Concise report

Inflammatory profile in cerebrospinal fluid of patients with headache as a manifestation of neuropsychiatric systemic lupus erythematosus

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Abstract

Objective. The objective of this study was to define the cytokine and chemokine profiles in cerebrospinal fluid (CSF) from patients with headache as neuropsychiatric systemic lupus erythematosus (NPSLE).

Methods. In a post hoc analysis, seven patients hospitalized because of headache were included. Patients were evaluated at hospitalization and 6 months later and a CSF sample was obtained. As controls, CSF from 27 patients with other NPSLE syndromes, 16 SLE patients without a history of NP manifestations (non-NPSLE) and 25 patients with non-autoimmune diseases were studied. Soluble molecules including cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-α and IFN-γ) and chemokines [monocyte chemo tactic protein-1, RANTES (regulated on activation normal T cell expressed and secreted), IL-8, monokine induced by IFN-γ (MIG), and IFN-γ-induced protein 10 (IP-10)] were measured with the use of cytometric bead array kits or luminometry.

Results. Patients with headache had increased CSF values in the following molecules compared with non-NPSLE and non-autoimmune diseases patients, respectively: IL-6 (208.5, 3.0, 3.0 pg/ml, P < 0.004 and P < 0.001), IL-8 (406.6, 30.0, 19.7 pg/ml, P < 0.05 and P < 0.004), IP-10 (4673, 329.7, 113.6 pg/ml, P = 0.02 and P < 0.002), RANTES (7.5, 2.5, 2.2 pg/ml, P < 0.003 for both) and MIG (944.7, 11.4, 3.5 pg/ml, P = 0.02 and P = 0.001). No clear difference was observed between patients with headache and other NPSLE. Higher levels of inflammatory molecules were found in patients with headache from intracranial hypertension and intractable non-specific headache than patients with migraine. Six months later, when the headache had resolved, all the elevated molecule levels had decreased significantly.

Conclusion. Headache from intracranial hypertension and intractable non-specific headache, but not migraine, share the inflammatory profile in CSF observed in other NPSLE syndromes.

Key words: SLE, neuropsychiatric systemic lupus erythematosus, headache, intracranial hypertension, intractable non-specific headache, IL-6, cytokines, chemokines, cerebrospinal fluid, CNS.

Introduction

Neuropsychiatric (NP) manifestations in SLE patients (NPSLE) are still poorly understood. In order to standardize their study, the ACR has defined 19 neuropsychiatric syndromes, from headache and cognitive dysfunction to seizures and psychosis [1].

Independent of their severity, the attribution of these syndromes may become a complex task, since there is neither a serological test nor one in cerebrospinal fluid (CSF) that unequivocally defines whether any of these syndromes is or is not secondary to SLE. For now, their attribution depends on clinical criteria and on the exclusion of competing causes [2, 3].

The ACR glossary of NP manifestations attributes five different types of headache to SLE [1]. However, a population-based study proposes that headache should be excluded, since it is not related to lupus and its inclusion
reduces specificity to the NPSLE criteria [4]. This recommendation has been adopted by the largest cohort on NPSLE [3]. On the other hand, the two major indices to assess lupus activity include headache as a manifestation of lupus and assign it a high activity weight [5, 6]. Unfortunately, no study has formally addressed whether intractable non-specific headache, headache from intracranial hypertension and cluster headache are related to lupus.

Our group carried out a series of studies in serum and CSF aiming to clarify the pathogenesis of NPSLE manifestations. We analysed cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ) and chemokines (monocyte chemotactic protein (MCP)-1/CCL2, IL-8/CXCL8, IFN-γ-induced protein 10 (IP-10)/CXCL10, monokine induced by IFN-γ (MIG)/CXCL9 and regulated on activation normal T cell expressed and secreted (RANTES)/CCL5), a variety of autoantibodies and even a blood-brain barrier lesion marker such as the protein S100B [7–10]. In CSF an inflammatory profile was identified with high levels of RANTES, MIG, IP-10, IL-6 and IL-8 during the acute event that decline when clinically in remission [7].

Due to the controversy stated above, we aimed to define the inflammatory profile in the CSF of lupus patients with intractable/refractory headache, benign intracranial hypertension and migraine and compare it with SLE patients with other major NP manifestations, non-NPSLE patients and patients with non-autoimmune diseases.

Patients and methods

We conducted a post hoc analysis focusing on headaches from data published previously [7]. In that study we included 34 patients with SLE diagnosed according to the ACR criteria [11] who were hospitalized at our institution because of NP manifestations and from whom a CSF sample was obtained. All the patients were evaluated by the participating rheumatologists and neurologists at the hospitalization and 6 months later according to a standardized protocol. The study was approved by our hospital’s institutional review board (Comité Institucional de Investigación Biomédica en Humanos) and all patients gave their written informed consent.

In this analysis we identified 7 patients with headache and 27 with other NPSLE manifestations (seizures, 13; acute confusional state, 8; cerebrovascular disease, 4; psychosis, 1; transverse myelitis, 1), classified according to the ACR nomenclature and case definitions for NPSLE. Manifestations were attributed to SLE based on the absence of exclusion factors for the attribution of NP manifestations [1].

A CSF sample was obtained from all patients upon their admission to the hospital and a second sample was obtained 6 months later from four of the seven patients with headache. As controls, we studied CSF samples from 16 SLE patients without current or past NP manifestations (non-NPSLE group) and 25 patients with non-autoimmune diseases and without NP manifestations (non-autoimmune disease group) who, during the study period, underwent elective surgery that required spinal block and gave their written permission for collection of a CSF sample. The surgeries were bone marrow donation in seven patients, hysterectomy in six, Tenckhoff catheter placement in three, hydrocele in two, lower limb amputation due to type 2 diabetes mellitus in two, saphenectomy in two, circumcision in one, umbilical hernia in one and inguinal hernioplasty in one.

The CSF was centrifuged at 12 000 g and the supernatant was immediately frozen (in all instances in <30 min) at −86°C until assayed for cytokine and chemokine content.

Flow cytometric detection of cytokines and chemokines

Soluble molecules were measured by cytometric bead array kits (BD Biosciences, San Diego, CA, USA) according to the manufacturer’s recommendations. These included (i) cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IFN-α) and (ii) chemokines (CCL2/MCP-1, CCL5/RANTES, CXCL8/IL-8, CXCL9/MIG and CXCL10/IP-10). Fifty microliters of CSF per test were used. Samples were analysed in a FACscan flow cytometer (Becton Dickinson, San Jose, CA, USA) using the BD CBA software (BD Biosciences). IFN-α was measured using Luminex bead-based technology according to the manufacturer’s recommendation (Invitrogen, Carlsbad, CA, USA). Results are expressed as picograms per millilitre. As the levels of IL-2, IL-4, IL-10, TNF-α and IFN-γ were low or undetectable among all groups studied, IFN-α, IL-6, CCL2, CCL5, CXCL8, CXCL9 and CXCL10 were the only molecules included in this post hoc analysis.

Statistical analysis

Continuous variables were analysed using Student’s t-test, Mann–Whitney U-test, or one-way analysis of variance. Cytokine and chemokine values are presented as the median and interquartile range. P-values < 0.05 (two-tailed) were considered significant. Analysis was performed using the SPSS 12.0 computer program (SPSS, Chicago, IL, USA).

Results

Population characteristics

Eighty-four per cent of the SLE patients were females. The mean age (s.d.) in each group is as follows: headache group, 27.6 (5.8) years; NPSLE group, 32.4 (13.3); non-NPSLE group, 37.7 (9.8) and non-autoimmune diseases group, 37.5 (15.3). SLE duration [mean (s.d.)] was 2.5 (2.9) years in the headache group, 4.0 (4.9) in the other NPSLE group and 8.8 (6.5) in the non-NPSLE group (P = 0.01). Disease activity was severe in patients with headache and other NPSLE manifestations [SLEDAI-2K score 10.6 (3.4) and 16.1 (8.8), respectively] in contrast to non-NPSLE patients in whom lupus activity was mild [SLEDAI-2K score 3.8 (1.55) (P < 0.001) [5]. The Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index (DI) score was 0.14 (0.4) in the headache
group, 0.6 (1.2) in the other NPSLE group, and 0.8 (0.4) in the non-NPSLE group ($P = 0.21$) [12].

**Headache characteristics**

The clinical diagnoses were intractable refractory headache in three patients, migraine without aura in two and benign intracranial hypertension and pseudotumor cerebri in one patient each. Except in the two patients with migraine in whom the headache responded with NSAIDs, in all the others headache improved after high doses of corticosteroids were administered. Other clinical characteristics and the levels of inflammatory molecules in each patient are shown in supplementary Table S1, available at *Rheumatology* Online.

**Chemokine and cytokine levels**

We initially compared individually the levels of the studied chemokines, IL-6 and INF-$\alpha$, between patients with headache and NPSLE vs patients with non-autoimmune diseases. The results showed a significant increase in the levels of IP-10, IL-8, MIG, RANTES and IL-6 in patients with headache ($P < 0.005$). Also, NPSLE patients had a significant increase in the levels of IFN-$\alpha$, IP-10, MCP-1, IL-8, IL-6, MIG and RANTES ($P < 0.02$).

Subsequently we analysed the levels of the inflammatory molecules between patients with headache and non-NPSLE patients; again, patients with headache showed a significant increase in the levels of IL-6, IL-8, RANTES, MIG and IP-10 ($P < 0.05$). Finally, the levels of the inflammatory molecules studied were similar between patients with headache and patients with NPSLE, except for IFN-$\alpha$, which was higher in NPSLE, and for MIG, which was higher in headache patients (see Table 1).

**Cytokine and chemokine levels at the time of hospitalization and 6 months later in patients with headache**

Six months after hospitalization a second CSF sample was obtained from four of the seven patients with headache. The diagnoses in these four patients were intractable refractory headache in two and pseudotumor cerebri and migraine without aura in one patient each. Except for the patient with migraine, all the other patients received prednisone (or its equivalent) at a dose of $\geq 45$ mg/day. At the time of the 6-month follow-up visit, headache had resolved in all patients and the SLEDAI-2K score had decreased significantly [median (minimum–maximum) at baseline 9 (8–18), at 6 months 2 (0–6)].

Fig. 1 shows the individual data per type of headache for the molecules whose levels decreased significantly between baseline and 6 months. Patients with intractable refractory headache showed a clear-cut decrease in IL-6, IP-10, IL-8, MIG and RANTES. The patient with pseudotumor cerebri had a decrease in MCP-1, IP-10 and MIG. However, the levels of IL-6 and chemokines did not vary in the patient with migraine.

**Discussion**

In this post hoc analysis, patients with headache showed higher levels of IL-6 and chemokines in CSF than non-NPSLE and non-autoimmune patients; the levels were similar to those of patients with seizures, acute confusional state, cerebrovascular disease, psychosis and transverse myelitis. Levels of IL-6, IL-8, IL-10, MIG and MCP-1 in patients with intractable refractory headache, benign intracranial hypertension and pseudotumor cerebri were higher than in patients with migraine. Six months later, when the headache had resolved, the levels of the inflammatory molecules showed a notable reduction in

**Table 1 Inflammatory molecule levels in CSF from SLE patients with headache, other NP manifestations and controls**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Headache ($n = 7$)</th>
<th>NPSLE ($n = 27$)</th>
<th>$P^*$</th>
<th>Non-NPSLE ($n = 16$)</th>
<th>$P^*$</th>
<th>Non-AU ($n = 25$)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-$\alpha$</td>
<td>14.2 (3.2–25.3)</td>
<td>38.9 (15–74.9)</td>
<td>0.04</td>
<td>15 (6.7–40.1)</td>
<td>0.45</td>
<td>17.4 (3.7–28.7)</td>
<td>0.63</td>
</tr>
<tr>
<td>IP-10</td>
<td>4673 (853.5–5636.7)</td>
<td>1014.7 (214.1–2094.6)</td>
<td>0.09</td>
<td>329.7 (190.1–583.6)</td>
<td>0.02</td>
<td>133.6 (84.2–164.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>MCP-1</td>
<td>333 (77.9–948.7)</td>
<td>566.2 (204.55–1518.7)</td>
<td>0.28</td>
<td>257.9 (165.1–391.5)</td>
<td>0.87</td>
<td>136.9 (89–177.6)</td>
<td>0.35</td>
</tr>
<tr>
<td>IL-8</td>
<td>406.6 (32.2–874.2)</td>
<td>106.8 (35.6–211.1)</td>
<td>0.56</td>
<td>30 (21.4–48.5)</td>
<td>0.05</td>
<td>18.7 (13.6–24.9)</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-6</td>
<td>208.5 (5.7–358.5)</td>
<td>18.8 (2.6–107.6)</td>
<td>0.24</td>
<td>3 (1.32–5.75)</td>
<td>0.004</td>
<td>3 (2.1–3.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>MIG</td>
<td>944.7 (18–4957.8)</td>
<td>27.8 (9.4–99.2)</td>
<td>0.05</td>
<td>11.4 (5.7–36.9)</td>
<td>0.02</td>
<td>3.5 (2–6.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>RANTES</td>
<td>7.5 (3.2–18.8)</td>
<td>3.8 (3–5.4)</td>
<td>0.18</td>
<td>2.5 (2–3.4)</td>
<td>0.003</td>
<td>2.2 (1.9–4.1)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are given as median (interquartile range) and are expressed as picograms per millilitre. NPSLE: other neuropsychiatric manifestations; non-NPSLE: non-neuropsychiatric manifestations; non-AU: non-autoimmune patients. *vs headache (Mann-Whitney $U$-test).
patients with intractable refractory headache and pseudotumor cerebri, but not with migraine. Overall, these results support that intractable refractory headache, headache secondary to benign intracranial hypertension and pseudotumor cerebri in patients with SLE are actually inflammatory and truly NPSLE manifestations, but not migraine.

Several studies have shown that intrathecal cytokines such as IL-6 and chemokines such as IL-8 are useful for the diagnosis of NPSLE [13, 14]. Also, MCP-1/CCL2 and IP-10/CXCL10 have been detected in these patients, suggesting an inflammatory reaction mediated by these molecules at the CNS level [15, 16].

We described a peculiar profile of cytokines and chemokines in the CSF of NPSLE patients [7]. In this post hoc analysis with a focus on patients with headache attributable to lupus, the results corroborate the presence of such an inflammatory profile, suggesting the occurrence of an inflammatory process in its pathogenesis since this response is similar to that found in other NPSLE patients. The potential role of these molecules within the pathogenesis of NPSLE is as chemoattractants, but they also have a regulatory role in the intensity and quality of the inflammatory response [17].

We are not aware of any other study where inflammatory molecules have been measured in the CSF of patients with headache as NPSLE. The single report of cytokines and chemokines in the CSF of patients with tension-type headache, migraine and cervicogenic headache showed an increase in the levels of IL-1Ra, MCP-1 and TGF-β in comparison to controls, but it was a very low-grade increase compared with those often accompanying serious neurological conditions, and may represent a mild response to pain [18].

We acknowledge some limitations of our study. It is a post hoc analysis of a larger study where the inflammatory
profile of NPSLE patients was identified, however, we believe there is no circularity in the results since we focused on a specific manifestation in comparison to all the others. The number of patients with headache is small to derive a robust conclusion about its pathogenic mechanism. We did not include lupus patients with tension-type or cluster headache, so we do not know if these inflammatory molecules are also elevated in the CSF of patients with these syndromes. This analysis offers new information that may be useful to properly characterize one of the most common and heterogeneous NPSLE manifestations whose attribution has been questioned recently. This study included lupus patients with tensional headache or migraine but not other types included in the ACR glossary of NPSLE [4], therefore the recommendation to withdraw headache as an NPSLE manifestation seems erroneous.

Based on the results of this analysis, we conclude that, as defined by the ACR glossary, headache from intracranial hypertension and intractable non-specific headache are of an inflammatory nature and should remain as NPSLE syndromes; however, migraine is non-inflammatory and might be excluded from this nomenclature.

**Rheumatology key messages**

- In SLE, headache from intracranial hypertension and intractable non-specific headache are of an inflammatory nature.
- In SLE, migraine has a non-inflammatory nature and might be removed from the NPSLE nomenclature.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

**References**