Fear the Sphere

Fellow’s Conference
Kalyan Banda
04/22/16
Shape Matters
Case

• 24 yo man presents with cholelithiasis, multiple episodes of biliary colic, and cholecystitis
• Undergoes cholecystectomy
• Presents a few months later to a new PCP with with a bad URI and extreme fatigue
• Labs indicate a Hb 10 (baseline 14-15), absolute reticulocyte count 342, indirect hemoglobin of 4, and splenomegaly
• Patient reveals family history of splenectomy in mother, maternal uncle, cousin and maternal grandmother for “anemia”
Diagnosis?
Shape And Geometry Of An Erythrocyte

- Biconcave shape
- Dimensions: $\sim 8 \times 2 \ \mu m$
- Volume: $90 \ \mu m^3$
- Surface area: $140 \mu m^2$
- Biconcave shape - maximum surface area: volume ratio
The Many “Shapes” Of An Erythrocyte

Reticulocyte

Mature RBC

Spleen

High velocity flow

Narla Mohandas, and Patrick G. Gallagher Blood 2008;112:3939-3948
The Many “Shapes” Of An Erythrocyte

(a) Biconcave
(b) “Slipper”
(c) “Parachute”

An Erythrocyte Undergoes Multiple Movements “Slippering”
An Erythrocyte Undergoes Multiple Movements “Snaking”

An Erythrocyte Undergoes Multiple Movements “Tumbling”
‘Deformability’ - the ability to change shape in response to mechanical forces and recover

Ju Li et al. PNAS 2007;104:4937-4942
“when he was greatly disordered, the globules of his blood appeared hard and rigid, but grew softer and more pliable as his health returned: whence he infers that in a healthy body they should be soft and flexible, that they may be capable of passing through the capillary veins and arteries, by easily changing their round figures into ovals, and also reassuming their former roundness when they come into vessels where they find larger room.”

Van Leeuwenhoek
Deformability Is Dependent On The Cell Membrane

- 100 times more elastic than latex
- Stronger than steel
- Linear dimensions can change up to 250%

Changes to surface area are poorly tolerated.
- 3% increase in surface results in lysis

Copyright © The McGraw-Hill Companies, Inc. All rights reserved.
Etiology of Hereditary Spherocytosis

Table 45-2. Erythrocyte Membrane Protein Defects in Inherited Disorders of Red Cell Shape

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disorder</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankyrin</td>
<td>HS</td>
<td>Most common cause of typical dominant HS</td>
</tr>
<tr>
<td>Band 3</td>
<td>HS, SAO, NIHF, HAc</td>
<td>“Pincered” HS spherocytes seen on blood film presplenectomy; SAO results from 9 amino acid deletion</td>
</tr>
<tr>
<td>β-Spectrin</td>
<td>HS, HE, HPP, NIHF</td>
<td>“Acanthocytic” spherocytes seen on blood film presplenectomy; location of mutation in β-spectrin determines clinical phenotype</td>
</tr>
<tr>
<td>α-Spectrin</td>
<td>HS, HE, HPP, NIHF</td>
<td>Location of mutation in α-spectrin determines clinical phenotype; α-spectrin mutations most common cause of typical HE</td>
</tr>
<tr>
<td>Protein 4.2</td>
<td>HS</td>
<td>Primarily found in Japanese patients</td>
</tr>
</tbody>
</table>

The Red Blood Cell Membrane and Its Disorders: Williams Hematology, 8e, 2010
Pathophysiology Of Hereditary Spherocytosis

• Hallmark defect – Loss of surface area of the RBC
• Sphere – occupies least surface area for a fixed volume
What Degree Of Loss Of Surface Area Is Significant?

Loss of 18% of surface area (or 27% of areas: volume ratio) results in significant retention in spleen.

Clinical severity directly correlates with degree of loss of surface area

<table>
<thead>
<tr>
<th>Laboratory Findings</th>
<th>HS Trait or Carrier</th>
<th>Mild Spherocytosis</th>
<th>Moderate Spherocytosis</th>
<th>Moderately Severe Spherocytosis</th>
<th>Severe Spherocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>Normal</td>
<td>11–15</td>
<td>8–12</td>
<td>6–8</td>
<td>&lt;6</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1–2</td>
<td>3–8</td>
<td>4± 8</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0–1</td>
<td>1–2</td>
<td>4± 2</td>
<td>2–3</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Spectrin content (% of normal)</td>
<td>100</td>
<td>80–100</td>
<td>50–80</td>
<td>40–80§</td>
<td>20–50</td>
</tr>
<tr>
<td>Blood film</td>
<td>Normal</td>
<td>Mild spherocytosis</td>
<td>Spherocytosis</td>
<td>Spherocytosis</td>
<td>Spherocytosis and poikilocytosis</td>
</tr>
<tr>
<td>Osmotic fragility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh blood</td>
<td>Normal</td>
<td>Normal or slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
</tr>
<tr>
<td>Incubated blood</td>
<td>Slightly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
<td>Distinctly increased</td>
<td>Markedly increased</td>
</tr>
</tbody>
</table>

Clinical features
Diagnostic Tests

Osmotic fragility

- Sensitivity: 68-81%
- No longer recommended

- Acid Glycerol Lysis Test (AGLT) sensitivity of > 90%

Diagnostic Tests

EMA (eosin-5’-maleimide) -binding test

- Flow cytometry
- MFI- estimates surface area
- < 18% decrease from normal – negative test
- > 21 loss of surface area – positive
- 18-21% indeterminate
- Sensitivity – 93%
- EMA + AGLT- sensitivity 100%

*HS Patient coloured Pink and the Controls Blue*
Treatment – Splenectomy (Total or partial)

Partial splenectomy – one pole left behind
Long Term Follow Up After Subtotal Splenectomy

- Sustained rise in Hb
- Decrease in reticulocytosis
- Spleen can grow!
- Peaks 5 years after surgery
- Relapse of anemia seen ~11%
Conclusions

• Biconcave shape - maximum surface area: volume ratio
• RBCs are the most deformable cells that exist
• Loss of surface area prevents deformations
• Loss of surface area results in spherical shape
• Key to pathophysiology, clinical manifestations, and diagnostics tests used in hereditary spherocytosis
Acknowledgements

• Bob Richard
• Pam Becker