ISOLD: A New Highly Sensitive Interleukin Score for Intraocular Lymphoma Diagnosis

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ISOLD: a new highly sensitive Interleukin Score for intra-Ocular Lymphoma Diagnosis

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Intra-ocular lymphomas (IOL) are rare and aggressive subsets of primary cerebral tumors. Few is known about the pathogenesis. This is explained by the scarcity of patients and the tiny amount of ocular fluid sampled for biological analyses. In most cases, IOL is misdiagnosed as its clinical features can mimic other ocular conditions. To date, no independent biological tool is able to firmly diagnose IOL; the combination of cytological examination, immunochemistry, flow cytometry and molecular analysis is required. The determination of the interleukin (IL)-10 and IL-6 profile in ocular fluids appears to be a promising alternative.[1, 2] IL-10 increase is related to IOL and has immunomodulatory effects contributing to cell proliferation. Inflammatory conditions are associated with increased levels of IL-6. Reevaluation of the decisional thresholds is necessary for more efficient management.

Our study is retrospective, multicenter-case series, non-interventional, designed to assess the contribution of IL-10 and IL-6 quantifications in aqueous humor (AH) and vitreous to the IOL diagnosis.

Data were collected from patients undergoing eye fluid sampling, before any treatment, over 33 months in 23 French, Belgian and Swiss hospitals. The final diagnoses: IOL or other etiologies unrelated to malignant neoplasm (non-IOL) were obtained analyzing the clinical charts. A first cohort (training) was conducted to develop a diagnostic score. A total of 352 patients (398 AH and vitreous) were included in the training cohort. IOL was proven in 86 samples. 34 patients underwent both AH and vitreous sampling. A second cohort (validation) was carried out to test the score and included 93 patients (86 AH and 26 vitreous) exclusively recruited at *La Pitie-Salpetriere* Hospital over 15 months. The standalone validation cohort included IOL was diagnosed in 25 samples. Of the 93 patients, 16 gave both AH and vitreous samples. Bilateral IOL was documented in 5 patients. Overall, we evaluated 510 samples (445 patients) for IL-10 and IL-6 were measured using a sensitive *Cytometric*

Bead Array® kit *(CBA,* BD Biosciences™*)* on a FACSCantoII cytometer, with a limit of quantification of 2.5 pg/ml. IL-10 and IL-6 quantifications were statistically analyzed and were compared with the definitive diagnosis.

It is widely accepted that high levels of IL-10 in ocular samples are an indirect marker of IOL.[3] Nevertheless, no threshold is clearly established because of a "gray zone" making it difficult to differentiate IOL from non-IOL. To obtain higher sensitivity, the IL-10:IL-6 ratio previously reported is routinely calculated.[4] In our study, despite the significant difference in the median IL-10:IL-6 ratios between the 2 groups, a ratio <1 did not necessarily exclude the IOL diagnosis. These results clearly demonstrate that the ratio failed to detect some IOL (9 cases). Altogether, the use of the ratio is controversial and should only be considered for screening purposes.[5]

Thus, we developed a score: the Interleukin Score for intra-Ocular Lymphoma Diagnosis (ISOLD) coupled with a probability of "having an IOL". Patients were classified into four ordinal groups ranging from "certainly not IOL" to "certainly IOL". For AH, the ISOLD formula is: -12.871 + 5.533 x log(IL-10 + 1) – 1.614 x log(IL-6 + 1). For vitreous, the ISOLD formula is: -12.208 + 4.648 x log(IL-10 + 1) – 1.669 x log(IL-6 + 1). Each ISOLD value is associated with a probability calculated using the following function: Probability(IOL) = 1 / (1 + exp (-ISOLD)).

ISOLD is associated with a probability of IOL and classifies patients in order to give easily interpretable results. In the first category, samples with scores <-4.6 (>99% probability) are considered free of IOL. None of the IOL cases had a score <-4.6. Thus, ISOLD does not underdiagnose any IOL. On the other hand, a score >+4.6 (>99% probability) is highly indicative of IOL. These two "certainty" clusters exclusively contain either non-IOL or IOL patients and represent 94% of all samples. In the intermediate zone, ranging from -4.6 to +4.6,

ISOLD has to be considered only when coupled with the probability. Between -4.6 and 0, 12 non-IOL samples were associated with <50% probability and were considered as well-classified (table). The range 0 to +4.6 contained 6 IOL but also 2 non-IOL samples. These 2 samples came from patients with primary cerebral lymphoma who died before further investigation. ISOLD was powerful in correctly re-classifying the discrepancies obtained from the IL-10:IL-6 ratio. Thereby, these high probabilities are valuable and strongly guide the diagnosis.

The accuracy of ISOLD was confirmed in the validation cohort, with 92% of samples placed in the certainty zones. In the intermediate range, all IOL samples were properly shifted towards IOL diagnosis (ISOLD>0) (table). These findings confirm the strong predictive ability of ISOLD. Of note, 2 patients were referred for ocular toxoplasmosis, which is known to increase IL-6 and IL-10. On both cohorts, the sensitivity and specificity were respectively estimated at 93% and 95%.

Cytology remains the main reliable criterion for IOL diagnosis. However, tumor cells are fragile and difficult to distinguish from reactive cells. Despite the rarity of IOL, we managed to conduct two large cohorts of 445 patients. For a more accurate diagnosis of IOL, we designed this ISOLD score. The real breakthrough is that the IL-10 and IL-6 values are not given as raw data but in association with a probability. This probability is a major revolutionary tool. In the 2 certainty zones of the score (probability>99%), ISOLD correctly categorized 94% of the training cohort samples and 92% of the validation cohort samples. In both cohorts, we demonstrated that ISOLD is a powerful tool to diagnose 100% of IOL cases (probability>50%).

ISOLD was developed to strictly detect B-cell IOL as T and NK-cell IOL are very rare. However, we also report 5 cases of non B-cell IOL out of which, four were associated

with high IL-10 levels, raising our suspicion regarding the capacity of NK-cells to secrete IL-10.

Our study allowed us to build a novel strong diagnostic score based on easily measurable IL-10 and IL-6 levels. ISOLD is a simple yet powerful method with high sensitivity and specificity for detecting B-cell IOL. This innovative approach could be extremely useful to optimize patient's management.

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