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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*, 2016, 123 (7), pp.1626-8. <10.1016/j.opthta.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: 19th 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-

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

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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.^{1, 2} 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.³ 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.⁴ 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.⁵

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.

143

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