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Tear analysis in clinically isolated syndrome as new multiple sclerosis criterion

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Abstract

In clinically isolated syndrome (CIS), the detection of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) is critical for space dissemination validation when magnetic resonance imaging (MRI) diagnostic criteria are not fulfilled. However, lumbar puncture for CSF collection is considered relatively invasive. Previous studies have demonstrated applicability of OCB detection in tears to the diagnosis of multiple sclerosis (MS). The objective of the present study was to assess concordance between OCB detection in tears and in CSF. We have prospectively included patients with CIS and compared results of CSF and tear OCB detection by isoelectric focusing (IEF). Tears were collected using a Schirmer strip. We included 82 patients. For 69 of them, samples were analysable. OCBs were detected in CSF for 63.8% and in tears for 42% of patients. All patients with tear OCBs had CSF OCBs. We suggest that tear OCB detection may replace CSF OCB detection as a diagnostic tool in patients with CIS. This would circumvent the practice of invasive lumbar punctures currently used in MS diagnosis.

Keywords

cerebrospinal fluid, immunology, isoelectric focusing, multiple sclerosis, oligoclonal bands, tears

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Introduction

Cerebrospinal fluid (CSF) analysis constitutes an important tool in multiple sclerosis (MS) diagnosis. Since the publication of McDonald's criteria, the importance of CSF analysis for MS diagnosis has slightly decreased in favour of magnetic resonance imaging (MRI).[1] The most reliable MS marker in CSF is immunoglobulin G (IgG) oligoclonal band (OCB) detection.[2] CSF OCB presence in clinically isolated syndrome (CIS) is considered by many authors to be predictive of conversion to MS: their presence denotes increased conversion risk to clinically defined MS, especially when MRI is normal.[3,4]

As lumbar puncture is considered relatively invasive, several studies have addressed the possibility of detecting OCB in tears, with somewhat conflicting results. Coyle and Sibony were the first to assess increased IgG concentration in MS patient tears.[5] However, further studies did not find OCB in tears.[6–8] In 2001,

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Devos et al. studied 123 patients, in which 60 suffered from MS and 63 had other neurological disorders.[9] They found a concordance of 83% between tear and CSF for OCB presence or absence.

The aim of this study was to assess concordance of OCB detection results between CSF and tears for patients presenting with CIS. The secondary objectives were to study relations between tears/CSF isoelectric focusing (IEF) profiles and IgG rates, as well as Link's IgG index, disease symptoms, and MRI findings.

Patients and methods

The study plan was to include prospectively CIS patients from 10 French medical centres to compare IgG IEF in three fluids: CSF, tears and blood.

Subjects

CIS inclusion criteria were: a first neurological event suggestive of MS lasting for at least 24 hours and with symptoms and signs indicating at least one lesion, with an onset at no more than 3 months before inclusion; at least two T2-weighted MRI white matter hyperintensities of at least 3 mm that could not explain clinical symptomatology and in which one should be suggestive of MS (ovoid or periventricular). Exclusion criteria included remitting–relapsing MS (RRMS) or primary/secondary progressive MS (PPMS or SPMS) criteria fulfilment, Asian origin, contact lens prescription (to avoid any potential reflex secretion), eye infection, corticotherapy prescription for current symptoms and treatment by immunomodulatory drugs. Patients signed a consent form.

Technical procedures

Tears were collected using a Schirmer strip placed in the external cul-de-sac of each inferior eyelid. Collected tear volume did not exceed one to two graduations (5–10 ml). The dry end of the Schirmer strip was cut, and the sample was placed in a test tube (to avoid humidification by ambient air).

Ten to 15 CSF drops were collected in a dry tube by classical lumbar puncture. At the same time, a blood sample (2 ml) was collected from each patient into a dry tube and was then centrifuged at 3000 rpm for 15 minutes in order to extract serum. Samples were mailed to a single laboratory, which performed all analyses. When immediate shipping was not possible, samples were stored at −80°C.

OCB detection was carried out by IEF on agarose gel, using a SPIFE Combo® analyser (Helena Biosciences®, UK), followed by immunoblotting, according to

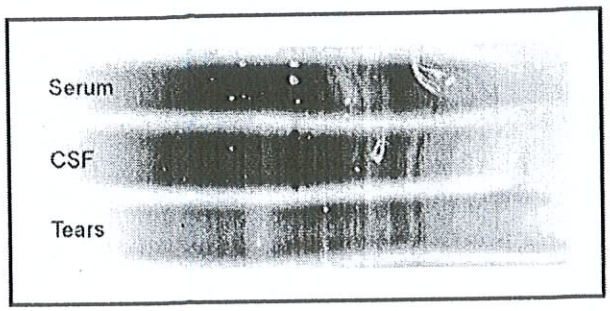

Figure 1. Example of isoelectric focusing (IEF) with immunoblotting. Oligoclonal bands (OCBs) present in tears and CSF are pointed out.

recommended criteria.[10] The first step of the analysis was to quantify IgG levels in CSF and serum and to standardize them by diluting serum with saline isotonic solution. IgG levels were quantified using Siemens Turbitimer®. Five microlitres of CSF and of serum were then loaded onto agarose gel. Tears were rehydrated with 50 µl of isotonic saline solution and 10 µl were picked up and loaded onto agarose gel without standardizing IgG levels. IEF was carried out for 60 minutes in the presence of anode (0.3M CH_3COOH) and cathode (1M NaOH) solutions, followed by protein transfer to a nitrocellulose membrane. Membranes were subsequently immunoblotted by blocking proteins (bovine milk proteins), followed by incubation with a peroxidase-conjugated goat anti-human-IgG serum. Finally, membrane signals were developed with chromophore and air-dried. Samples were analysed by two independent biologists. To read tear OCBs, a magnifying glass with incidental light is needed. The presence of at least three OCBs was required for a positive call (Figure 1). Non-interpretable samples were excluded. In case of disagreement, a dialogue was established between the assessors.

MRI was read by neuroradiologists blinded to diagnosis. The numbers and sizes of white matter hyperintensities were counted. For all patients, MRI was captured on a 1.5 Tesla machine and results were interpreted as fulfilling Barkhof's criteria or not.[11]

Statistical analysis

Clinical data were collected in case report form by treating neurologist. Demographic data, inclusion and exclusion criteria, clinical signs, duration of signs, previous history, therapeutics and MRI results were included. Global concordance was assessed by kappa coefficient. Fisher's exact test was used to study the association between each pair of CSF IEF and tear IEF and symptoms topography or MRI results. We analysed IEF results with other CSF values (IgG index and rates) using a Kruskal–Wallis test with the Bonferroni method and Mann–Whitney tests.

Results

Patients description

We included 82 patients and excluded 7 patients (because test tubes had broken during travel). Among the 82 patients included, 29 were male and 53 were female. First signs onset age was 33.6 ± 10 years (mean $\pm$ standard deviation). Samples were collected between September 2004 and December 2008. For 13 patients, tear samples were not analysable because of dilution, and they were therefore excluded from statistical analyses, so a total of 69 patients were included.

Concordance between CSF and tear IEF (Table 1)

The concordance rate between CSF OCB and tear OCB detection was 78.3% with a kappa coefficient of 0.58. Adjusted kappa reaches 1. Important imbalances between discordant results were assessed by MacNemar chi-squares test ($X^2MN = 13.07$, $P = 0.0003$). All patients with tear OCBs (29/69) had CSF OCBs (Table 1). OCBs have the same pattern in tears and in CSF, although for some patients less OCBs are detected in tears than in CSF. However, the number of bands is always enough to define OCBs (at least three). There was no OCB mirror pattern between CSF and serum, whereas two patients had OCB in serum and in CSF, but not in mirror.

Table 1. Isoelectric focusing (IEF) results in tears and CSF

	Tears−[a]	Tears+[b] (%)	Total (%)
CSF−[a]	25	0	25
CSF+[b]	15	29	44 (63.8%)
Total	40	29 (42%)	69

[a]CSF (or tears) = the absence of OCBs in CSF (or tears).
[b]CSF+ (or tears+) = the presence of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) (or tears).

Clinical signs, MRI findings and CSF IgG rates and indexes influence

Groups formed by a combination of tear IEF and CSF IEF results were not significantly different with regard to clinical signs topography (Fisher's exact test: $P = 0.49$); see Table 2. Similarly, no significant difference was found in MRI findings between groups (Fisher's exact test: $P = 0.14$). Thirty-seven CIS patients (53.6%) did not fulfil McDonald's criteria for space dissemination before CSF analysis (these patients presented less than three of Barkhof's criteria and non-plurifocal signs).[11]

We also studied IgG rates and IgG index variations between these same three groups. IgG rates comparison using a Kruskal–Wallis test with the Bonferroni method showed significant difference between all groups ($P < 0.0001$). We compared groups in pairs using a Mann–Whitney test (Figure 2): IgG rates were significantly higher in oligoclonal CSF groups than in non-oligoclonal groups, whether tear IEF was oligoclonal or not. IgG rates in contrast were not significantly different between oligoclonal and non-oligoclonal tear

Table 2. In each group of cerebrospinal fluid (CSF) and tear isoelectric focusing (IEF) results: repartition by gender, topography of clinically isolated syndrome (CIS) symptoms, MRI findings (defined by Barkhof's criteria fulfilment or not)

	CSF+ tears+[a]	CSF+ tears−[a,b]	CSF− tears−[b]	Total
Number of patients	29	15	25	69
Gender				
Male	8	8	10	26
Female	21	7	15	43
Symptoms				
Optic neuritis	4	1	6	11
Brainstem symptoms	8	6	6	20
Spinal cord symptoms	14	6	7	27
Polyregional symptoms	3	2	6	11
MRI results				
Barkhof−[c]	12	8	17	37
Barkhof+[d]	17	7	8	32

[a]CSF+ (or tears+) = the presence of oligoclonal bands (OCBs) in CSF (or tears).
[b]CSF− (or tears−) = the absence of OCBs in CSF (or tears).
[c]Barkhof− = Barkhof's criteria not fulfilled.
[d]Barkhof+ = Barkhof's criteria fulfilled.

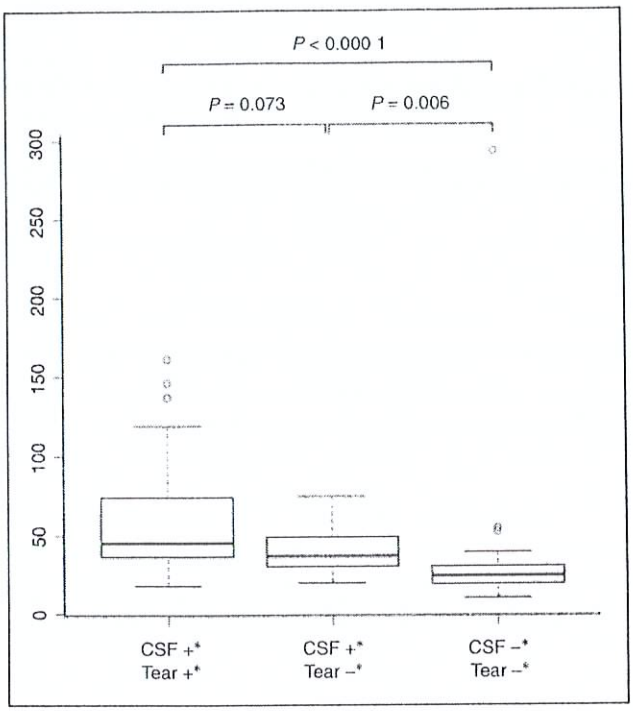

Figure 2. Immunoglobulin G (IgG) rate (mg/l). *CSF + (or Tear +) = the presence of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) (or tears); CSF − (or Tear −) = the absence of OCBs in CSF (or tears).

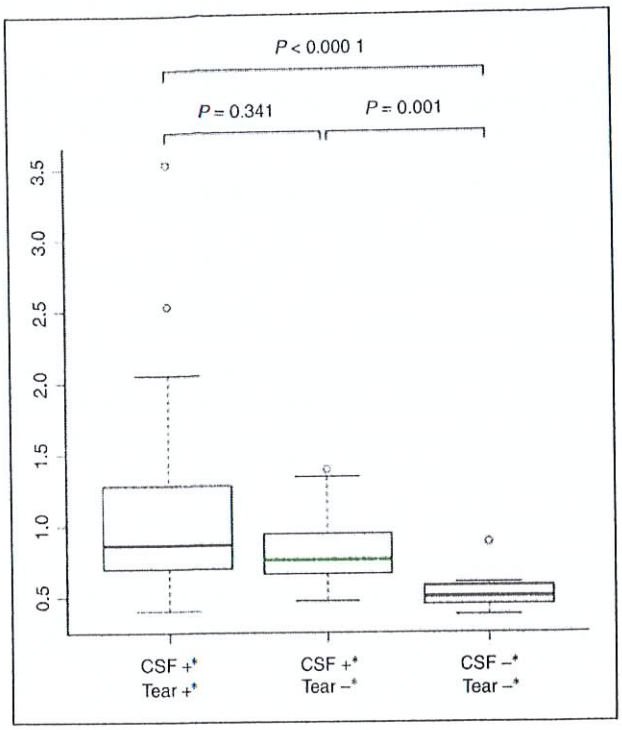

Figure 3. Immunoglobulin G (IgG) index. *CSF + (or Tear +) = the presence of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) (or tears); CSF − (or Tear −) = the absence of OCBs in CSF (or tears).

groups if CSF was oligoclonal. Similarly, IgG index were different between these three combinations, by Kruskal–Wallis analysis with a Bonferroni method ($P < 0.0001$), and a comparison of groups in pairs found a difference between each group, except between the oligoclonal tear group and non-oligoclonal tear group if CSF was oligoclonal (Figure 3).

Discussion

Concordance

This study has demonstrated concordance between tear IEF and CSF IEF in CIS patients. Furthermore, all patients with tear OCBs have also CSF OCBs. These results complement Devos et al.'s study (which included MS patients, patients with other neurological inflammatory diseases and other neurological patients) by targeting CIS patients exclusively, because these patients are most likely to require lumbar puncture for diagnostic purposes.[9] In their study, Coyle and Sibony analysed only tears and thus lacked a comparison with CSF results.[5] The analyses of Mavra et al. and Liedtke et al. did not reveal concordance between CSF IEF and tear IEF,[6,7] which can probably be attributed to the different sample collection techniques employed in those studies: these authors used tearing induction with onions and tear collection with capillary tubes, whereas the Schirmer strip technique was used by Devos et al. and in this study. That former method leads to substantial sample dilution, which probably makes it impossible to detect OCB. In these same studies, OCB presence in the tears of patients suffering from systemic disorders could potentially be explained by the frequent occurrence of xerophthalmia in those patients, resulting in sample concentration.

Our CIS patient population seems to match the descriptions in the literature closely. Even though CSF OCBs are found in 95% of patients with definite MS, they can be detected in only 61–63% of CIS patients[3,4] and their presence is predictive of conversion to MS.[12] Our finding that 63.8% of CIS patients have CSF OCBs is consistent with those findings. The kappa coefficient was not as high as expected, given the great imbalance between groups caused by the number of patients with OCBs in CSF but not in tears, as assessed by MacNemar chi-squared test. Kappa adjusted value also shows this asymmetric predictability.

Relation with MRI and CSF parameters

In contrast to previous reports,[4] in this study we did not find any association between CSF or tear IEF results and MRI fulfilment of Barkhof's criteria. CSF OCBs are known to be correlated with IgG rates and with Link's

IgG index.[2] Our study found similar association trend between OCBs (in tears or in CSF) and IgG rates or IgG index in CSF, which were not different between patients with CSF OCBs who had tear OCBs and those with CSF OCBs without tear OCBs. This finding supports the immunologic similitude of these two groups of patients.

Practical implications

According to the literature, on average rarely more than 50% of MRI performed in CIS patients fulfils Barkhof's spatial dissemination criteria at baseline.[12–14] This is close to our finding of 46.4% of CIS patients who fulfilled them and to our finding of 53.6% of CIS patients who fulfilled McDonald's criteria for space dissemination. Therefore, establishment of diagnosis for these patients requires CSF analysis. Furthermore, some authors suggest that CSF OCB absence may lead to MS diagnosis reevaluation.[15]

The tear OCB detection technique described here can easily be implemented in laboratories trained to conduct IEF. The only difference from classic the CSF OCB detection protocol consists of the dilution method, as described in the Methods section. Tear collection is also easily achieved using Schirmer strip following the procedure described above. We suggest that the best way to prevent dilution is to limit sample collection to a maximum of 10 ml, generally collected in less than 1 or 2 minutes. Further studies are needed to confirm the assumption that tear IEF is not interpretable because of dilution, due to a prolonged collection which may induce a reflex secretion.

Lower detection of OCBs in tears than in CSF is perhaps due to a lower sensitivity of the detecting method in tears (because of a lower proteic concentration). The technical difficulty of assessing the presence or absence of OCBs on nitrocellulose membrane after immunoblotting, because of its photosensitivity, represents a shortcoming of this study: results should be read quickly using incident light. It was not possible to make a precise numeration of OCBs with the naked eye using our technique. We have planned to measure OCB quantitatively with a camera in a future study.

A tear IEF approach can avoid lumbar puncture in more than one-third (42%) of CIS patients, according to results obtained in this study. This technique could be used as a first test, for example, in outpatient clinics, especially when McDonald's criteria are not met. In this way, lumbar puncture would only be used in cases showing negative lachrymal analysis.

Physiopathogenic hypothesis

Several hypotheses could explain tear OCB origin. A major explanation seems to be the presence of lymphoid follicles in the lachrymal gland that are close to the central nervous system's follicles. This may be supported by a compartmentalized immune response theory established regarding the presence of lymphoid follicle-like structures in cerebral meninges of some SPMS patients.[16,17] Other hypotheses include lymphoid cells or IgG direct migration through the blood–lachrymal barrier.

Perspectives

Preliminary results reported here are a starting point for further investigations studying the applicability of tear OCB detection to MS diagnosis. We plan to follow our patients by collecting new tear samples 1 and 2 years after first collection, to study the stability of tear IEF results. Tear OCB predictive values regarding CIS disease conversion to MS needs to be assessed in a global population and according to each clinical subgroup of patients. Furthermore, comparisons between tear OCB values and MRI findings should also be established, as well as between pathologic and sane eyes in optic neuritis. Another perspective is to study tear OCB detection in other diseases known to show CSF OCBs frequently, such as chronic meningitis, Lyme disease and other chronic central nervous system infections, lupus and other inflammatory diseases, and paraneoplastic syndromes. It would also be interesting to collect tears in patients with PPMS. Indeed, in this disease, OCB detection is an important diagnosis criterion.[18]

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