0.91	0.04	4.93		
Between-day	Serum (IQC)	IgG (g/L)	28	7.1	0.28	3.86	5	5
			28	13.6	0.40	2.96	5	5
	Serum (IQC)	Albumin (g/L)	29	30.07	1.25	4.16	5	8
			29	58.74	2.12	3.61	4	8
	CSF (IQC)	IgG (mg/L)	30	29.8	1.2	4.04		8
			30	85.8	2.9	3.38		8
	CSF (IQC)	Albumin (mg/L)	29	325.8	9.35	2.87		10
			29	752.5	27.32	3.63		10

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