# Cerebrospinal fluid interleukin (IL)-10 and IL-10:IL-6 ratio as biomarkers for small B-cell lymphoproliferations with leptomeningeal dissemination

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# CSF IL-10 and IL-10:IL-6 ratio as biomarkers for small B-cell lymphoproliferations with leptomeningeal dissemination

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Identifying the etiology of neurological symptoms in blood malignancies is still a challenging issue. Lymphomatous meningitis (LM) is mainly described in aggressive systemic lymphomas (diffuse large B-cell (DLBCL) and Burkitt lymphomas) [1, 2]. Leptomeningeal involvement in small B-cell lymphoproliferative disorders (CLPD) is a rare, poorly described condition, only mentioned in a few case-reports [3, 4]. The diagnosis is suspected in patients presenting non-specific central nervous system (CNS) symptoms and non-specific results on medical imaging. It is confirmed by the detection of tumor cells in the cerebrospinal fluid (CSF). At present, conventional cytology (CC) can confirm the diagnosis for high tumor burden with excellent specificity but its sensitivity remains low. During the past decade, flow cytometry analysis (FCM) has been reported as a highly sensitive tool to detect lymphomatous cells. To overcome early cell mortality, the adjunction of a stabilizing cell solution to CSF can be used [5]. Unfortunately, these tests are not always efficient enough to diagnose LM. New complementary approaches are required. Soluble biomarkers seem to be potent candidates since they can be processed using multiplex techniques on quite small volumes; hence overcoming cellular pitfalls. Among the various screened candidates, interleukin (IL)-10 is promising[6, 7]. It is a pleiotropic, anti-inflammatory, cytokine involved in B-cell differentiation. This marker has been largely used to diagnose primary vitreoretinal lymphoma (PVRL) [8, 9]. More recently, its role in primary CNS lymphoma (PCNSL) and systemic DLBCL with CNS involvement has been documented [10-13]. It has also been proposed in dual with IL-6 level to determine the IL-10:IL-6 ratio [7, 14]. The aim of our study was to evaluate the diagnostic value of IL-10 and IL-6 levels combined with the IL-10:IL-6 ratio in CSF in CLPD with LM.

Thirty patients were recruited over 4 years from the two hematology centers in Paris: Pitie Salpetriere and Saint Louis university hospitals. All patients suffered from small B-cell LM: 7 chronic lymphocytic leukemias (CLL), 6 mantle-cell lymphomas (MCL), 14 Waldenström macroglobulinemias (WM), 1 follicular lymphoma (FL), and 2 unclassified B-cell non-Hodgkin lymphomas (u B-NHL). All patients presented CNS symptoms and medical imaging was evocative of an LM. Leptomeningeal dissemination was revealed by CC, FCM or immunoglobulin heavy chain rearrangement analyses (IGHV), before intrathecal chemotherapy, either at the diagnostic stage or at the time of the relapse. IL-10 and IL-6 were measured by the Cytometric Bead Array® technique (human IL-10 and IL-6 CBA kits; BD Biosciences™, Pont de Claix, France) on a FACSCantoII flow cytometer (BD Biosciences™) following the manufacturer's recommendations. This method is correlated with the standard sandwich enzyme immunoassay (ELISA) technique [7] and the limit of sensitivity is 2.5 pg/ml. Data were analyzed on the FACSDiva software (BD Biosciences™). This is a retrospective medical record review study performed with approval from the Pitie-Salpetriere hospital committee. All existing data were processed without any additional tests and were treated confidentially.

Patients were mostly male (male:female ratio of 2) with a median age of 71 (range [49-88]). Notably, IL-10 levels were generally low. Indeed, approximately half of the patients (n=17) had an undetectable IL-10 level (<2.5 pg/ml) along with undetectable or low levels of IL-6 [2.5-14 pg/ml], leading to an IL-10:IL-6 ratio <1 or *undeterminable* (group n°1). All the other patients had detectable IL-10 levels [4-39 pg/ml] with heterogeneous levels of IL-6 [4-540 pg/ml] (table). The IL-10 cutoffs previously described in three studies on PCNSL and DLBCL with LM fluctuated (3, 9.5 and 16.5 pg/ml) [10, 11, 13, 15]. Therefore, IL-10 level is not enough as an isolated marker for aggressive lymphomas with LM. We applied the IL-

10:IL-6 ratio with a threshold set at 1, as described in PRVL[9], to divide patients into 2 additional subgroups: group n°2 with ratios ≤1 (n=9) and group n°3 with ratios >1 (n=4). In the vast majority of the patients, CLPD with LM were associated with an IL-10:IL-6 ratio of ≤1 (groups n°1 and n°2). All these patients with ratios IL-10:IL-6 ≤1 presented CLPD with LM, but no evidence of aggressive B-cell lymphoma. Surprisingly, even in patients with MCL which is considered to be an aggressive lymphoma, IL-10:IL-6 ratios in the CSF were still low. Interestingly, in WM patients with LM (n=14), also called Bing Neel syndrome[16], IL-6 levels tend to be higher (median value: 7.5 pg/ml]) than in CLL (median value: 4 pg/ml) and MCL patients (median value: 4.5 pg/ml). The 2 WM patients who had the highest cell count in the CSF (#25 and 26) also presented the highest IL-6 levels. High levels of IL-6 in the CSF could be an additional argument for BNS as IL-6 plays a central role in the WM microenvironment[17]. Moreover, further investigations on IL-6 levels seem to be interesting in BNS to examine its role in WM pathophysiology. Group n°3 includes patients suffering from both CLPD and another aggressive lymphoma. Two patients had PCNSL simultaneously diagnosed in the CSF. For patient #29, these results were confirmed by the IGHV analysis on the brain biopsy and the CSF, showing 2 different tumoral clones. Patient #27 presented a blastoid variant of MCL. Patient #30 had a transformed WM into DLBCL in the CNS during follow-up. Additionally, these 4 patients also presented an increased IL-10 level (≥10 pg/ml) (table). Furthermore, an elevated IL-10 level associated with an IL-10:IL-6 ratio >1 occurring in patient with indolent lymphoma LM should potentially require further investigations to detect any concomitant large B-cell lymphoma, as revealed by our cohort. In these difficult cases, the contribution of IGHV analysis could be essential to confirm the co-existence of multiple tumoral clones. Other soluble biomarkers such as CXCL13 [10], soluble CD19 [18] or soluble CD23 [19] could be relevant in this context.

In conclusion, we here report for the first time that low levels of IL-10 do not exclude LM in CLPD. Unexpectedly, IL-10 levels and IL-10:IL-6 ratio in CLPD differed from the levels observed in DLBCL. We report the usefulness of adding the IL-10:IL-6 ratio in order to potentially reveal more aggressive lymphomas: either a transformation or an association with another "hidden" lymphoma such as PCNSL.

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Table

Patient clinical characteristics and CSF data

BNS: Bing Neel syndrome, CC: conventional cytology, CLL: lymphocytic leukemia, FCM: flow cytometry, FL: follicular lymphoma, IGHV: immunoglobulin heavy chain variable region genes, IL: interleukin, MCL: mantle-cell lymphomas, NP: not performed, PCNSL: primary central nervous system lymphoma, u B-NHL: unclassified B-cell non-Hodgkin lymphoma, WBC: white blood cells count, WM: Waldenström macroglobulinemia

| Groups | Patients | Diagnosis | WBC/mm$^3$ | CC | FCM | IGHV | IL-10 *(pg/ml)* | IL-6 *(pg/ml)* | IL-10:IL-6 ratio |
|---|---|---|---|---|---|---|---|---|---|
| | #1 | CLL | 10 | - | + | + | <2.5 | <2.5 | *Undeterminable* |
| | #2 | CLL | 0 | - | + | + | <2.5 | <2.5 | *Undeterminable* |
| | #3 | CLL | 0 | - | + | + | <2.5 | <2.5 | *Undeterminable* |
| | #4 | MCL | 6 | - | + | + | <2.5 | <2.5 | *Undeterminable* |
| | #5 | WM (BNS) | 7 | - | + | - | <2.5 | <2.5 | *Undeterminable* |
| | #6 | WM (BNS) | 0 | - | - | + | <2.5 | <2.5 | *Undeterminable* |
| | #7 | CLL | 30 | - | + | + | <2.5 | 7 | <1 |
| | #8 | CLL | 2 | - | + | *NP* | <2.5 | 14 | <1 |
| 1 | #9 | MCL | 3 | - | + | *NP* | <2.5 | 5 | <1 |
| | #10 | WM (BNS) | 0 | - | + | + | <2.5 | 3 | <1 |
| | #11 | WM (BNS) | 3 | + | - | + | <2.5 | 4 | <1 |
| | #12 | WM (BNS) | 1 | + | + | + | <2.5 | 4 | <1 |
| | #13 | WM (BNS) | 0 | - | + | *NP* | <2.5 | 9 | <1 |
| | #14 | CLL | 14 | - | + | - | <2.5 | 4 | <1 |
| | #15 | MCL | 0 | - | + | + | <2.5 | 4 | <1 |
| | #16 | WM (BNS) | 4 | - | + | *NP* | <2.5 | 10 | <1 |
| | #17 | MCL | 2 | - | - | + | <2.5 | 12 | <1 |
| | #18 | MCL | 0 | - | - | + | 3 | 3 | <1 |
| | #19 | FL | 24 | + | - | - | 3 | 4 | <1 |
| | #20 | CLL | 15 | + | + | + | 3 | 5 | <1 |
| | #21 | u B-NHL | >100 | + | + | *NP* | 4 | 4 | 1 |
| | #22 | WM (BNS) | 32 | + | + | + | 4 | 11 | <1 |
| 2 | #23 | WM (BNS) | 5 | - | + | *NP* | 5 | 8 | <1 |
| | #24 | WM (BNS) | 38 | - | + | + | 7 | 32 | <1 |
| | #25 | WM (BNS) | >100 | + | + | *NP* | 36 | 540 | <1 |
| | #26 | WM (BNS) | >100 | + | + | *NP* | 39 | 359 | <1 |
| | #27 | MCL | >100 | + | + | + | 24 | 5 | >1 |
| | #28 | u B-NHL + PCNSL | 0 | - | + | *NP* | 10 | <2.5 | >1 |
| 3 | #29 | WM (BNS) + PCNSL | 1 | - | + | + | 18 | 4 | >1 |
| | #30 | Transformed WM | 6 | + | + | *NP* | 21 | 7 | >1 |