Tear analysis in a radiologically isolated syndrome as a new tool to predict the risk of clinical conversion

C LEBRUN*, G FORZY, N COLLONGUES***, M COHEN*, J de SEZE***, P HAUTECOEUR** on behalf CFSEP and RISConsortium***

* neurologie, CHU de Nice

** Institut catholoique de Lhomme

*** neurologie, CHU Strasbourg

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Correspondance:

LEBRUN christine

Service de neurologie. Hôpital Pasteur. 30 voie romaine. 06002. Nice. France.

LEBRUN.c@chu-nice.fr

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Abstract:

Although Radiologically isolated syndrome (RIS) is a newly defined entity, incidental findings of T2 hypersignals on brain MRI can lead to misdiagnosis or useless investigations. The detection of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) is a major indicator that confirms diagnosis of infra clinical inflammatory disease of the central nervous system, but lumbar puncture still remains an invasive option. We have prospectively included patients with RIS and compared the results of CSF and tear OCB detection by isoelectric focusing (IEF) and assessed concordance between OCB detection in tears and in CSF. Tears were collected using a Schirmer strip. In 45 recruited RIS patients, OCBs were detected in CSF for 55% (25/45) and in tears for 50% (21/42) of samples. We suggest that tear OCB detection may replace CSF OCB detection as a diagnostic tool in patients with RIS.

Introduction:

Radiologically isolated syndrome (RIS) is a recently defined entity that describes the incidental discovery of lesions suggestive of multiple sclerosis (MS) on brain magnetic resonance imaging (MRI), with demonstration of dissemination in space without symptom expression, with a normal neurological examination, and no better medical explanation to account for the observed anomalies. In RIS, the detection of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) is critical for space dissemination validation associated with MRI multiple sclerosis (MS) diagnostic criteria, as published by Barkhof in 1997 (1). It gives strength to RIS diagnosis compared with other incidental white matter T2 lesions. However, lumbar puncture for CSF collection is considered relatively invasive. Previous studies have demonstrated applicability of OCB detection in tears to the diagnosis of MS and CIS (2, 3).

Materials and methods

All participating subjects met strict entry criteria for RIS as defined previously and underwent annual clinical assessment, brain and spinal MRI scans as part of an observational, prospective, multi-center, longitudinal study protocol that included 3 recruiting sites specialized in central nervous system (CNS) inflammatory diseases in France (see Appendix) (4, 5). All participants gave their informed consent and were asked for a screening diagnosis. At baseline, all RIS patients had paraclinical analyses (CSF, visual evoked potentials – VEP, spinal MRI) and biological screening (serological panel, antinuclear and antiphospholipid antibodies). Informed written consent was obtained from all patients followed in MS centers, which use EDMUS software (European Database for MS). All patients were given a unique EDMUS identification number. According to French national regulations (CNIL), EDMUS databases were declared and no additional approval was specifically needed from the ethics committee or institutional board for this kind of descriptive study. Data were analyzed anonymously.

A total of 100 consecutive RIS subjects were identified, of which 45 patients were enrolled for the specific tear analysis. The objective was to study the relationship between tear/CSF isoelectric focusing (IEF) profiles and IgG rates, as well as the Link's IgG index. 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 (2ml) was collected from each patient into a dry tube, which was then centrifuged at 3000 rpm for 15 minutes in to obtain the serum. Samples were mailed to a single laboratory, which performed all the analyses. Quantification of IgG levels in CSF and serum was performed as previously described (6). The presence of at least three OCBs was required for a positive result. Non-interpretable samples were excluded. The number of bands to define a OCBs positive status was at least three. Univariate analysis was performed with SPSS 20.0 software for Mac OS X.

Results

We included prospectively 45 patients. For 3 of them, samples were non analyzable due to an insufficient quantity of tears. OCBs were detected in CSF for 55% (25/45) and in tears for 50% (21/42) of patients. The concordance rate between CSF OCB and tear OCB detection was 69% with a kappa coefficient of 0.95.

All patients with tear OCBs had CSF OCBs. One patient had a positive CSF and negative tears (figure 1). 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 protein concentration). The technical difficulty of assessing the presence or absence of OCBs on nitrocellulose membranes after immunoblotting, because of its photosensitivity, represents a shortcoming of this study: results should be read quickly using incidental light.

Discussion

Coyle and Sibony were the first to assess the increased IgG concentration in MS patients' tears (7). The presence of CSF OCB in RIS is considered to be predictive of conversion to a clinical event, which was demonstrated earlier in CIS (Calais). In a previous study, the CSF criteria were statistically significant when associated with 9 or more T2-hyperintense lesions on the first MRI (5). Intrathecal immunoglobulin synthesis and oligoclonal bands were found in more than 90% of patients with MS. In our previous study, 72.8% of patients had at least 1 abnormal result on CSF analysis (either an increased IgG index or the presence of OCB). MRI and CSF criteria had a high specificity but less sensitivity and accuracy. These results reinforced the role of examination of CSF in predicting the risk of clinical conversion.

The examination of tears has demonstrated concordance between tear IEF and CSF IEF in CIS patients. Furthermore, all patients with tear OCBs also had CSF OCBs.

Our RIS patient population seems to match closely descriptions in the literature. Associated with spatial dissemination MRI criteria, the OCB detection denotes a statistically significant increased conversion risk to clinical conversion. For OCB negative patients, a follow-up of tears could help early detection of CSF conversion.

Several hypotheses could explain the tear OCB origin. A probable explanation seems to be the presence of lymphoid follicles in the lachrymal gland that are close to the central nervous system's follicles (8). We suggest that tear OCB detection may replace CSF OCB detection as a diagnostic tool in patients with RIS. The tear OCB detection technique described here can easily be implemented in laboratories trained to do IEF.

This would circumvent the practice of invasive lumbar puncture and would probably increase facilities for patients and neurologists to accept MRI T2 hypersignal investigations.

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Conflict of interest

None declared. The authors declare that they have no conflict of interest in relation to the present study.

Appendix:

RISConsortium: D Okuda, À Siva, O Kantarci, D Pelletier

CFSEP: Olivier Heinzlef, Jérôme de Seze, Sophie. Pittion,Hélène Zephyr, Olivier Anne, Bertrand Audouin , Eric Berger, David Brassat, Bruno, Brochet, Bertrand Bourre, Philippe Cabre, Jean-Philippe Camdessanche, Olivier Casez, Pierre Clavelou, Nicolas Collongues, Marc Coustans, Alain Créange , Marc Debouverie, Nathalie Derache, Gilles Defer, Dominique Dive , Agnès Fromont, Riadh Guider, Jérôme Grimeau, Arnaud Kiatkoswki, Pierre Labauge, David Laplaud, Christine Lebrun, Emmanuelle Le Page, Romain Marignier, Thibaut Moreau, Jean-Christophe Ouallet, Caroline Papeix, Jean Pelletier, Lucien Rumbach, Myriam Schluep, Pierrette Seeldrayers, Ilham Slassi Sennou, Bruno Stankoff, Frédéric Thaite, Ayman Tourbah, Eric Thouvenot, Patrick Vermersch, Sandra Vukusic, Sandrine Wiertlewski

Figure 1: representation of patients with positive concordance between cerebrospinal (csf) and tears in oligoclonal bands (ocb) detection (csf-/tears- and csf+/tears+). Only one patient had a positive CSF and negative tears and 3 patients were non analyzable. These cases were probably due to dilution or lack of tears.