Hevylite®: a New Serum Test for Assessing Patients with Multiple Myeloma

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Why is Hevylite important?

- Not all patient m-proteins are easily identified by SPE
- Hevylite can sometimes quantify samples that SPE cannot
- New: Hevylite can measure the involved (IgAk) and uninvolved (IgAl) isotypes separately
IgG, A and M assays measure IgXk and IgXl isotypes together

Now they can be measured independently with Hevylite
What is HevyLite?

- Hevylite antibodies (assays) recognize conformational epitopes between heavy and light chains
- Can distinguish:
  - IgA\(_\kappa\) v. IgA\(_\lambda\)
  - IgG\(_\kappa\) v. IgG\(_\lambda\)
  - IgM\(_\kappa\) v. IgM\(_\lambda\)
Not all m-spikes are easily identified by SPE (14 IgA patients)
HL IgAκ/l assays can identify unclear SPE peaks

n=210

Mirbahai et al. Presented at AACC 2011
Hevylite can sometimes quantify samples that SPE cannot.

<table>
<thead>
<tr>
<th>Isotype</th>
<th>No. samples not able to be quantified by SPE/total no. tested</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>26/56</td>
<td>46</td>
</tr>
<tr>
<td>IgG</td>
<td>4/100</td>
<td>4</td>
</tr>
</tbody>
</table>

All samples were able to be quantified by Hevylite.

Ludwig Leukemia 2013
Only β2M and HLCR remain signif. Upon MV analysis for PFS

**Table 2.** Significance of markers for progression-free survival assessed by univariate and multivariate Cox regression analysis

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Univariate analysis</th>
<th>Multivariate analysis (n = 242)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del:13</td>
<td>( P = 0.03^a ) (n = 283)</td>
<td>( P = 0.546 )</td>
</tr>
<tr>
<td>t4-14</td>
<td>( P = 0.05^a ) (n = 252)</td>
<td>( P = 0.515 )</td>
</tr>
<tr>
<td>Del:17p</td>
<td>( P = 0.08 ) (n = 277)</td>
<td>( P = 0.457 )</td>
</tr>
<tr>
<td>β2-M &gt; 5.5 mg/l</td>
<td>( P = 0.51 ) (n = 308)</td>
<td>( P = 0.407 )</td>
</tr>
<tr>
<td>β2-M &gt; 3.5 mg/l</td>
<td>( P = 0.001^a ) (n = 308)</td>
<td>( P = 0.045^a )</td>
</tr>
<tr>
<td>Albumin &lt;35 g/l</td>
<td>( P = 0.153 ) (n = 302)</td>
<td>( P = 0.828 )</td>
</tr>
<tr>
<td>FLC tertiles</td>
<td>( P = 0.589 ) (n = 307)</td>
<td>( P = 0.689 )</td>
</tr>
<tr>
<td>Monoclonal Ig tertiles(^b)</td>
<td>( P = 0.16 ) (n = 300)</td>
<td>( P = 0.748 )</td>
</tr>
<tr>
<td>HLC ratios of &lt;200 to &gt;0.01 vs more extreme values</td>
<td>( P = 0.017^a ) (n = 308)</td>
<td>( P = 0.001^a )</td>
</tr>
</tbody>
</table>

Abbreviations: β2-M, β2-microglobulin; FLC, serum free light chain; HLC, immunoglobulin heavy/light chain; Ig, immunoglobulin. n = number of results available for the analysis. \(^a\)Significant result at \( P < 0.05\). \(^b\)Monoclonal immunoglobulins as measured by SPEP densitometry.
Nomenclature

• IgAk MM patient
• Involved HLC isotype: IgAk
• Uninvolved HLC isotype: IgAl
• Immunosuppression can be in:
  – Uninvolved HLC isotype: IgAl
  – Systemic immunoglobulin suppression: IgG + IgM
  – Both
Key driver: elevated monoclonal AND suppressed uninvolved HLC

HLC inv./uninvolved ratios

Fig 2 b

Blue = more normal ratios
Red = more abnormal ratios

Cutpoint: ratio 0.01 or 200

Involved HLC

Fig 3 a

Blue = less involved HLC
Red = more involved HLC

Uninvolved HLC

Fig 3 b

Blue = less suppressed uninvolved HLC
Red = more suppressed uninvolved HLC

Red n = 116
Blue n = 209
Uninvolved HLC isotype suppression and systemic immunoglobulin suppression

No suppression

Suppression of the noninvolved isotypes

Suppression of the HLC pair of the involved isotype
Hevylite chain ratio (HLCR) a hypothesis:

- HLCR Appears to be a unique, significant marker when monitoring MGUS, SMM and MM

- Hypothesis: Suppression of the uninvolved HLC isotype polyclonal proteins are a biologically important factor
  - resulting from tumor-stroma interactions and/or microenvironment properties
  - Not simply crowding out in marrow by myeloma cells

- Evidence that supports this
  - suppression seen in MGUS (<10% plasma cells)
  - HLC isotype suppression has been reported as being prognostic for PFS and OS
  - Systemic Ig suppression “does not appear to be as strong”
Frequently Asked Question (FAQ)

• Q: Now that we have HL do we need FL?
• Answer: YES!
• Here’s why . . .
sFLC and HL are independent tumor markers...

![Graph](image-url)

- *n* = 164 IgG<sub>K</sub> MM
- $R^2 = 0.0223$

Freelite sFLC (mg/L) vs. Hevylite IgG<sub>K</sub> (g/L)
Immunoglobulin and Free Light Chain Production by Plasma Cells

Kappa

Lambda
IFE and HLC ratio normal at the same time
HLC ratio became abnormal indicating relapse when IFE was still normal
IFE remained normal for further 5.5 months
Laboratory relapse was confirmed by IFE
Later clinical relapse was noted.

Ludwig Leukemia 2013
Monitoring Jan 2008 – Jan 2011

- Early indication of relapse?
- Polyclonal increase in IgA due to infection?
**IgAκ/IgAλ HLC ratio NEVER normalised**

- Early indication of relapse?
- Polyclonal increase in IgA due to infection?

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Summary: Importance of Hevylite

• Not all patient m-proteins are easily identified by SPE
• Hevylite can sometimes quantify samples that SPE cannot
• New: Hevylite can measure the involved (IgAk) and uninvolved (IgAl) isotypes separately
• Cases: HL adds value not seen with other tests
Extra Slides
Free light chain escape

Figure 2

Keats et al 2012 Blood
The clone you present with is not the clone that kills you – Mayo Scottsdale

Keats et al 2012 Blood
Free Light Chain Escape

• Prevalence: 2.5% (10/407 pts 2004-7)

• Patient Characteristics
  – Stable by intact Ig monitoring
  – But developed severe organ dysfunction
  – Median anti-MM cycles 6
  – sFLC reliable markers

Kuhnemaund et. al. J Cancer Res Clin Oncol 2009
Kuhnemund et al 2009 Table 1

<table>
<thead>
<tr>
<th></th>
<th>Pt #1</th>
<th>Pt #2</th>
<th>Pt #3</th>
<th>Pt #4</th>
<th>Pt #5</th>
<th>Pt #6</th>
<th>Pt #7</th>
<th>Pt #8</th>
<th>Pt #9</th>
<th>Pt #10</th>
<th>Median</th>
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<tbody>
<tr>
<td><strong>Pt age (y) at MM diagnosis (ID)</strong></td>
<td>65</td>
<td>52</td>
<td>61</td>
<td>60</td>
<td>37</td>
<td>50</td>
<td>74</td>
<td>75</td>
<td>75</td>
<td>66</td>
<td>63</td>
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<tr>
<td>Gender, m versus f</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>f</td>
<td>m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM type</td>
<td>IgAκ</td>
<td>IgAλ</td>
<td>IgGκ</td>
<td>IgGλ</td>
<td>IgGκ</td>
<td>IgGκ</td>
<td>IgGκ</td>
<td>IgGκ</td>
<td>IgGλ</td>
<td>IgGλ</td>
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<td>Preceding MGUS before MM</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>D&amp;S; ISS stage at ID of LCE-MM</td>
<td>IIIB; IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
<td>IIIA; II</td>
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<tr>
<td>Karyotype (n vs. other)</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>13q14</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>t(11;14)</td>
<td></td>
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<tr>
<td>Prior LC elevation before LCE-MM</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Urinary B JP prior to LCE-MM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Urinary B JP with LCE-MM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>SFLC before LCE-MM (mg/L)</strong></td>
<td>863</td>
<td>80</td>
<td>212</td>
<td>1,020</td>
<td>189</td>
<td>17</td>
<td>7</td>
<td>428</td>
<td>419</td>
<td>20</td>
<td>201</td>
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<tr>
<td><strong>SFLC w LCE-MM (mg/L)</strong></td>
<td>2,000</td>
<td>2,000</td>
<td>5,220</td>
<td>7,260</td>
<td>8,940</td>
<td>1,390</td>
<td>111</td>
<td>1,210</td>
<td>1,673</td>
<td>180</td>
<td>1,837</td>
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<tr>
<td>Max. SFLCs w LCE-MM (mg/L)</td>
<td>4,390</td>
<td>7,100</td>
<td>11,600</td>
<td>7,260</td>
<td>9,700</td>
<td>1,390</td>
<td>4,500</td>
<td>7,980</td>
<td>1,673</td>
<td>1,170</td>
<td>5,800</td>
</tr>
<tr>
<td>Min. SFLCs w LCE-response (mg/L)</td>
<td>0.6</td>
<td>500</td>
<td>60</td>
<td>950</td>
<td>561</td>
<td>10</td>
<td>24</td>
<td>12</td>
<td>115</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>Max. SFLC decline (fold change)</td>
<td>7,317</td>
<td>14</td>
<td>193</td>
<td>7</td>
<td>17</td>
<td>139</td>
<td>188</td>
<td>665</td>
<td>15</td>
<td>117</td>
<td>128</td>
</tr>
<tr>
<td><strong>Symptoms of LCE-MM</strong></td>
<td>DCC, RI</td>
<td>DCC</td>
<td>DCC</td>
<td>DCC, A, TBP, RI</td>
<td>DCC, RI</td>
<td>DCC, A, BP</td>
<td>DCC, BPF, RI</td>
<td>DCC, RI</td>
<td>DCC, A, TBP</td>
<td>DCC, EM-MM</td>
<td></td>
</tr>
<tr>
<td><strong>KI (%) before and with LCE-MM</strong></td>
<td>80 → 50</td>
<td>80 → 60</td>
<td>90 → 60</td>
<td>80 → 70</td>
<td>80 → 70</td>
<td>70 → 50</td>
<td>70 → 50</td>
<td>80 → 50</td>
<td>80 → 50</td>
<td>80 → 50</td>
<td></td>
</tr>
<tr>
<td><strong>Cycle # (lines) before LCE-MM</strong></td>
<td>0 (0)</td>
<td>15 (3)</td>
<td>13 (5)</td>
<td>7 (5)</td>
<td>5 (3)</td>
<td>4 (2)</td>
<td>3 (2)</td>
<td>8 (2)</td>
<td>5 (3)</td>
<td>8 (3)</td>
<td>6 (3)</td>
</tr>
<tr>
<td><strong>Cycle # (lines) after LCE-MM</strong></td>
<td>10 (2)</td>
<td>2 (1)</td>
<td>4 (1)</td>
<td>2 (1)</td>
<td>4 (2)</td>
<td>4 (1)</td>
<td>7 (3)</td>
<td>14 (4)</td>
<td>2 (1)</td>
<td>6 (3)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Best response of LCE-MM</td>
<td>PR</td>
<td>SD</td>
<td>SD</td>
<td>PD</td>
<td>SD</td>
<td>PR</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td><strong>Time of LCE-MM after ID of MM (m)</strong></td>
<td>36</td>
<td>189</td>
<td>60</td>
<td>93</td>
<td>104</td>
<td>101</td>
<td>21</td>
<td>32</td>
<td>14</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td><strong>Time of LCE-MM to death (m)</strong></td>
<td>19</td>
<td>6</td>
<td>19</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>11</td>
<td>NA</td>
<td>NA</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Disease duration from ID (m) [y]</td>
<td>55</td>
<td>195</td>
<td>79</td>
<td>97</td>
<td>+122</td>
<td>+127</td>
<td>32</td>
<td>460</td>
<td>+15</td>
<td>22</td>
<td>70 [58]</td>
</tr>
<tr>
<td>Alive = 1, dead = 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Death due to</td>
<td>PD</td>
<td>PD</td>
<td>PD</td>
<td>PD</td>
<td>NA</td>
<td>NA</td>
<td>PD</td>
<td>NA</td>
<td>NA</td>
<td>PD</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>sCR</td>
<td>VGPR</td>
<td>PR</td>
<td>SD</td>
<td>PD</td>
<td></td>
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</tr>
<tr>
<td>Neg. s and uIFE</td>
<td>CR +</td>
<td>Clarifications for CR and VGPR when only measurable disease is by sFLC</td>
<td>If s and u m-protein not measurable</td>
<td>Don’t meet criteria for other categories</td>
<td>Patients w/o measurable s and u m-prot</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;5% PC in bone marrow</td>
<td>Normal FLC ratio+ Absence of clonal PCs by IHC or 2-4 color Flow cytometry</td>
<td>A decrease in &gt;90% between involved and uninvolved sFLC levels is required</td>
<td>A decrease in &gt;=50% between involved and uninvolved sFLC levels is required</td>
<td>Difference between involved and uninvolved FLC levels must increase by &gt;10 mg/dL</td>
<td></td>
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Rajkumar Blood 2011
<table>
<thead>
<tr>
<th>Protein</th>
<th>Half Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>20–25 days</td>
</tr>
<tr>
<td>IgA</td>
<td>6 days</td>
</tr>
<tr>
<td>IgM</td>
<td>6–8 days</td>
</tr>
<tr>
<td>Free Kappa</td>
<td>2–4 hours</td>
</tr>
<tr>
<td>Free Lambda</td>
<td>3–6 hours</td>
</tr>
</tbody>
</table>
Multiple Myeloma – Diagnosis

1. >10% plasma cells in the bone marrow

2. Monoclonal protein in either serum or urine (or abnormal FLC ratio)

3. End Organ Damage (CRAB)
   - Calcium elevation in the blood (*hypercalcemia*)
   - Renal Insufficiency (*kidney damage*)
   - Anemia (*blood disorder*)
   - Bone lesions (*bone damage*)
Reductions in SPEP and iHLC in Patients with Quantifiable SPEP

\[ y = 1.0009x + 0.0011 \]
\[ R^2 = 0.9214 \]

% reduction in SPEP

% reduction in involved Hevylite

Therefore IMWG guidelines apply
iHLC (involved Ig)

uHLC (uninvolved Ig, isotype matched suppression)

rHLC (Ig’κ/Ig’λ ratio)

dHLC (estimation of monoclonal Ig)
Relationship of SPE to qIgs

Impact of SPE non-linearity on qIgG

SPE M-Ig = 44.9g/L

A 30% reduction in M-Ig will be missed using SPE due to dye saturation

Total IgG 66.7g/L