

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

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► **To cite this version:**

Myrto Costopoulos, Valérie Touitou, Jean-Louis Golmard, Adil Darugar, Sylvain Fisson, et al.. ISOLD: A New Highly Sensitive Interleukin Score for Intraocular Lymphoma Diagnosis. *Ophthalmology: Journal of The American Academy of Ophthalmology*, Elsevier, 2016, 123 (7), pp.1626-1628. 10.1016/j.ophtha.2016.01.037 . hal-01290416

**HAL Id: hal-01290416**

**<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01290416>**

Submitted on 18 Mar 2016

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

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36 Meeting Presentation: 19<sup>th</sup> European Haematology Association Annual congress, Milan, June

37 2014

38 Financial Support: None

39 The authors have no financial conflict to declare.

40

41 Author Contributions:

42 Conception and design: Costopoulos, Merle-Beral, Le Garff-Tavernier

43 Analysis and interpretation: Costopoulos, Golmard, Bonnemye, Merle-Beral, Le Garff-

44 Tavernier

45 Data collection: Costopoulos, Darugar, Fisson, Bonnemye, Le Lez, Soussain, Cassoux, Lamy,

46 Le Hoang, Bodaghi

47 Obtained funding: none

48 Overall responsibility: Costopoulos, Touitou, Merle-Beral, Le Garff-Tavernier

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

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

65 Data were collected from patients undergoing eye fluid sampling, before any  
66 treatment, over 33 months in 23 French, Belgian and Swiss hospitals. The final diagnoses:  
67 IOL or other etiologies unrelated to malignant neoplasm (non-IOL) were obtained analyzing  
68 the clinical charts. A first cohort (training) was conducted to develop a diagnostic score. A  
69 total of 352 patients (398 AH and vitreous) were included in the training cohort. IOL was  
70 proven in 86 samples. 34 patients underwent both AH and vitreous sampling. A second cohort  
71 (validation) was carried out to test the score and included 93 patients (86 AH and 26 vitreous)  
72 exclusively recruited at *La Pitie-Salpetriere* Hospital over 15 months. The standalone  
73 validation cohort included IOL was diagnosed in 25 samples. Of the 93 patients, 16 gave both  
74 AH and vitreous samples. Bilateral IOL was documented in 5 patients. Overall, we evaluated  
75 510 samples (445 patients) for IL-10 and IL-6 were measured using a sensitive *Cytometric*

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

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

87 Thus, we developed a score: the Interleukin Score for intra-Ocular Lymphoma  
88 Diagnosis (ISOLD) coupled with a probability of “having an IOL”. Patients were classified  
89 into four ordinal groups ranging from “certainly not IOL” to “certainly IOL”. For AH, the  
90 ISOLD formula is:  $-12.871 + 5.533 \times \log(\text{IL-10} + 1) - 1.614 \times \log(\text{IL-6} + 1)$ . For vitreous, the  
91 ISOLD formula is:  $-12.208 + 4.648 \times \log(\text{IL-10} + 1) - 1.669 \times \log(\text{IL-6} + 1)$ . Each ISOLD  
92 value is associated with a probability calculated using the following function:  
93  $\text{Probability(IOL)} = 1 / (1 + \exp(-\text{ISOLD}))$ .

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

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

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

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

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

124 with high IL-10 levels, raising our suspicion regarding the capacity of NK-cells to secrete IL-  
125 10.

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

130

### 131 **ACKNOWLEDGMENTS**

132 The authors thank the clinicians from all of the participating hospital centers in France,  
133 Belgium and Switzerland, in alphabetical order: Karine Augeul Meunier (Nantes), Edoardo  
134 Baglivo (Genève), Emmanuel Barreau (Bicêtre), Mpari Bedel (Amiens), Pierre Blaise  
135 (Liège), Jean-Louis Bourges (Paris), Frédéric Davi (Paris), Alice Degoumois (Caen), François  
136 Devin (Marseille), Bénédicte Dupas (Paris), Marie-Hélène Errera (Paris), Philippe Gohier  
137 (Angers), Julie Gueudry (Rouen), Jérôme Guyomarch (Fort de France), Valérie Klinger  
138 (Mulhouse), Grégory Lazarian (Bobigny), Jean-Pierre Marolleau (Amiens), Hélène Massé  
139 (Rennes), Jean-Come Méniane (Fort de France), Bruno Mortemousque (Rennes), Frédéric  
140 Mouriaux (Caen), Pierre-Yves Robert (Limoges), Michel Ticchioni (Nice), Michel Weber  
141 (Nantes). The authors also thank Martine Brissard and Stéphanie Peuvion for valuable  
142 technical contributions.

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