Interlaboratory Quality Control Round of MPL Mutation Detection in Fourteen European Laboratories: A MPN&MPNr-EuroNet Study

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BACKGROUND

The Myeloproliferative Leukemia Virus (MPL) encodes the thrombopoietin receptor and is a key factor for the growth of megakaryocytes. Somatic mutations occur in exon 10 and particularly at codon 515 in 15% of JAK2-V617F negative myeloproliferative neoplasms (MPN): W515L, W515K and the rare W515A variant are the most frequent. A hereditary mutation, S505N, is also associated with familial thrombocytosis (Pikman et al., PLOS Medicine, 2006; Tofferti, Leukemia, 2010).

MPL mutation detection is a helpful new tool to detect clonality in JAK2 V617F negative MPN and to establish the diagnosis of MPN. As many laboratories use very different methods and interpretations, the results need to be comparable, requiring comprehensive testing.

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AIMS

• To determine the diversity of methods used to detect MPL mutations in different European labs.
• To determine specificity and sensitivity of the different methods.
• To initiate a first step towards guidelines for MPL mutation screening and standardization of MPL mutation detection and quantification.

METHODS

29 Randomized samples with concentrations between 100% and 1% of the four mutations MPL W515L, W515K, W515A and S505N introduced in a a MPI plasmid were analyzed in 13 European laboratories, using 14 different methods.

The following methods were used: Allelic discrimination (home-made method (2 labs) or MutaScreen W515L/K Kit (Ipsogen, France) (4 labs), high resolution melting (HRM) curve analysis (6 labs) and sequencing (2 labs: 1 Sanger, 1 pyrosequencing).

RESULTS

• The allelic discrimination assays (home-made or MutaScreen) detected the mutations W515L and W515K down to 1-2%.
• The sensitivity of the 2 different HRM methods was 2 to 10% for all 4 mutations.
• The detection limit of Sanger sequencing and pyrosequencing was 5-10%, with the pyrosequencing assay not being designed for the S505N mutation.
• There were no false positive results in any of the labs.

CONCLUSIONS

• All methods used for MPL mutation detection yielded high specificity with varying sensitivity.
• Smaller clones (below 5-10%) are missed by the less sensitive methods, the impact of which remains to be defined.
• More extensive inter-laboratory testing, including analysis of patient samples, is needed to identify the most robust assays suitable for diagnostic mutation detection and particularly for quantification of mutated alleles.

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