Clinical Validity of Beta-glucosidase and Alpha-iduronidase Enzyme Analysis in Dried Blood Spots

Teresa Thompson, BS, MB(ASCP)CM

Lysosomal storage disorders (LSDs)
- Deficiency of enzyme or cofactor responsible for breaking down complex macromolecules in lysosome
- Glycosaminoglycans, oligosaccharides, lipids, etc accumulate in lysosomes of specific body tissues & disrupts cell function
- Infantile or late onset
- Approximately 40 different disorders with diverse clinical features

Accumulation (storage)

Typical diagnostic strategy for LSDs
1. Clinical suspicion
2. Urine screening
   - Available for all MPS disorders & some glycoprotein disorders
   - False-negative & false-positive results well documented
3. Enzymatic testing
   - "Gold-standard" for diagnostic testing for LSDs
   - Testing in leukocytes (must be isolated within 48 hr of blood draw) or fibroblasts (requires skin biopsy)
4. Molecular testing
   - Confirmation of abnormal enzyme result
   - Diagnosis of disorders without a clinically available enzyme assay
   - Prenatal testing
Enzyme testing using 4-MU substrates

Benefits of diagnostic enzyme testing in DBS
- Less invasive
- Lower sample volume requirement
- Increased sample stability vs. whole blood
- Decreased shipping costs (overnight delivery not required)
- BUT, for enzyme analysis to be diagnostic in DBS, must demonstrate 100% sensitivity and near 100% specificity like the “gold-standard” leukocyte assay

MS/MS DBS enzyme analysis

Study design

• Goal was to evaluate whether enzyme analysis in DBS can be used as a diagnostic test
• DBS card made from whole blood samples (sodium heparin) sent for lysosomal enzyme testing prior to leukocyte isolation
• Beta-glucosidase (Gaucher disease) and alpha-iduronidase (Hurler syndrome) enzymes analyzed in leukocytes and DBS from the same peripheral blood sample for ~190 patients
  -- Includes both affected and unaffected individuals

Beta-glucosidase enzyme analysis

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes (nmol/hr/mg)</th>
<th>DBS (nmol/mL/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher</td>
<td>0.096 - 1.13</td>
<td>0.025 - 0.86</td>
</tr>
<tr>
<td>Unaffected</td>
<td>2.98 - 33.5</td>
<td>2.84 - 28</td>
</tr>
</tbody>
</table>

Alpha-iduronidase enzyme analysis

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes (nmol/hr/mg)</th>
<th>DBS (nmol/mL/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hurler</td>
<td>0 - 0.95</td>
<td>0 - 0.84</td>
</tr>
<tr>
<td>Unaffected</td>
<td>8.55 - 71.0</td>
<td>1.76 - 26.8</td>
</tr>
</tbody>
</table>
Alpha-iduronidase pseudo-deficiency

• Unaffected individuals have low enzyme activity when measured in vitro
• Was historically thought to be very rare
• NBS for Hurler syndrome using DBS started in Missouri in January 2013
• Large number of babies with positive NBS have alpha-iduronidase activity below normal range, but above affected range in leukocytes when sent for diagnostic follow-up testing
• IDUA gene sequencing found several recurrent changes ("pseudo-deficiency alleles") in patients with intermediate enzyme activity

DBS alpha-iduronidase activity in patients with pseudo-deficiency

7/22 patients with intermediate alpha-iduronidase activity in leukocytes have deficient activity in DBS

Conclusions

• DBS enzyme analysis via MS/MS technology was able to accurately identify all patients affected with Hurler syndrome and Gaucher disease (100% sensitivity)
• No overlap between activities of affected patients and unaffected individuals with clinical suspicion of an LSD (100% specificity)
• DBS enzyme analysis can not distinguish all patients with alpha-iduronidase pseudo-deficiency (detected by NBS) from affected patients- leukocyte enzyme analysis or molecular testing would be required
Acknowledgements

• Greenwood Genetic Center Biochemical Diagnostic Laboratory
  – Tim Wood
  – Laura Pollard
  – Jenny Miller
  – Christina Shouse
• Hui Zhou (Centers for Disease Control)