W13: Detection of *MYD88* L265P mutation by digital PCR in peripheral blood samples from IgM monoclonal gammopathy patients

Nieves Garcia-Gisbert[1, 2], Eugenia Abella[3], Concepción Fernandez-Rodriguez[1, 4], Ana Ferrer[4], Maria del Carmen Vela[4], Laura Camacho[1, 4], Xavier Calvo[4], Sara Montesdeoca[4], Leonor Arenillas[4], Eva Gimeno[3], Carles Besses[1, 3], Antonio Salar[1, 3], Beatriz Bellosillo[1, 2, 4]

[1]Grup de Recerca Clínica Aplicada a Neoplàsies Hematològiques, IMIM, Barcelona, Spain
[2]Pompeu Fabra University, Barcelona, Spain
[3]Hematology Department, Hospital del Mar-IMIM, Barcelona, Spain
[4]Pathology Department, Hospital del Mar-IMIM, Barcelona, Spain

Fundamentals. Assessment of *MYD88* p.L265P mutation has been implemented in clinical routine as a diagnostic tool in IgM monoclonal gammopathy. Although bone marrow sample is usually available at diagnosis, the study of peripheral blood is useful for less invasive follow-up or in absence of bone marrow sample. However, the low representation of mutated cells in peripheral blood makes the detection difficult, leading to a false negative result. A strategy to overcome this situation is to enrich the target population and to use high sensitivity techniques. Enrichment of the mutated cells in the sample can be done by depletion of CD3-positive lymphocytes (as the expression of CD19 is variable in the B-cell clonal population). Digital PCR is a technology that allows the detection and quantification of mutated DNA, even when it is present in a low proportion of total DNA.

Objectives. To analyze the mutational status of *MYD88* p.L265P in peripheral blood samples by real-time allele-specific PCR (AS-qPCR) and digital PCR in a series of patients with IgM monoclonal gammopathy, as well as to determine the utility of the enrichment of the CD3-negative population for detecting this mutation.

Patients and methods. 41 peripheral blood samples were collected from 36 patients with IgM monoclonal gammopathy (28 with Waldenström macroglobulinemia (WM) and 8 with IgM monoclonal gammopathy of undetermined significance (IgM MGUS)) who were known to be *MYD88*-mutated in bone marrow. Depletion of CD3-positive lymphocytes was performed and DNA was obtained from the fraction of total mononuclear cells and from the CD3-negative subfraction. The *MYD88* p.L265P mutation was analyzed by AS-qPCR (7500Fast, Applied Biosystems) and digital PCR (QuantStudio 3D, Applied Biosystems).

Results. When the total mononuclear cell fraction was analyzed, *MYD88* p.L265P was detected in 22 from the 41 samples studied (53.65%) using AS-qPCR and in 31/41 samples (75.6%) by digital PCR. The analysis of the CD3-negative fraction allowed the detection of the *MYD88* p.L265P in 24/41 samples (58.5%) by AS-qPCR and in 33/41 (80.5%) by digital PCR. These results are summarized in Table 1. In the CD3-negative fraction, the average mutational load was of 1.72% (range: 0.05%-16.75%), which was significantly higher than the observed in the total mononuclear cell population: mean 1.13% (range: 0.05%-11.21%), p=0.004. The samples detected only by digital PCR corresponded to cases with low mutational load (<0.25%). No significant differences were observed in the allelic load between WM and IgM MGUS cases. As a whole, *MYD88* p.L265P was detected in peripheral blood in 29 from the 36 patients studied, 6/8 (75%) IgM MGUS and 23/28 (82%) WM patients. This last percentage rose up to 91% (21/23) when only untreated WM patients were considered.

Table 1. Number of positive samples for the *MYD88* p.L265P mutation according to the cell population studied and the technique used.

	AS-qPCR	digital PCR
PBMC	22/41 (53.7%)	31/41 (75.6%)
CD3- PBMC fraction	24/41 (58.5%)	33/41 (80.5%)

PBMC: peripheral blood mononuclear cells.

Conclusions. In patients with MW and IgM MGUS, the application of high sensitivity techniques such as digital PCR and, to a lesser extent, the enrichment of the sample in tumoral cells by T-cell depletion, improve the detection of *MYD88* p.L265P mutation.