NEW TEST ANNOUNCEMENT
CALRETICULIN MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

The UR Medicine Labs-Strong Memorial Hospital Molecular Diagnostics Laboratory has received conditional approval from the New York State Department of Health to offer a test to detect mutations in exon 9 of the calreticulin gene (*CALR*) to aid in the diagnosis of the myleoproliferative neoplasms (MPN) essential thrombocythemia (ET) and primary myelofibrosis (PMF). A recent publication proposes that *CALR* mutations be included as a major criterion in the WHO diagnostic criteria for ET and PMF.[1]

The most common mutation in the MPNs polycythemia vera, ET and PMF is *JAK2* V617F.[2] More than half of the cases of ET and PMF lacking *JAK2* V617F have an insertion or deletion in exon 9 of the *CALR* gene.[3, 4] The *CALR* mutations are also found in some cases of myelodysplasia of the refractory anemia subtype, but have not been found in polycythemia vera, lymphoid malignancies or in non-hematopoietic cancers.[4] The mutations have been shown to be an early somatic event in all cases that have been evaluated, and are, with a few exceptions, heterozygous.[3, 4] Patients with a *CALR* mutated MPN had a higher level of platelets, lower hemoglobin, a lower rate of thrombosis and overall better outcome than patients with a *JAK2* or *MPL* mutation.[3, 5-7] The discovery of this mutation is still fairly new, with the initial reports in December 2013, so there is still much to be learned about it.[3, 4]

The mechanism by which mutations in *CALR* cause disease is unknown. All the mutations create a frameshift equivalent to a one-bp deletion and the same carboxy-terminal 37 amino acids. This finding suggests that the mutations create a new function. There is evidence that cells with the *CALR* MPN mutations activate signal transduction downstream of JAK2 and are susceptible to inhibition by JAK2 inhibitors suggesting that the mutations cause activation of the JAK2 tyrosine kinase, similar to *JAK2* V617F.[3]

After *JAK2* V617F and frameshift mutations in exon 9 of *CALR*, mutation at codon 505 or 515 of the *MPL* gene, which is found in a small fraction of ET and PMF cases, may be useful to aid in diagnosis. Mutations in several other genes, including *ASXL1*, *DNMT3A*, *TET2*, etc., are found in ET and PMF, although mutations in these genes are also found in other hematopoietic malignancies and are not specific for ET and PMF.[8] We plan to expand our test menu to include other mutations to aid in the diagnosis and care of patients with the MPNs as their potential for clinical utility becomes evident. Suggestions for prioritization concerning future clinical molecular testing are welcome.

The purpose of the *CALR* mutation test is to aid in the diagnosis of ET and PMF. The presence of the mutation provides evidence for a clonal process in distinction from a reactive process or other pathology. The test is done as a reflex after a negative test result for the *JAK2* V617F mutation, when requested because of a suspicion of ET or PMF. The absence of a pathogenic mutation in the *JAK2*, *CALR* and *MPL* genes does not rule out a diagnosis of ET or PMF.

The test is done on peripheral blood or bone marrow. DNA is purified from the specimen, subjected to PCR using a fluorescently-labeled primer, and then the PCR products are separated by capillary electrophoresis. Specimens containing evidence for an insertion or deletion mutation are studied by Sanger sequence analysis to confirm the insertion or deletion and identify the exact mutation. The test has a limit-of-detection (analytical sensitivity) of 20% on a per cell basis for a heterozygous mutation. The optimal sensitivity for this test has not been established; consequently we do not know the risk of a false negative result due to a mutation in a small fraction of cells below the analytical sensitivity of the assay.

For additional information about the test, please contact Dr. Paul Rothberg, Director of the Molecular Diagnostics Laboratory (273-2229; Paul_Rothberg@urmc.rochester.edu).

References

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