MYD88^{L265P} mutation in IgM-MGUS patients: high detection rates by droplet digital PCR

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Conventional molecular techniques, as allele-specific real time quantitative polymerase chain reaction (ASqPCR), are able to detect MYD88^{L265P} in up to 50% of patients with IgM monoclonal gammopathy of undetermined significance (IgM-MGUS). Patients with IgM-MGUS, in particular if carriers of MYD88^{L265P} mutation, are at risk for development of lymphoid malignancies. We recently described a droplet digital PCR (ddPCR) assay for the identification of the MYD88^{L265P} mutation in patients with WM, suitable for screening and MRD monitoring in bone marrow (BM), peripheral blood (PB) and cell-free DNA from plasma (cf-DNA).

This study aims to investigate MYD88^{L265P} mutational load by ddPCR in IgM-MGUS, in comparison to those of WM. Two retrospective samples series of patients with IgM-MGUS (as defined by Owen, 2003) and WM were collected at the Hematology Units of Torino and Pavia. All patients provided written informed consent for the research use of biological samples, in accordance with Helsinki's declaration. ddPCR and ASqPCR were performed both on mononuclear cells (MNC), collected after red cells lysis or Ficoll-Hypaque, and on cfDNA from plasma samples, as described (Drandi D et al, Varettoni M et al).

To demonstrate the higher sensitivity of ddPCR compared to ASqPCR, 55 samples were blindly analyzed in parallel, 38 IgM-MGUS and 17 WM (42 BM and 13 PB). ddPCR revealed MYD88^{L265P} in 41/55 samples (75%), while ASqPCR only in 20 (36%), $p<0.001$ and $r^2=0.78$. Of note, samples ASqPCR-negative/ddPCR-positive showed a median MYD88^{L265P} MUT/WT ratio of 4.90E-04 (range: 1.23E-03 - 3.6E-04), while samples ASqPCR-positive/ddPCR-positive showed a median ratio of 3.53E-03 (1.10E-02 - 1.16E-03), pointing out the different limit of detection between the two methods.

Overall, 158 BM and PB samples from 33 IgM-MGUS and 82 WM patients (50 and 108 samples, respectively) were evaluated by $MYD88^{L265P}$ ddPCR. Median M-protein level of IgM-MGUS patients was 1,1 g/dl (range: 0,23-2,4 g/dl). All the IgM-MGUS (29/29) and 97% (69/71) of WM scored $MYD88^{L265P}$ in BM. $MYD88^{L265P}$ MUT/WT ratios in BM were about 1 log lower in IgM-MGUS than WM controls (Figure 1: median 3.75E-03 vs 2.81E-02, ranges: 1.10E-02 - 3.44E-04 vs 7.26E-01 - 3.60E-04) and did not correlate with M-protein (r^2=0,12). Conversely, only 3 out of 9 (33%) IgM-MGUS patients scored $MYD88^{L265P}$ in PB (detailed quantifications: 4.20E-03, 3.98E-03 and 3.80E-04), in contrast to the 100% detection rates in IgM-MGUS BM samples (median 3.75E-03, range: 1.10E-02 - 3.44E-04) and to the 82% (28/34) rate in PB of the WM series (median 2.79E-03, range: 8.31E-02 - 3.40E-04). Notably, all these 3 IgM-MGUS patients scored positive in cfDNA, too.

In conclusion, all the analyzed IgM-MGUS patients of our series carry the $MYD88^{L265P}$ mutation in MNC BM samples, despite overall lower ddPCR quantitative values, if compared to WM patients. However, preliminary data showed lower $MYD88^{L265P}$ detection rates in PB samples of IgM-MGUS, that need to be confirmed on wider series. Finally, the deeper sensitivity of the ddPCR approach in comparison with ASqPCR was confirmed in this retrospective cohort. As the study is currently ongoing, more extensive results will be presented during the meeting.