JAK2 Mutation Screening for Diagnosis of Polycythemia Vera and Other Chronic Myeloproliferative Disorders

In early 2005, several groups of investigators reported a somatic acquired point mutation in the JAK2 (Janus kinase 2) protein in the blood and bone marrow of patients with BCR/ABL-negative chronic myeloproliferative disorders. JAK2 is a tyrosine kinase which plays an important role in normal hematopoietic growth factor signaling, and the mutation results in activation of the kinase and deregulated intracellular signaling with cell proliferation that is independent of normal growth factor control.

The JAK2 mutation has been reported to be present in 65-100% of cases of polycythemia vera, 23-57% of cases of essential thrombocytopenia, and 35-57% of cases of chronic idiopathic myelofibrosis. This variation in prevalence in different studies is most likely related to differences in diagnostic criteria for these disorders as well as differences in assay sensitivity. Homozygosity for the mutant allele has been described in one third of polycythemia vera patients, and is rare in the other chronic myeloproliferative disorders. The mutation has also been reported in rare cases of other clonal myeloid disorders, including myelodysplastic syndromes, acute myeloid leukemia, systemic mastocytosis, and hypereosinophilic syndrome. In contrast, JAK2 mutation has not been described to date in any patient with BCR/ABL-positive chronic myeloid leukemia, any acute or chronic lymphoid disorders, any healthy person, or any patient with secondary polycythemia or a reactive blood count elevation. These findings suggest that the presence of the JAK2 mutation is specific for diagnosis of a clonal myeloid lineage proliferative disorder. This feature promises to be of significant diagnostic value, in view of the considerable clinical and hematological overlap between clonal myeloproliferative disorders and reactive cellular proliferations.

This new assay may be included in the recommended algorithm for laboratory investigation of polycythemia, as shown below. The polycythemia vera – related clinical and laboratory features referred to in the algorithm include persistent leukocytosis or thrombocytosis, microcytosis, splenomegaly, pruritus after bathing, thrombosis or erythromelalgia.

Laboratory Evaluation of Polycythemia

- Rule out volume depletion
- relative erythrocytosis
- Serum EPO
- JAK2 mutation analysis

- EPO decreased
- and/or JAK2 positive
- PV likely
- Bone marrow
- Present
- PV possible
- Bone marrow
- Evaluate for secondary erythrocytosis
- PV unlikely
- Arterial O2 sat.
- Red cell O2 dissociation
- Hb electrophoresis
- Inappropriate EPO secr.

- EPO increased
- and JAK2 negative
- PV unlikely
- PV unlikley
- Evaluate for secondary erythrocytosis

It is worth keeping in mind that a positive JAK2 mutation is not specific for polycythemia vera; the differential diagnosis for a positive test includes essential thrombocytopenia and chronic idiopathic myelofibrosis.

Currently, we are sending samples for JAK2 mutation analysis to a reference laboratory, however within the near future this testing will be performed by Saint Luke’s Reference Laboratory. Sample requirement for this assay is either peripheral blood (one lavender-top tube containing at least 3mL of whole blood) or bone marrow (3mL in a lavender-top tube).

Improved Infectious Vaginitis Testing

The three most common types of acute vaginitis, accounting for up to 90% of cases, are bacterial vaginosis, candidiasis and trichomoniasis. Differentiation of the causes of vaginitis generally
includes microscopic examination of vaginal secretions, commonly termed the wet mount.

Bacterial vaginosis (BV) is the most common cause of acute vaginitis & is believed to be caused by a shift in the normal bacterial flora from a predominance of lactobacilli to mixed flora, including Gardnerella, mycoplasmas, and anaerobes. The diagnosis of BV is suggested by the presence of watery discharge, vaginal pH greater than 4.5, presence of amine odor and "clue cells" on the wet mount. Although vaginal culture is not useful for the diagnosis of BV, candidiasis can be detected either by culture or by presence of hyphae on the wet mount. Likewise, visualization of motile trichomonads on the wet mount is diagnostic of trichomoniasis.

Although the wet mount is a mainstay of diagnosis for vaginitis syndromes, the sensitivity is suboptimal. For example, the sensitivity of wet mount for vaginal candidiasis is reportedly 50% overall, while sensitivity for trichomoniasis is 45-60%. The sensitivity of the wet mount for clue cells is 62 to 93%. The value of microscopic examination for these pathogens is further decreased when there is a delay in specimen transportation.

Beginning in October, Saint Luke’s Regional Laboratories will begin replacing the wet mount exam with a DNA probe hybridization test for simultaneous detection of Gardnerella, Candida, and Trichomonas (Affirm VPIII). This test offers significant improvements over microscopy with 92% sensitivity, 99.7% specificity for Trichomonas; 95% sensitivity, 100% specificity for clinically significant levels of Gardnerella; and 82% sensitivity, 98% specificity for Candida.

The new DNA probe test should be ordered as “vaginitis panel.” Vaginal swab specimens should only be submitted in the Affirm Ambient Temperature Transport System, supplied by SLRL. The test will be performed on a 24/7 basis with routine results available within 24 hours. STAT testing is available with results reported approximately one hour after receipt of the sample in Microbiology.

### Reference Range Changes

Due to new instrumentation in the laboratory, new reference ranges have been implemented at Saint Luke’s Hospital, Saint Luke’s East-Lee’s Summit and Anderson County Hospital. These same changes will be introduced at the other SLHS hospitals at a later date.

<table>
<thead>
<tr>
<th>Test</th>
<th>Old Reference Range</th>
<th>New Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>0 – 5 ng/mL</td>
<td>0 – 6.3 ng/mL</td>
</tr>
<tr>
<td>Troponin I Normal</td>
<td>0 – 0.15 ng/mL</td>
<td>0 – 0.05 ng/mL</td>
</tr>
<tr>
<td>Troponin I Increased Cardiac Risk</td>
<td>0.16 – 1.5 ng/mL</td>
<td>0.06 – 0.5 ng/mL</td>
</tr>
<tr>
<td>Troponin I Myocardial Infarction</td>
<td>&gt;1.5 ng/mL</td>
<td>&gt; 0.5 ng/mL</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>175 – 940 pg/mL</td>
<td>180 – 914 pg/mL</td>
</tr>
<tr>
<td>T uptake</td>
<td>22 – 37%</td>
<td>32 – 48%</td>
</tr>
<tr>
<td>Insulin</td>
<td>3 – 25 mIU/mL</td>
<td>2 – 23 mIU/mL</td>
</tr>
<tr>
<td>Cortisol 0800</td>
<td>5 – 25 ug/mL</td>
<td>6.7 – 22.6 ug/mL</td>
</tr>
<tr>
<td>Cortisol 1600</td>
<td>2 – 17 ug/mL</td>
<td>0 – 10.0 ug/mL</td>
</tr>
<tr>
<td>Estradiol male</td>
<td>10 – 40 pg/mL</td>
<td>20 – 75 pg/mL</td>
</tr>
<tr>
<td>Estradiol female Premenopausal</td>
<td>15 – 350 pg/mL</td>
<td>24 – 273 pg/mL</td>
</tr>
<tr>
<td>Estradiol female Postmenopausal</td>
<td>0 – 10 pg/mL</td>
<td>20 – 88 pg/mL</td>
</tr>
</tbody>
</table>

Please note that there are significant changes in the Troponin I value for risk stratification and for acute MI. The new Troponin I cutoff value for risk stratification represents the 99th percentile of a control group of normal individuals and has a precision of <10% as recommended by the European Society of Cardiology, the American College of Cardiology and the American Heart Association.