

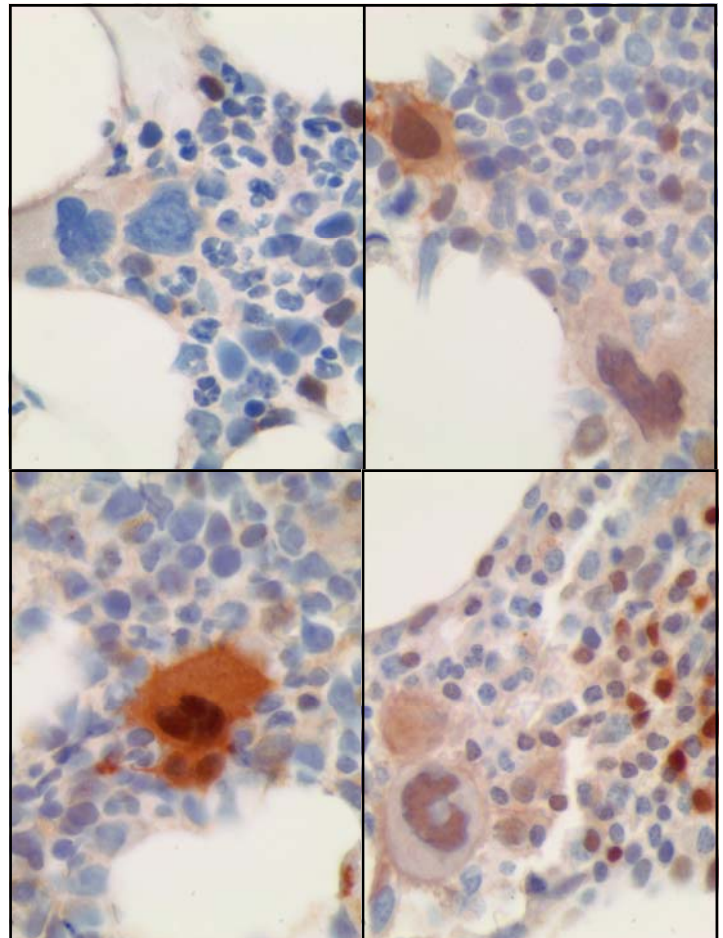
Phospho-STAT5 Immunostains as a Surrogate Marker for JAK2 V617F Mutations in non-CML Chronic Myeloproliferative Disorders

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Chronic myeloproliferative disorders (CMPD) encompass a variety of entities, including chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF). For many years, it has been known that CML is associated with a specific chromosomal abnormality, the t(9;22)(q34;q11), resulting in an abnormal BCR/ABL1 fusion gene. More recently, a number of studies have found that roughly 80% of PV patients, 38% of ET patients, and 46% of CIMF patients harbor a specific mutation involving the JAK2 gene, where the normal valine present at residue 617 is replaced by phenylalanine (JAK2 V617F mutation). This month, we call attention to an article published in the February 2007 edition of *The American Journal of Surgical Pathology*, that describes results of a study performed at the Cleveland Clinic involving the use of antibodies to phosphorylated STAT5 (pSTAT5) as a surrogate marker of the JAK2 V617F mutation.

The JAK2 gene codes for a tyrosine kinase that is involved in mediation of signals from a number of hematopoietic growth factor receptors. Binding of a growth factor to its receptor leads to activation of the JAK2 tyrosine kinase, and the enzyme subsequently phosphorylates a number of downstream effector molecules, including some from the "Signal Transducer and Activator of Transcription (STAT)" family. When STAT5 is phosphorylated (pSTAT5), it moves from the cytoplasm into the nucleus, where it binds to DNA and activates a number of genes involved in cell proliferation. The JAK2 V617F mutation results in continuous activation of the JAK2 gene, with subsequent enhanced cell proliferation. Because of its central role in driving abnormal cell



pSTAT5 immunostains on non-CML patients with chronic myeloproliferative disorders. The top left case was negative for the JAK2 V617F mutation, and did not show any positive staining of megakaryocyte nuclei. The remaining 3 stains are from patients with the JAK2 V617F mutation, and show conspicuous nuclear reactivity in megakaryocytes. Note that scattered background erythroid cells are also positive in the lower right photo, providing an internal positive control.

proliferation, the JAK2 V617F gene mutation may at some point become a therapeutic target for patients with CMPD.

Because STAT5 is an important mediator of JAK2 activation, the authors hypothesized that antibodies to pSTAT5 may allow immunohistochemical detection of this activated form of STAT5 in the nucleus of cells that harbor the JAK2 mutation.

Paraffin-embedded bone marrow biopsies and aspirates from 73 patients with non-CML CMPD were studied, including 27 with PV, 20 with ET, and 26 with CIMF. A control group of 39 patients with benign bone marrow biopsies was also examined, obtained from patients who had lymphoma staging procedures, cytopenias, or other non-neoplastic conditions. For the purposes of data analysis, the authors defined a "pSTAT5 positive" case as one that showed conspicuous nuclear staining of megakaryocytes in >10% of the megakaryocytes examined. The intensity of the stain varied substantially within individual cases, with some megakaryocytes staining strongly and others weakly. Cytoplasmic staining was ignored for the purposes of interpreting a case as "pSTAT5 positive" or "pSTAT5 negative". In addition to performing pSTAT5 immunostains on the cases, the aspirates were also analyzed for the presence of the JAK2 V617F mutation.

The control group of 39 patients showed nuclear pSTAT5 staining in about 25% of normoblasts, and reactivity tended to occur in small clusters. Myeloid elements only rarely showed reactivity, which was usually cytoplasmic. Occasional megakaryocytes showed cytoplasmic reactivity, but nuclear pSTAT5 reactivity was noted in only 2 of the 39 control patients, and interestingly, both of these patients were receiving growth factor therapy immediately before the biopsy. As expected, none of the control patients harbored the JAK2 V617F mutation.

In the CMPD group, the JAK2 V617F mutation was found in 85% (23/27) of the PV patients, 65% (13/20) of the ET patients, and 65% (17/26) of the

CIMF patients. As in the normal control group, nuclear reactivity was noted in a minority of erythroid cells, usually in clusters, and cytoplasmic (but not nuclear) reactivity was identified in only rare myeloid cells. However, abnormal staining of megakaryocyte nuclei was found in 85% (23/27) of the PV patients, 75% (15/20) of the ET patients, and 77% (20/26) of the CIMF patients. The percentage of positive megakaryocyte nuclei in these patients varied up to 70%, although most cases showed 20-30% positive megakaryocytes. All of the patients who had the JAK2 V617F mutation were pSTAT5 positive, although pSTAT5 positive immunostains were also found in 2 of 7 ET patients and 3 of 9 CIMF patients who lacked the JAK2 V617F mutation. As such, the sensitivity of megakaryocyte nuclear staining for pSTAT5 as a surrogate marker for the JAK2 V617F mutation was 100%, with a positive predictive value of 88%. The negative predictive value was 100%.

At the March 2007 meeting of the United States and Canadian Academy of Pathology, additional investigators from the Cleveland Clinic reported that nuclear pSTAT5 expression was present in 21 of 23 patients with systemic mastocytosis, but not in non-neoplastic mast cells (abstract # 1224).

In summary, abnormal staining of >10% of megakaryocyte nuclei with antibodies to pSTAT5 can serve as a valuable surrogate marker of the JAK2 V617F mutation, particularly in a patient that has not had recent hematopoietic growth factor therapy. It may also be of assistance in recognizing cases of systemic mastocytosis. Undoubtedly additional applications for this marker will be found in the future. Immunostains for pSTAT5 are now available at ProPath.

Reference: Aboudala S, Murugesan G, Szpurka H et al: Bone Marrow Phospho-STAT5 Expression in Non-CML Chronic Myeloproliferative Disorders Correlates With JAK2 V617F Mutation and Provides Evidence of In Vivo JAK2 Activation. *Amer J Surg Pathol* 31(2): 233-239, Feb 2007.
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