P24. MOLECULAR DIAGNOSIS IN MYELOPROLIFERATIVE NEOPLASMS JAK2 POSITIVE: EFFICIENCY OF DETECTION METHODS.

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V617F mutation in Januss kinase 2 (JAK2) gene is detected in more than 50% of patients with myeloproliferative neoplasms (MPN), such as: polycythemia vera, essential trombocythemia and idiopathic myelofibrosis. This mutation results in self inhibition of the JAK2 activity from cytoplasm, an enzyme with a role of signal transduction from growth factor receptors, resulting in increased cell proliferation in hematopoietic bone marrow and periphereal blood.

Numerous new molecular genetics methods are presently used for V617F mutation detection in JAK2 gene. Our initial implemented method used, Amplification Refractory Mutation System (ARMS-PCR), described by Baxter et al., has been re-designed and optimized in our laboratory for the concomitant detection of the mutant allele and the wild type gene. The detection sensitivity by this method is 1%.

In our laboratory were analysed 620 samples of periphereal blood from the patients diagnosed and ambiguous for MPN. Half of the positive samples were evaluated as low level positives. Mutant allelic burden is expressed as ratio of JAK2 V617F / JAK 2 wild type. A ratio of 1.5% or less is considered as negative result; a ratio between 1.5% and 5% is considerred ambigous; a ratio more than 5% is considered as positive result.

In some individuals are pressent some positive clones with JAK2 V617F, but these clones remain as a subpopulation of cells, because such clones do not have sufficient advantaje for growth and hence do not develop further.

The ambiguous results from these cases may be caused by:

- Previous cytoreductive therapy
- Presence of two MPN clones
- Presence of an alternative mutation in exon 14
- Presence of an hereditary polymorphism which affects the binding of ARMS primers.

These cases should be interpreted in a clinical context, considering other diagnostic criteria also.

Comparatively, for some low level pozitive samples, there was performed the mutation detection with JAK1 ACE Genotyping kit (Seegene) (sensitivity up to 10%), the results being „negative".

For confirmation, some low level pozitive samples were sequenced by Next Generation Sequencing (MiSeq - illumina) method with sensitivity between 1 to 5%. The results were confirmed the presence of V617F mutation in exon 14 of JAK2 gene.

For a semiquantitative evaluation that can be approached in molecular monitoring of therapy, a high accurate and sensible method of detection – High Resolution Melting temperature (HRM) based system (i-densy - Arkray) was performed. This method performs extraction, PCR amplification and melting curves analyzes in the same reaction cartridge within 2 hours.

The results of this study approach provides useful information for the most advantageous methods in JAK2 V617F mutation detection for a high professional therapeutic decision.