K30 Case Conference: 6MP Metabolism

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**HPI**

- 14 year old caucasian female
- Diagnosed with Crohn’s disease, presenting with severe activity
- 8 episodes of bloody diarrhea per day
- 20 pound weight loss over 7 weeks
- Fevers to 102 degrees
- Complaints of weakness, malaise
- Urgency, tenesmus
PEx

• Wt 54.3kg, T 36.5, BP 106/66, R 21, P 106, Pox 98%
  General: thin female; +pallor; multiple episodes of excusing herself to have a BM
  HEENT: PERRRL, NCAT, oropharynx clear, may have small ulcer along R post oropharynx, however difficult to fully appreciate; sclera anicteric, dry lips, mmm
  CHEST: CTA b/l, unlabored respirations
  CV: S1, S2 normal, RRR, no audible murmur
  ABD: Soft, NT/ND, +BS, No palpable mass
  Rectal exam: deferred
  Skin: excoriations along L antecubital fossa, otherwise no rashes
  Ext: W/WP, cap refill<2sec
Labs

- Per previous records reported: OB positive stools. Stool cultures negative for Campylobacter, Salmonella, or Shigella. Negative for Clostridium, for ova and parasites. Stool is notable for many white blood cells and many red blood cells. Na 138, K3.8, Cl 101, bicarb 28, Bun 8, Cr 0.5, Ca 84; UA negative
- CBC 10.2/11.1/33.3/355 (P76%, L 16%) C-reactive protein of 4.17
# IBD Serologies

## Patient Test Result

- **X** IBD Predicted
- **☐** IBD Not Predicted
- **☐** Ulcerative Colitis Predicted
- **X** Crohn's Disease Predicted

## General Test Information

<table>
<thead>
<tr>
<th></th>
<th>IBD</th>
<th>CD</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>93%</td>
<td>88%</td>
<td>93%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>95%</td>
<td>98%</td>
<td>97%</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>96%</td>
<td>96%</td>
<td>89%</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>90%</td>
<td>93%</td>
<td>98%</td>
</tr>
</tbody>
</table>

**PROMETHEUS® Predictive Algorithm Description:**

- Utilizes Smart Diagnostic Algorithm (SDA) technology to characterize complex relationships between multiple markers to produce a diagnostic prediction with greater accuracy than simple comparison of assay results to a reference range.
- Developed (n=1313; 38% CD, 24% UC, 20% IBS, 20% normal) and validated (n=500; 38% CD, 21% UC, 41% normal) using serology results for samples with a known diagnosis.
- Patent Pending.

## Patient Assay Information

<table>
<thead>
<tr>
<th>Assay</th>
<th>ASCA IgA ELISA</th>
<th>ASCA IgG ELISA</th>
<th>Anti-OmpC IgA ELISA</th>
<th>Anti-CBir1 ELISA</th>
<th>Neutrophil-Specific Nuclear AutoAntibodies (NSNA) (IBD specific pANCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Value</td>
<td>28.0 EU/ml</td>
<td>30.1 EU/ml</td>
<td>2.3 EU/ml</td>
<td>27.7 EU/ml</td>
<td>AutoAntibody ELISA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>44.2 EU/ml</td>
</tr>
</tbody>
</table>

### Note:
Patient test results are based on the Smart Diagnostic Algorithm which interprets patterns between the 7 assay values. Assay and reference values are provided for prognostic interpretation.
Endoscopy/Colonoscopy

- Terminal Ileum (biopsies A And B):
  - Small bowel mucosa with focal mild acute enteritis.
  - No villous blunting, chronic enteritis, infectious microorganisms, or granulomas present.
- Colon, from cecum to rectum (biopsies C-I):
  - Moderate to severe acute colitis with features of chronic mucosal injury.
  - No infectious microorganisms or granulomas present.
- Duodenum, 2nd portion (biopsy A):
  - Chronic duodenitis with moderate activity and pyloric metaplasia.
  - Uninvolved duodenal mucosa with submucosal granuloma.
Inflammatory Bowel Disease

• IBD is a common phenotype of many pathways
• Results from an abnormal response to normally occurring gut constituents
  – Involves both genetic and environmental components
    • Genetic: bacterial sensing, clearance mechanisms
    • Environmental: components of commensal bacteria
• Loss of gut homeostasis (physiologic inflammation)
Hospital course

- Induction with IV Solumedrol 60 mg qd
- Responded after 5 days with decrease in number of diarrheal stools, absence of blood
- Obtained TPMT (thiopurine methyltransferase) phenotype
- TPMT enzyme activity 31.2 enzyme units (normal activity)
TPMT activity

Test Result: Normal Activity

<table>
<thead>
<tr>
<th>Assay</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROMETHEUS TPMT Enzyme Activity</td>
<td>31.2 EU</td>
<td>Defined Below*</td>
</tr>
</tbody>
</table>

* PROMETHEUS TPMT Enzyme Activity Ranges:
  > 23.6 EU - Normal Activity
  6.7 - 23.8 EU - Intermediate Activity
  < 6.7 EU - Low Activity

The highest TPMT enzyme activity level observed in the Prometheus validation studies of normal individuals was between 60-70 EU.

All PROMETHEUS TPMT Enzymes Activity Results for This Patient

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Sample ID</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>04/06/2007 11:15 AM</td>
<td>S04070095</td>
<td>31.2 EU</td>
</tr>
</tbody>
</table>

PROMETHEUS TPMT Enzyme Activity

- Normal Activity
- Intermediate Activity
- Low Activity

Collection Date 04/06/07
6MP metabolism

- 6-thiouracil
  - Xanthine oxidase
  - HPRT
  - Non-enzymatic

- AZA → 6-MP → Thioinosinic acid (TIMP) → 6-thioguanine (6-TG)

- TPMT

- 6-MMP
  - TPMT
  - 6-MMP ribonucleotides
TPMT deficiency: Pharmacogenomics

- 10% of Caucasian and AA population has intermediate activity
- 1 in 300 deficient activity (autosomal recessive), treatment with 6MP can be fatal
- TPMT 1 is wild type allele
- Most common mutant alleles among Caucasians are 3A
- African americans have 20% lower median activity (3C)
TPMT deficiency: Pharmacogenomics

- Guidelines written by AGA (Am Gastro Assn) recommend checking TPMT phenotype or genotype prior to initiating therapy
- Cost effective, quicker time to therapeutic level, prevents hospitalization for leukopenic side effects
- Recommended to start at _ usual dose of 6MP in those with intermediate activity
Mutant TPMT alleles

TPMT Alleles in Various World Populations

- Mayo/Europe: 3A, 3C, 2, 3B, 3D, 4, 5
- St. Jude: 3A, 3C, 2
- Caucasians: 3A, 3C, 2
- African American: 3A, 3C, 2, 8
- UK: 3A, 3C, 2
- France: 3A, 3C, 2, 7
- Norway: 3A, 3C
- S.W. Asia: 3A
- Ghana: 3C
- Kenya: 3C
- Thailand: 3C
- Japan: 3C
- China: 3C
- Korea: 3C, 6
Patient’s course

- Was started on 2 mg/kg of 6MP prior to leaving the hospital
- 6MP metabolites checked 2 weeks after initiated on therapy
- 6TG metabolite 641 (therapeutic range being 230 to 400 and 641 being associated with a higher risk of leukopenia and a higher likelihood of response)
- 6MMP metabolite is 11,155 which is associated with a higher risk of hepatotoxicity
- Upon receiving these results, I called mom on the same day and told her to decrease dose of 6MP to approximately 1.25 mg per kg.
Correlative labs

- WBC 2.7, hemoglobin of 9.1, hematocrit of 27.7, platelets of 416.
- MCV is 84, and RDW is 16.8
- 35% neutrophils, 20% bands
- AST of 13, ALT of 34, alk phos of 55, direct bili of 0.1, total bili of 0.4, total protein 7.2, and albumin of 4.0.
## Test Results

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Result (Units: pmole/8 x 10^8 RBC)</th>
<th>Reference Range</th>
<th>Result Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-MMPN</td>
<td>7122</td>
<td>&lt; 5700</td>
<td>Higher Risk of Hepatotoxicity.</td>
</tr>
</tbody>
</table>

## Cumulative PROMETHEUS Thiopurine Metabolites Patient Results

### 6-TGN Metabolite Levels

- Higher Risk of Leucopenia.
- Higher Likelihood of Response.

### 6-MMPN Metabolite Levels

- Higher Risk of Hepatotoxicity

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Sample ID</th>
<th>6-TGN Level</th>
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</thead>
<tbody>
<tr>
<td>05/21/2007 04:28 PM</td>
<td>SI05240386</td>
<td>505</td>
</tr>
<tr>
<td>05/04/2007 08:59 AM</td>
<td>SI05070251</td>
<td>641</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6-MMPN Level</th>
<th>Drug</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>7122</td>
<td>6-MERCAPTOPURINE</td>
<td>75 mg/day</td>
</tr>
<tr>
<td>11155</td>
<td>6-MERCAPTOPURINE</td>
<td>50 mg/day</td>
</tr>
</tbody>
</table>
Most recent labs

- Total protein 7.1, albumin 4.5, globulin 2.6, albumin-to-globulin ratio 1.7, total bilirubin 0.5, direct 0.1, indirect 0.4, alkaline phosphatase 57, AST 13, ALT 9
- Sed rate was 6.
- White blood count was 3,600. Hemoglobin 8.8, hematocrit 26.6 with the MCV being 87.5, RDW 27.3. Platelet count is 158