

**Title: Clinical spectrum of vitreoretinal lymphoma and its association with *MyD88*
L265P mutation**

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Editor,

Vitreoretinal lymphoma (VRL) is a rare extranodal lymphoma, usually of the B-cell lineage that originates in the eye (primary vitreoretinal lymphoma, PVRL) or due to secondary ocular involvement from a primary central nervous system lymphoma (PCNSL) or in systemic lymphoma (SL). (Coupland & Damato 2008) The diagnosis of vitreoretinal lymphoma (VRL) is particularly challenging because of the typically low yield of high-quality material for pathological evaluation from vitrectomy specimens. (Davis et al. 2005)

Myeloid differentiation primary response 88 (*MyD88*) is a protein involved in the innate immune response. It has recently been described in several series of VRL, (Bonzheim et al. 2015; Pulido et al. 2015; Raja et al. 2016) leading to the suggestion that testing for the *MyD88*-L265P mutation may have an important role to play in the diagnosis of VRL. (Bonzheim et al. 2015; Raja et al. 2016)

Vitreous and chorioretinal biopsies from patients in a single tertiary referral centre diagnosed with VRL were retrospectively analysed for the presence of the *MyD88*-L265P mutation. The initial diagnosis was based on morphological assessment and immunohistochemistry. Clonality analysis was performed when sufficient sample was available. However, demonstration of clonality was not considered essential for diagnosis. The *MyD88*-L265P mutation was detected using an allele-specific PCR on target DNA extracted from a formalin fixed paraffin embedded tissue block.

Eighteen patients diagnosed with VRL were included in the study. Table 1 summarises the distribution in gender, age, and clinical characteristics for each patient as well as the type of lymphoma (PCNS, PVRL or SL). Seven patients were classified as PVRL at the time of diagnosis as there was no radiological or clinical suggestion of extra-

ocular disease; 6 patients had secondary ocular involvement from PCNSL and 5 patients were either known to have systemic lymphoma at the time of the ocular diagnosis, or this was confirmed within 6 months of diagnosis. From the 7 patients initially diagnosed as PVRL, 4 patients subsequently developed CNS involvement during follow-up (the mean time for the diagnosis of CNS involvement in these 4 cases was 14.5 ± 9.9 months). The other 3 patients with no evidence of CNS involvement had a shorter duration of follow-up. In total, the *MyD88-L265P* mutation was found in 14/18 patients. A second biopsy to reach a diagnosis was necessary in 7 cases; the *MyD88-L265P* mutation was identified in 4 cases and a sample from the first equivocal biopsy was also available for us to test, 3/4 of which were positive.

In a case with confirmed SL of the testes with associated VRL and positive *MyD88-L265P* mutation disease relapse was identified during the follow-up by detection of *MyD88* mutation from a paucicellular intraocular sample. Eleven patients died during the follow-up and 4 were lost to follow-up.

These data support the notion that the presence of the *MyD88-L265P* mutation with an adequate clinical correlation may be a clinico-pathological adjunct to the diagnosis of lymphoma for cases in which the morphological assessment and immunohistochemistry remain inconclusive. Similarly, we postulate that the presence of the *MyD88-L265P* mutation has potential to be used to diagnose early recurrence of VRL in patients with previous confirmed mutation-positive disease. This would be particularly relevant in very early recurrences where conventional pathology is likely to be inconclusive due to very low cellularity of the sample.

Although we are limited by a small sample size, CNS involvement in the cases of PVRL was universal in patients with more than 3 months follow-up, but may take more than 2 years surveillance to be detected.

In our series conventional clonality analysis was also only possible in 7 cases and positive in one case. Hence, detection of this mutation has potential utility in the early diagnosis of VRL, especially when the outcome of traditional cytomorphologic or clonality analyses are inconclusive.

We additionally observed that in VRL associated with SL, the 3 patients who were positive for the *MyD88-L265P* mutation had a lymphoma of an immune privileged site (2 testes and 1 skin), leading us to speculate that there may be a predilection of SL cases harboring the *MyD88-L265P* mutation to selectively involve immune privileged sites.

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