Journal of Neuroimmunology 263 (2013) 116–120

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Determination of kFLC and K Index in cerebrospinal fluid: A valid alternative to assessintrathecal immunoglobulin synthesis

Fabio Duranti [a], Massimo Pieri [a], Diego Centonze [b], Fabio Buttari [b], Sergio Bernardini [a], Mariarita Dessi [a,*]

[a] Department of Experimental Medicine and Surgery, "Tor Vergata" University Hospital, Rome, Italy
[b] Department of Systems Medicine, "Tor Vergata" University Hospital & IRCCS Fondazione Santa Lucia, Rome, Italy

ARTICLE INFO

Article history:
Received 10 May 2013
Received in revised form 4 July 2013
Accepted 8 July 2013

Keywords:
kFLCs
CSF
Nephelometry
OCBs
kFLC Index
Multiple sclerosis

ABSTRACT

Intrathecal immunoglobulin synthesis is observed in several disorders of the central nervous system, but its detection by current laboratory tests is relatively insensitive and operator depending. We assessed the diagnostic accuracy of a nephelometric assay for k free light chain determination in cerebrospinal fluid and serum. The patients were grouped according to clinical and laboratory criteria. ROC curves for all methods were performed to find the best cut-off value. kFLC Index seems to be more accurate than other parameters. Our data indicate that nephelometric assay for kFLCs in CSF reliably detect intrathecal immunoglobulin synthesis and discriminate multiple sclerosis patients.

1. Introduction

Intrathecal immunoglobulin synthesis is commonly observed in inflammatory disorders of the central nervous system (CNS) of either infectious or autoimmune origin (DeCarli et al., 1987), and has been shown to be of high diagnostic value. Small amounts of immunoglobulins typically pass into the cerebrospinal fluid (CSF) by passive transfer through the blood–CSF barrier, so it is necessary to differentiate the origin of Ig in the CSF before intrathecal immunoglobulin synthesis can be diagnosed (Reiber and Felgenhauer, 1987). This can be achieved either by calculation of the CSF/serum ratios of immunoglobulins compared with the CSF/serum ratio of albumin (Q_{alb}), which is not synthesized intrathecally, or by the detection of so-called oligoclonal immunoglobulin bands (OCBs) in CSF (Mattson et al., 1982; Walker et al., 1983; Luxton et al., 1990). In general, CSF/serum ratios and quotient diagrams are not sufficiently sensitive to reliably differentiate intrathecal immunoglobulin synthesis from passive transfer across the blood–CSF barrier, and the analysis of OCBs is time-consuming, not quantitative, and subject to investigator bias (Luxton, McLean, 1990).

In theory, the determination of free light chains (FLCs) might be a sensitive alternative to the above-mentioned approaches. Immunoglobulin light chains are typically secreted together with intact Ig by plasma cells. Although the half-life of kFLCs in serum is very short (2–4 h) because of rapid renal elimination, this clearance pathway is not available from CSF; therefore, is possible that the half-life of kFLCs in CSF is comparable to that of other proteins. Thus, even small amounts of intrathecal immunoglobulin synthesis with concomitant kFLC secretion will disproportionately increase the CSF concentration of kFLCs, making them a potentially sensitive marker of intrathecal immunoglobulin synthesis.

Attempts to determine FLCs in CSF have been made previously (Eickhoff and Heipertz, 1978; Lamers et al., 1995; Krakauer et al., 1998). We perform an automated nephelometric assay for the detection of FLCs based on specific monoclonal antibodies against epitopes that are hidden in intact immunoglobulins (Bradwell et al., 2001). We applied this test to CSF/serum pairs and evaluated its diagnostic accuracy in the detection of intrathecal immunoglobulin synthesis in comparison with the determination of OCBs, which is considered to be the most sensitive procedure and was taken as the reference standard for our study (Caudie et al., 2000; Richard et al., 2002).

2. Materials and methods

2.1. Patients

CSF/serum pairs from 80 patients with different clinically well-documented neurological disorders were collected; all patients had undergone an elective spinal puncture at the Neuroscience Department of the Tor Vergata University Hospital between September

Abbreviations: Ig, immunoglobulin; FLC, free light chain; CSF, cerebrospinal fluid; CNS, central nervous system; OCBs, oligoclonal immunoglobulin bands; MS, Multiple Sclerosis; Q_{alb}, CSF/serum ratio of albumin.

* Corresponding author at: University Hospital Tor Vergata, Viale Oxford 81, 00133 Rome, Italy. Tel.: +39 062 090 2359.
E-mail address: mariarita.dessi@uniroma2.it (M. Dessi).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.07.006

2012 and January 2013. The study protocol complied with the declaration of Helsinki (1964).

Samples were excluded if artificial blood contamination of CSF or monoclonal bands in both CSF and serum were present (n = 7). The patients were allocated to one of the following groups depending on the neurological diagnosis: non inflammatory disorders (group 1, 33 patients), inflammatory disorders, excluding Multiple Sclerosis (MS) (group 2, 24 patients) and MS (group 3, 23 patients).

Diagnosis of MS was established according to the McDonald criteria (Polman et al., 2011) at the end of the hospitalization period. After their admittance, all these patients underwent, in sequence, brain (and in selected cases also spinal) magnetic resonance imaging (MRI) scan and CSF withdrawal within 24 h. Corticosteroids or other MS-specific immunoactive therapies were initiated later when appropriate.

12/28 patients were in the acute phase of the disease, defined based on the presence of gadolinium-enhancing lesions at the MRI (n = 8) and/or a clinical relapse (n = 7). Relapses were defined as the development of new or recurrent neurological symptoms not associated with fever or infection lasting for at least 24 h.

The patient characteristics are shown in Table 1.

3. Methods

Immunoglobulin and albumin concentrations were measured by nephelometry (BN Prospec, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) in fresh CSF and serum samples.

OCBs were determined by immunofixation (Hydragel9 CSF Isofocusing; Sebia) on a semi-automated agarose electrophoresis system (Sebia Hydrasys) (Caudie, Allausen, 2000; Richard, Miossec, 2002). The system can detect the OCBs presence in a concentration range of 30–125 μg/L. Analysis was performed by laboratory technicians, and the results were read by an experienced staff physician and reported as positive or negative. After analysis of immunoglobulin concentrations and OCBs, samples were aliquoted and stored at −20 °C until further use.

Nephelometric measurement of kFLCs was performed with the N Latex FLC kappa Kit (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) that uses monoclonal antibodies for determination.

FLCs in CSF and serum samples were measured according to the manufacturer's protocol on the BN Prospec automated analyzer; the lower detection limit for kFLCs is 0.035 mg/L. Calibrators and controls were provided by the manufacturer and consisted of stabilized human sera containing polyclonal kFLCs; calibrators and controls were diluted to the appropriate concentrations for serum and CSF determinations.

As FLCs are also present in serum, they are able to passively diffuse into the CSF. CSF/serum quotients are calculated to evaluate this diffusion. An additional factor to consider is the permeability of the blood–CSF barrier. It is well known that the FLCs concentration increases with blood–CSF barrier permeability. The CSF/serum quotient of the FLCs was therefore plotted against the Q_{alb}. This is because albumin is only synthesized in liver and diffuses just passively into the CSF. The hyperbolic function commonly used to detect the limit between diffusion and synthesis of FLCs in the CSF is:

$$Q_{FLC} = \frac{a}{\sqrt[b]{(Q_{alb}^2 + b^2) - c}}$$

According to Reiber (Reiber, 2001), an optimal fit of the hyperbolic curve may be achieved using the following parameters: a/b = 6.5; $b^2 \times 10^6 = 5$; $c \times 10^3 = 1$.

Data were analyzed with Origin 7 (Origin Lab Corporation, Northampton, USA) and SPSS 17.0 (IBM Corporation, New York, USA) software. ANOVA followed by Bonferroni's test was used to determine the significance of the differences between the three groups. A p value of <0.05 was considered significant.

4. Results

4.1. Patients

CSF/serum pairs of 80 patients of the neurological clinic were examined. According to their diagnoses the patients were divided into three groups: group 1 (33 patients, 21 female and 12 male) patients having diseases without inflammation, patients with inflammatory diseases other than MS group 2 (24 patients, 15 female and 9 male) and patients with definitive MS group 3 (23 patients, 14 female and 9 male).

Patient characteristics and patient groups are shown in Table 1.

4.2. Detection of kFLCs

The median CSF concentration of kFLCs in group 1 was 0.07 mg/L (range, 0.04–0.33 mg/L). kFLCs were detectable in group 2, with median of 0.095 mg/L (range, 0.02–2.31 mg/L) and in group 3 with median of 1.08 (range, 0.08 – 11.7 mg/L) (Table 2).

The upper outlier in group 1 was a patient with vertigo. In group 2 the two upper outliers are a patient with leukoencephalopathy being study for MS and the other has an inflammation of cranial nerves (Fig. 1).

CSF/serum ratios were calculated for all patients and values ranged from 0.003 to 0.045 in group 1, from 0.001 to 0.955 in group 2 and from 0.01 to 2.12 in group 3. However we must considerer that these ratios are an overestimation for the cases with kFLCs in CSF below the lower detection limit.

Kappa CSF/serum ratios were plotted against the Q_{alb} to determine the influence of the blood CSF barrier (Fig. 2). Using the quotient diagram, intrathecal kFLCs production was identified in four patients from group 2.

All patients from group 1 lie below the hyperbolic dividing line, and all patients except one from group 3 lie above the hyperbolic dividing line. The only patient in group 3 that lies below Reiber hyperbolic function was an MS patient with no laboratory evidence of intrathecal immunoglobulin synthesis.

The use of kFLC Index calculated as $\frac{kFLC_{CSF}/kFLC_{serum}}{Qalbumina} \times 1000$ that takes into account the function of the blood–CSF barrier is tentative to increase the diagnostic accuracy of kFLC determination and to better

Table 1
Characteristics of the patient groups.

	Group 1 (n = 33)	Group 2 (n = 24)	Group 3 (23)
Sex (F/M)	21/12	15/9	14/9
Mean (minimum–maximum) age, years	50 ± 14 (23–79)	47 ± 15 (19–72)	39 ± 11 (21–58)
Cerebrovascular disease/bleeding, n	5		
Neurodegenerative diseases, n	7		
Neoplastic diseases, n	7		
Morbus Alzheimer, n	1		
Psychiatric/mentaldisorders, n	5		
Vertigo, n	1		
Focalpartialepilepsy, n	2		
Emicrania, n	2		
Miateniagravis, n	2		
Meningitis, encephalitis, n		2	
Leukoencephalopathy, n		7	
Cranialneuritis, n		2	
Polyneuropathy		5	
Myelitis/myelophaties, n		7	
Opticneuritis, n	1	1	
MS, n			23
OCBs positive, n	1	4	18
IgG Index positive, n	0	2	9