Please Don’t Say HTLA

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High Titer, Low Avidity

- **Definition**
  - High titer: 32 or higher
  - Low Avidity: speed & intensity of antibody binding
- Never were a “blood group”; no longer considered a category of antibodies
- Some serologists refuse to drop the term
"...serologists frequently use slang or colloquial statements that are intended to be descriptive of the general problem...These slang terms are not intended to define an antibody specificity, but rather roughly describe the serological results." quote by JJ Moulds
Serological Characteristics

- Weak agglutination at AHG
- Not enhanced by LISS or PEG
  Kn non-reactive by solid phase
- Cord cells weaker, fresh cells stronger
- IgG - don’t bind complement
- Variable reactions, hard to reproduce
# Antibody Titration Patterns

<table>
<thead>
<tr>
<th>Titer</th>
<th>Neat</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low titer</td>
<td>w+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Low avidity</td>
<td></td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High avidity</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High titer</td>
<td></td>
<td>1+</td>
<td>1+</td>
<td>w+</td>
<td>w+</td>
<td>w+</td>
<td>w+</td>
<td>m+</td>
<td>m+</td>
<td>m+</td>
</tr>
<tr>
<td>Low avidity</td>
<td>1+</td>
<td>1+</td>
<td>w+</td>
<td>w+</td>
<td>w+</td>
<td>w+</td>
<td>w+</td>
<td>m+</td>
<td>m+</td>
<td>m+</td>
</tr>
<tr>
<td>High titer</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>w+</td>
</tr>
</tbody>
</table>
Previous “HTLA” Antibodies

- Chido (Ch^a)
- Rodgers (Rg^a)
- Holley (Hy)
- Gregory (Gy^a)
- Cartwright (Yt^a)
- York (Yk^a)
- Cost (Cs^a)
- Knops (Kn^a)
- McCoy (McC^a)
- Swain-Langley (Sl^a)
Assignment to Blood Group Systems

- Cartwright ISBT 011
- Holley ISBT 014
- Gregory ISBT 017
- Chido ISBT 017
- Rodgers
- Knops ISBT 022
  - Knops
  - McCoy
  - Swain-Langley
  - York

- Cost – remains a collection
2 antigens, Yt^a & Yt^b
chromosome 7q22.1
C 1057 A, His 353 Asn

Located on GPI-linked protein, AChE
m.w.= 160 kD (dimer)

7-10,000 copies/RBC
Lacking on PNH III
red cells, no true null
Some delayed tx. rx.
# MMA Results for Anti-Yt<sup>a</sup>

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Anti-Yt&lt;sup&gt;a&lt;/sup&gt; Strength</th>
<th>Monocyte Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;, K, s</td>
<td>3+</td>
<td>0.5</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;, Fy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2+</td>
<td>1.5</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;, c, Jk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2+&lt;sup&gt;s&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1+</td>
<td>0.3</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;, Jk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>w+</td>
<td>4.7</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>neg</td>
<td>6.8</td>
</tr>
<tr>
<td>Yt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2+</td>
<td>5.7</td>
</tr>
</tbody>
</table>
JMH- ISBT 026

- 6 high incidence ags.
  JMH1 to JMH6
  (JMHK, JMHL, JMHG, JMHM, JMHQ)
- Carried on CD108
  Unknown RBC function
- No Tx. Rx. or HDFN
  Some are auto-abs.
  Anti-JMH1 are IgG₄
7 antigens: Do\textsuperscript{a}/Do\textsuperscript{b}, Hy, Jo\textsuperscript{a}, Gy\textsuperscript{a}, DOYA, DOMR

Found on 12p13.2 - 12.1

Do glycoprotein of unknown function;

m.w. = 47-58 kD

Null = Gregory negative;

absent from PNH III RBCs

Acute/delayed transfusion reactions; ?HDFN but +DAT
60 y.o. female with CLL
Admission: hgb 8.1/hct 24
Next day: hgb 6.7/ hct 20
Two units requested

PEG antibody screen negative
Two units compatible at AHG
Transfused one unit
“Coca cola colored” urine, hematuria
Serum sample hemolyzed
Increased LDH (623), bilirubin (2.6)
Decreased haptoglobin (5)

Suspected hemolytic transfusion reaction
ABO and Rh checked
DAT negative
Antibody screen negative in PEG & LoIon
Positive reactions in gel but no pattern

*Patient negative for E, K, Fy\(^a\), Fy\(^b\), S, Do\(^a\)*
Additional cells tested in gel

4/4 Do(a-) cells were negative
2/4 Do(a+) cells were positive

Future transfusions should be phenotyped (genotyped) matched
Chido/Rodgers (Ch/Rg)- ISBT 017

- Carried on C4d protein
- Total C4 deficient = null
  - Rg neg associated with SLE
- 9 antigens: Rg1, Rg2, Ch1-6, WH

*Anaphylactic reactions reported to transfusion of plasma products
9 known antigens: Kn\(^a/b\); McC\(^a/b\); SI 1,2,3; Yk\(^a\); KCAM

- carried on complement receptor one (CR1)
- polymorphisms= Knops, molecular weight & RBC expression
- Null = “Helgeson phenotype”, genetically low E-CR1

may also be acquired in AIHA, SLE, HIV, other diseases with increased IC
The Newest Knops Antigen- KCAM

- Antigen frequency:
  98% + in Caucasians, 20% + in West Africans

- KCAM neg. associated with the Helgeson phenotype

- Antibody often “misidentified” as another Knops specificity:
  4 of 9 anti-McCa were anti-KCAM
  6 of 19 anti-Kn/McC “ “ “ “
Arrrrgh… How do I deal with these?

Apply science to serology
Expression Level of CR1 Contributes to Variability in Antigen Strength

- Helgeson phenotype results from low E-CR1 #
  Average = 300-400 copies, Helgeson = 25-60
- “False negative” phenotypes occur in ~20% of Africans
- If Knops suspected type for multiple Kn antigens or perform a genotype
Titer is Dependent on Indicator Cell

<table>
<thead>
<tr>
<th>Anti-</th>
<th>Low E-CR1 #</th>
<th>Medium E-CR1 #</th>
<th>High E-CR1 #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn\textsuperscript{a}</td>
<td>64 (8)</td>
<td>&gt;1024 (34)</td>
<td>&gt;1024 (62)</td>
</tr>
<tr>
<td>McC\textsuperscript{a}</td>
<td>32 (5)</td>
<td>1024 (33)</td>
<td>&gt;1024 (45)</td>
</tr>
<tr>
<td>Sl1</td>
<td>8 (3)</td>
<td>64 (19)</td>
<td>&gt;1024 (48)</td>
</tr>
</tbody>
</table>
Technical Tips

- Use 60 min. saline-AHG antibodies are weaker in LISS & PEG
- Do not use saline with azide
  *Solid phase negative if membranes have azide added
- Use fresh cells for identification
- Use chemicals & enzymes to differentiate
  Ficin destroys Ch, Rg, JMH
  Trypsin & DTT destroy Knops & Dombrock
Other Useful Techniques

- Antibody neutralization:
  - Plasma inhibition for Ch/Rg
  - Recombinant sCR1 for Knops
- C4d coated RBCs for anti-Ch or anti-Rg
  

- Molecular typing:
  
  \[
  \text{KN} = \text{Kn}^a/\text{Kn}^b, \text{McC}^a/\text{McC}^b, \text{Sl1/2}
  \]
  
  \[
  \text{DO} = \text{Do}^a/\text{Do}^b, \text{Hy}, \text{Jo}^a
  \]
  
  \[
  \text{YT} = \text{Yt}^a/\text{Yt}^b
  \]
50 y.o. Black woman
No pregnancy history
Transfused in 1989
Dx= anemia

O positive R₁R₁
Antibody screen= IS neg, w+ @ 24° C
w+ @ AHG, neg auto
Serological Results

- Cold panel = 1+ at 4°C, AC neg
- Anti-\(Sd^a\) ?= not neutralized with urine
- Must be an HTLA = titer m+ to 128

- Panel = reactive 1+ at AHG with all cells including cord cells, reactive ficin panel
- Phenotype matched cells all reactive
- \(Js(b-)\) cells reactive
More Testing

- DTT treated cells = all reactive
- Anti-Ch/Rg? = not neutralized with plasma
- Helgeson cells (KN null) = reactive
- Maybe anti-Hy, order a genotype
Kn(a+b-), McC(a-b-), SI -1,2 (Sl^a neg)

Do(a+b+), Hy+, Jo(a+)

Yn(a-)

Cr(a-)
Safe Transfusion

- Get it into a system before you decide if it is “clinically insignificant”
- Exclude the presence of other allo-antibodies (including cold agglutinins)
- To find suitable blood for the patient
  - Crossmatch using LISS & short incubation
  - Use older donor units for Knops antibodies
- *When in doubt do an MMA*
Monocyte Monolayer Assay (MMA)

MI<5 = clinically insignificant
MMA Prayer

Dear Lord save us from hemolytic transfusion reactions
I Pray Every Day
It’s Not an HTLA