Understanding Freelite® and Hevylite®

Naveen Bangia, PhD
The Binding Site Inc.
What is Myeloma?

Symptoms & Diagnosis of Myeloma

Detecting Monoclonal Proteins

Why Urine?
Multiple Myeloma is a Monoclonal Gammopathy

MGUS 57%
Multiple Myeloma 18%
AL amyloidosis 9.5%
Lymphoproliferative 3%
SMM 4% (1,780)
Solitary or extramedullary 2%
Macro 2.5%
Other 4%

N = 46,739
Mayo Clinic Database, 1960–2012
Incidences may differ with other populations

3Kyle, R. 2013, personal commun.
Multiple Myeloma

Many ~ muelos (greek) = marrow
tumor

1844
Sarah Newbury

1873
Dr. Von Rusitzky

1879
Dr. Otto Kahler

Portions of sternum
Replaced with red substance
Eight bone marrow tumors
Multiple Myeloma is a disease of an immune cell, found in the blood and bone marrow.
Plasma cells make antibodies (immunoglobulin) to block bacteria and viruses.

B cell → SURVEILLANCE “RIGHT FIT”

Plasma cell → REPLICATION & MASSIVE ANTIBODY PRODUCTION
Plasma cells decrease after Infection cleared
Myeloma is a cancer of plasma cells

- Myeloma = too many plasma cells

- Each myeloma cell (clone) makes and releases one type of antibody (gamma globulin) into the circulation (monoclonal gammopathy)
Plasma cells secrete intact antibody and free light chains

Heavy Chain
- Kappa, $\kappa$
- Lambda, $\lambda$

Light Chain
- Kappa releasing
- Lambda releasing
Each patient with myeloma usually has one cancerous plasma cell clone that secretes a lot of one type of antibody

Antibody = Immunoglobulin = Ig

IgG kappa
IgG lambda
IgA kappa
IgA lambda
IgM kappa
IgM lambda
Different types of multiple myeloma secrete different products

Intact Ig Myeloma (~80%) (89% also secrete free light chains)

Light Chain Myeloma (15-20%) (κ or λ)

Non-secretory or oligosecretory (1-5%)
What is Myeloma?

Symptoms & Diagnosis of Myeloma

Detecting Monoclonal Proteins

Why Urine?
What is Myeloma?

Hematological Malignancy

- Monoclonal Protein – intact / FLC only
- Plasma cell dyscrasia
- Monoclonal Gammopathy

Bone Marrow (BM) – Anemia

- BM destruction - Pain, Fractures
- Suppression of Normal Antibodies - Infections
Symptoms
32%
What Brings People to the Doctor when they are Diagnosed with Multiple Myeloma

- Bone pain
- Anemia
- Fatigue
- Protein
- Kidney
- Infect
- Physical
- Other

IMF Patient Family Seminar
August, 2013
Philadelphia, PA
Multiple Myeloma – Diagnosis

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

2. >10% plasma cells in the bone marrow

3. End Organ Damage (CRAB)
   - Calcium elevation in the blood *(hypercalcaemia)*
   - Renal Insufficiency *(kidney damage)*
   - Anemia *(blood disorder)*
   - Bone lesions *(bone damage)*

MGUS (1 only)

Smoldering

MM (1 + 2)

MM (1 + 2 + 3)
MM diagnosis

- \( \geq 10\% \) clonal BMPCs
- \( \geq 60\% \) clonal BMPCs
- Involved/uninvolved Freelite ratio \( \geq 100 \)
- \( >1 \) focal lesion by MRI

CRAB
- HyperCalcemia
- Renal insufficiency
- Anemia
- Bone Lesions

SLiM

Rajkumar Lancet Oncol 2014;15:e538-48
MM diagnosis

≥10% clonal BMPCs

HyperCalcemia
Renal insufficiency
Anemia
Bone Lesions

Myeloma Defining Events

≥60% clonal BMPCs
Involved/uninvolved Freelite ratio ≥ 100
>1 focal lesion by MRI

Rajkumar Lancet Oncol 2014;15:e538-48
What is Myeloma?

Symptoms & Diagnosis of Myeloma

Why Urine?

Detecting Monoclonal Proteins
### Multiple Myeloma

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1844</td>
<td>Sarah Newbury</td>
</tr>
<tr>
<td>1848</td>
<td>Dr. Von Rusitzky</td>
</tr>
<tr>
<td>1873</td>
<td>Sir Henry Bence Jones</td>
</tr>
<tr>
<td>1879</td>
<td>Dr. Otto Kahler</td>
</tr>
<tr>
<td></td>
<td>Urine protein in patients with mollities ossium</td>
</tr>
</tbody>
</table>

**Key Figures:**
- **Sarah Newbury**
- **Dr. Von Rusitzky**
- **Sir Henry Bence Jones**
- **Dr. Otto Kahler**

**Timeline Events:**
- 1844: Sarah Newbury
- 1848: Dr. Von Rusitzky
- 1873: Sir Henry Bence Jones
- 1879: Dr. Otto Kahler

**Urine Protein:** Urine protein in patients with mollities ossium
Overproduction of one specific immunoglobulin in multiple myeloma
Different types of multiple myeloma secrete different products

Intact Ig Myeloma (~80%) (90% also secrete free light chains)

Light Chain Myeloma (15-20%) (κ or λ)

Non-secretory or oligosecretory (1-5%)
Multiple Myeloma – How is it diagnosed?

“Off hand, I'd say you're suffering from an arrow through your head, but just to play it safe, I'm ordering a bunch of tests.”
Separating and Detecting Serum Proteins to Detect Multiple Myeloma

Electrical current
Detecting the monoclonal protein in the serum of Multiple Myeloma Patients

 Serum Protein electrophoresis (sPEP)

Normal profile

Monoclonal protein = M-spike

MAYO CLIN PROC. 2001;76:476-487
© 2001 MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
Multiple Myeloma History

<table>
<thead>
<tr>
<th>Year</th>
<th>Person/Invention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1844</td>
<td>Sarah Newbury</td>
</tr>
<tr>
<td>1844</td>
<td>Dr. Von Rusitzky</td>
</tr>
<tr>
<td>1873</td>
<td>Sir Henry Bence Jones</td>
</tr>
<tr>
<td>1879</td>
<td>Dr. Otto Kahler</td>
</tr>
<tr>
<td></td>
<td>Urine Protein Electrophoresis</td>
</tr>
<tr>
<td></td>
<td>Serum Protein Electrophoresis</td>
</tr>
<tr>
<td>2001</td>
<td>Freelite</td>
</tr>
</tbody>
</table>
Different types of multiple myeloma secrete different products

Intact Ig Myeloma (~80%) (90% also secrete free light chains)

Light Chain Myeloma (15-20%) (κ or λ)

Non-secretory or oligosecretory (1-5%)
Detection of Multiple Myeloma

Intact Ig Myeloma (~80%)
(90% also secrete free light chains)

Light Chain Myeloma (15-20%)
(κ or λ)

SPEP + Freelite = 100%

(Katzmann et. Al. 2009, Clin. Chem. 55(8) 1517-1522)
Freelite®: Detects light chains of antibody – that are not bound to heavy chains.

- Kappa releasing
- Lambda releasing

Freelite does NOT detect Kappa, $\kappa$

Freelite detects Lambda, $\lambda$
Free light chains \( \neq \) Total Light Chains

Free Light Chains

milligram/liter (mg/l)

Total Light chains

grams/liter (g/l)
Why use Freelite?

• At initial diagnosis
  - SPEP+Freelite = 100% (467) of multiple myeloma patients
  - 96% (559/581) amyloidosis
    (Katzmann et. Al. 2009, Clin. Chem. 55(8) 1517-1522)

• To monitor response to treatment
• To adjust therapy if treatment not working
• When in remission, to monitor for relapse/regrowth of myeloma cells
Freelite® = Blood (serum) test for free light chains (FLC)

- Because everybody has plasma cells, we all have free light chains (both kappa and lambda) in our blood.

- **Normal ranges:**
  - Kappa: 3.3 - 19.4 milligrams per liter
  - Lambda: 5.7 - 26.3 milligrams per liter
  - Ratio of Kappa / Lambda = 0.26 – 1.65

What is Myeloma?

Why Urine?

Symptoms & Diagnosis of Myeloma

Detecting Monoclonal Proteins
Hevylite® = Blood (serum) test to monitor the monoclonal protein
Overproduction of one specific immunoglobulin in multiple myeloma

\[
\text{Ratio: } \frac{\text{IgA kappa}}{\text{IgA lambda}}
\]
Hevylite tests for IgAκ, IgAλ, IgGκ, IgGλ and IgMκ, IgMλ
Hevylite recognizes heavy and light chains that are bound together.

Distinguishes:
- IgG $\kappa$ vs IgG $\lambda$
- IgA $\kappa$ vs IgA $\lambda$
- IgM $\kappa$ vs IgM $\lambda$
Hevylite & Freelite are different tests
Hevylite May Detect Minimal Residual Disease

Following treatment patient achieves CR, normal HLCr & FLCr

IFE becomes positive 5.5 months after HLCr

At Complete Response (CR)

Monitor sFLCs

Monitor Intact Igs

Measure Freelite ratio
To define a stringent CR

Measure Hevylite ratio
Residual disease detection

Serological assessment
Bone Marrow Biopsy

At Complete Response (CR)
- Monitor sFLCs
- Monitor intact IgGs

Measure Freelite ratio
- Abnormal
  - Monitor with Freelite

Measure Hevylite ratio
- Abnormal
  - Monitor with Hevylite

Normal

Minimal residual disease detection (MFC, ASO-PCR, NGS)
Interpreting Freelite Lab Results
## Sample Freelite results 1

**Clinical Details** | **Serum FLC** | **Other laboratory tests**
--- | --- | ---
Fatigue | \( \kappa = 15 \text{ mg/L} \) | No monoclonal protein observed on Serum protein electrophoresis
| \( \lambda = 24 \text{ mg/L} \) |  |
| \( \kappa/\lambda = 0.7 \) |  |

### Comments:

According to free light chain results, no evidence of monoclonal light chains
**Sample Freelite results 2**

<table>
<thead>
<tr>
<th>Clinical Details</th>
<th>Serum FLC</th>
<th>Other laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Back pain, fatigue</td>
<td>( \kappa = 500 \text{ mg/L} )</td>
<td>Monoclonal protein detected on serum protein electrophoresis</td>
</tr>
<tr>
<td></td>
<td>( \lambda = 25 \text{ mg/L} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \kappa/\lambda = 20 )</td>
<td></td>
</tr>
</tbody>
</table>

**Comments:**
Freelite results indicate monoclonal \( \kappa \) FLCs
Sample Freelite results 3

<table>
<thead>
<tr>
<th>Clinical Details</th>
<th>Serum FLC</th>
<th>Other laboratory tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue, Bone pain,</td>
<td>( \kappa = 1.1 \text{ mg/L} )</td>
<td>Faint monoclonal protein observed on serum protein electrophoresis</td>
</tr>
<tr>
<td></td>
<td>( \lambda = 220 \text{ mg/L} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \kappa/\lambda = 0.005 )</td>
<td></td>
</tr>
</tbody>
</table>

### Comments:

Freelite indicates monoclonal \( \lambda \) FLCs.
Patient with Kappa light chain myeloma monitored weekly using free light chain testing.
IgG κ Multiple Myeloma Monitored with Freelite vs SPEP

Why use Freelite?

- At initial diagnosis
  - SPEP + Freelite = 100% (467) of multiple myeloma patients
  - 96% (559/581) amyloidosis
    (Katzmann et. Al. 2009, Clin. Chem. 55(8) 1517-1522)
- To monitor response to treatment
- To adjust therapy if treatment not working
- When in remission, to monitor for relapse/regrowth of myeloma cells
Why use Hevylite?

- To monitor response to treatment
- When in remission, to monitor for relapse/regrowth of myeloma cells
- Before bone marrow biopsy
Why did my Freelite values change so suddenly?

• Normal biological variation: 20 – 30% day-to-day variation

• Were your tests:
  run on a different lab machine?
  sent to a different laboratory?

• Yes → Run the new sample at both labs – new baseline
What is Myeloma?

Symptoms & Diagnosis of Myeloma

Detecting Monoclonal Proteins

Why Urine?
Why did the doctor ask for a urine test?
Light chains are filtered through the kidney and normally re-absorbed.

Intact Ig = 150 kDa
κFLC = 25 kDa
λFLC = 50 kDa

Glomerulus
40-60 kDa pores

Proximal tubule

10-30g/day reabsorption & breakdown

Urine
Kidney dysfunction leads to excretion of light chains in urine

Glomerulus
40-60 kDa pores

Proximal tubule

10-30g/day reabsorption & breakdown

Intact Ig = 150 kDa
κFLC = 25 kDa
λFLC = 50 kDa

Urine
Intact Ig can be found in the urine during kidney damage. Glomerular damage results in a daily excretion of 10-30g of Ig. The Ig is further broken down and reabsorbed in the proximal tubule. Intact Ig has a molecular weight of 150 kDa, while κ and λ light chains have molecular weights of 25 kDa and 50 kDa, respectively.
Why did the doctor ask for a urine test?

- To test for intact Ig (urine PEP)
- To test for kidney damage
Summary I:

- Monoclonal Gammopathies
- gamma globulin = Immunoglobulin = Ig = antibody …
- … made by plasma cells to fight infections
- Myeloma = too many of one plasma cell making one type of Ig
- Antibody structure – heavy and light chains
- Detected with serum protein electrophoresis (sPEP)
Summary II:

- **Freelite**:  
  - Blood test - Free light chains (kappa and lambda)  
  - Helps detect more cases of Multiple Myeloma, Amyloidosis and most monoclonal gammopathies  
  - The level of free light chains tells your doctor how much myeloma is present

- **Hevylite**:  
  - Blood test – Specifically measures the heavy chain and light chains that are bound together  
  - The amount of (involved) Hevylite tells your doctor how much myeloma is present  
  - Before a bone marrow biopsy  
  - During treatment: Helps monitor treatment efficacy  
  - In remission: May detect relapse earlier
www.thebindingsite.com
Overview of Monoclonal Gammopathies
Plasma cell disorders associated with cancers of the bone marrow

Multiple Myeloma (MM)
A cancer of the bone marrow plasma cells

Products

Freelite®
Freelite® aids the detection and monitoring of Multiple Myeloma and related diseases

Hevylite®
Hevylite® is a unique test for heavy and light chain isotype analysis

Monoclonal Gammopathies resources
The most recent resources available about Monoclonal Gammopathies.

eBOOK
View our Freelite Brochure

Webinar
Discover the Binding Site

Webinar
The Binding Site's 7th International Symposium presented the...

Webinar
Professor Kyle discusses the history of monoclonal proteins
What is Myeloma?

Symptoms & Diagnosis of Myeloma

Detecting Monoclonal Proteins

Why Urine?