ABO Discrepancies

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Classifications

- Incongruent results
  - Cell grouping
  - Serum grouping
- Unexpected reactions
  - Missing reactivity
  - Extra reactivity
Weak or Missing Reactivity in Cell Grouping Tests
Subgroups of A and B

- Weak expression of A or B antigens
  - Caused by rare allele inheritance
  - B subgroups infrequently encountered
- Traditionally characterized by reactivity with human polyclonal reagents
  - Monoclonal reagents have changed these classifications
- Subgroups weaker than $A_2$ not often seen
  - Most monoclonal reagents react well with $A_1 - A_x$ cells
Subgroups of A and B cont.

- Genotyping can be performed
  - ABO system extremely polymorphic
    - Over 100 ABO alleles for common and unusual phenotypes have been described
      - Ethnic diversity
      - Geographic diversity
  - DNA-based ABO grouping has limited clinical use at this time
Noted Subgroup Characteristics

- **A₃ subgroup**
  - Mixed-field pattern with anti-A from group O or B donors

- **Aₓ subgroup**
  - No agglutination with human anti-A from group B people
  - Agglutination with anti-A,B from group O people
  - May react with monoclonal anti-A
Noted Subgroup Characteristics cont.

- $A_{el}$ subgroup
  - Not agglutinated by anti-A or anti-A,B of any origin
  - A antigen demonstrable only by adsorption and elution studies

- Genomic analysis does not consistently correlate with serologic classification
Para-Bombay Phenotype

- $H$ and $Se$ genes produce fucosyltransferases
  - Expressed in different tissues
    - $Se$ – $H$ substance in secretions
    - $H$ – $H$ antigen on red cells
- Inheritance of $hh$ and $Se$ and $A$ or $B$ can result in small numbers of $H$ and $A$ or $B$ antigens on cells
  - Probably absorbed from plasma
Cis-AB Phenotype

- Rare chromosome encodes for an enzyme with both A and B transferase activity
  - A antigen reacts similarly to A_2 cells
  - B antigen weakly reactive
  - Anti-B and anti-A_1 can be present
Newborns

- Cells carry lower number of A and B antigen sites
  - Antigens attached to linear structures rather than branched chains
- Most monoclonal reagents will not demonstrate weak reactivity with cord cells
Leukemia

- Weak expression of A or B antigens can occur
  - Weak or mixed-field reactivity with routine reagents has been reported with some patients
Mixed-Cell Populations and Chimeras

- Mixed-field reactivity noted with routine reagents can be caused by:
  - Transient dual-cell population
    - Transfusion – most common
    - Allogeneic hematopoietic cells transplant
      - Can cause persistent chimerism
    - Fetomaternal hemorrhaging
  - Genetic chimerism – rare
    - In-utero exchange of progenitor cells in fraternal twins
    - Dispermy
Excessive Blood-Group Substance

- Neutralization of typing reagents can occur when testing unwashed cells
  - Caused by large amounts of soluble blood-group-specific substance in plasma
- Associated with:
  - Ovarian cysts
  - Carcinoma of the stomach
Extra Reactivity in Cell-Grouping Tests
Acquired B

- $A_1$ people can acquire a B-like antigen
  - Presumed to be caused by action of bacterial enzymes
    - A immunodominant sugar is deacetylated from $N$-acetyl-D-galactosamine to D-galactosamine
    - D-galactosamine is structurally close to D-galactose – group B determinant
      - Cells react with anti-B
Formation of Acquired B Antigen

D-galactose → OH

CH2OH

HO

N-acetyl-D-galactosamine

NH2

OH

D-galactosamine

Inactive OH

H, OH

NH3

HO

N-acetyl-D-galactosamine

Acq-B

OH

H, OH

D-galactosamine

B

OH

H, OH

CH2OH

A

OH

H, OH

CH2OH

NHCOCH3
Acquired B cont.

- Patient tests as AB but serum contains anti-B
  - Serum nonreactive with own cells
  - Serum nonreactive with cells from other acquired B patients
- Transient condition associated with disorders of gastrointestinal tract
What’s the cause?

- ES4 clone used to make mononclonal anti-B causes strong reactions with acquired B cells
  - Strength of reactivity weakened with reduced reagent pH
Resolution

- Review patient’s clinical history and historical blood type
  - Associated with colonic bacteria infections
- Test autocontrol or other acquired B cells
  - Will be negative if patient has acquired B
- Test cells with monoclonal anti-B reagent that does not detect acquired B
- Test red cells with human anti-B acidified to pH 6.0
B(A) and $A_1(B)$

- Monoclonal reagents detect minute quantities of antigens
  - Good for reduction of observed subgroups
  - Causes other problems
    - False positives

- Do not rule out reagents as cause for apparent typing problem
B(A) Phenomenon - TM

- **Reactions**
  - Cells react weakly with anti-A, strongly with anti-B
  - Serum reacts with $A_1$ red cells but not with B cells
- **Resolution**
  - Verify if anti-A contains MSO4 clone
    - Test with different anti-A
  - Test serum with $A_1$ and $A_2$ cells
    - Should agglutinate both
      - Won’t work with newborns or immunocompromised patients
    - Distinguishes B(A) from subgroup
Polyagglutination

- Use of monoclonal reagents has eliminated most problems with polyagglutinable cells
  - T-activated cells react with human serum but not with monoclonal reagents
  - Tn-activated cells react with human serum and some monoclonal reagents
    - One example of anti-A,B
    - Tn-activation destroyed by enzyme treatment
Positive DAT

- Strongly reactive DAT cells can spontaneously agglutinate with cell-grouping reagents
  - Most often seen with Rh typing reagents
  - Can occur with ABO reagents if coating antibody is cold-reactive
Resolution

- Wash cells with 37 C saline
- Incubate patient cell suspension at 37 C and wash with warm saline
- More power needed….
  - Elute antibodies from red cells with CDP or DTT
Contaminated Cord Blood Specimens

- Improperly collected samples may contain Wharton’s jelly
  - Hyaluronic acid in jelly causes cells to agglutinate spontaneously in typing reagents
    - “Stringy” agglutination
  - Resolution
    - Saline washes
    - Treat with hyaluronidase
    - Request new heel-stick specimen
      - Easiest solution
Unwashed Cells

- Patient antibodies to reagent components can react nonspecifically in anti-A and anti-B
- Plasma suspended cells may react with anti-B acidified to low pH due to activation of cold-reactive antibodies
- Rouleaux formation can also interfere
- *Washing solves all these problems!!*
Weak or Missing Reactivity in Serum Grouping Tests
Low Antibody Levels

- **Newborns**
  - Serum grouping should not be performed on babies less than 4 months old
  - Maternal antibodies may also interfere
- **Older patients**
  - Research has shown a certain age cannot be reliably used to predict this phenomenon
Missing Antibodies

- Immunocompromised patients may have little or no detectable anti-A or anti-B

Resolution
- Extended RT incubation for serum tests
- 4 C incubation for 15-30 minutes
  - Must include AC and group O tests
- Treat back typing cells with enzymes
  - Parallel test group O and autologous enzyme treated cells
Chimeras

- Persistent chimeras develop a tolerance to both cell populations

- *Check patient history!!!*
Subgroups

- Cells from A subgroup patients often typed as group O
  - Can appear to be missing an expected antibody
  - Example – $A_{el}$ that has not made anti-$A_1$
    - Forwards as O, reverses as A
Weak Reactivity with $A_1$ Cells

- Titer of anti-A is usually higher than that of anti-B in most group O individuals
  - Weak reactivity with $A_1$ cells in group O person should be investigated
    - Same is true for group B people
  - May be due to anti-$A_1$ or other alloantibodies and not by anti-A
  - Testing panel of $A_1$, $A_2$, and O cells can help determine if a discrepancy exists
Extra Reactivity in Serum Grouping Tests
Rouleaux

- Nonspecific pseudoagglutination
  - Cells appear as stacked coins
  - May also appear as irregular clumps that closely resemble agglutination
- Seen in patients with:
  - Elevated protein levels
  - Receiving high-molecular-weight volume expanders
  - Receiving fibrinogen
Resolution

- Saline replacement technique
  - Recentrifuge serum/cell mixture
  - Remove serum, leaving cell button undisturbed
  - Replace serum with equal volume of saline (2 drops)
  - Centrifuge
  - Resuspend cell button gently and observe for agglutination
    - Rouleaux will disperse
    - True agglutination remains
- Dilute serum 1:3 with saline
Cold-Reactive Antibodies

- Commercial serum grouping cells are pools of cells from several Rh-negative donors
  - Express most common blood group antigens – except D
  - Any RT reactive antibody can interfere with serum grouping
    - ABO system (e.g., A₁ or H)
    - Other BGS (e.g., Lewis, M, N, P₁)
    - Autoantibodies (e.g., anti-I)
- Passively acquired abs may be directed to ABO system ags
Resolution

- Testing with A₂, O, and autologous cells, in addition to tests with A₁ and B cells can help explain serum discrepancies
- “Mini-cold panel”
# Interpretation

<table>
<thead>
<tr>
<th>Patient Serum</th>
<th>A₁ Cells</th>
<th>B cells</th>
<th>A₂ cells</th>
<th>O cells</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- Patient forwards as A
- Results consistent with anti-A₁
- Tests should include three examples of A₁ and A₂ cells to meet probability requirements
Cold-Reactive Alloantibody

- Select antigen-negative reverse cells
- Raise temperature of test components to 30 to 37 C before mixing serum and cells, incubate for 1 hour, perform “settled” reading
  - May resolve if thermal amplitude of alloab is below temp at which anti-A and anti-B react
- If absc is neg, test serum against several A\textsubscript{1} and B cells
  - Serum may contain ab to low incidence ag
  - Ag will be absent from most randomly selected red cells
# Interpretation

<table>
<thead>
<tr>
<th></th>
<th>A\textsubscript{1} Cells</th>
<th>B cells</th>
<th>A\textsubscript{2} cells</th>
<th>O cells</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Serum</td>
<td>1+</td>
<td>4+</td>
<td>0</td>
<td>1+</td>
<td>0</td>
</tr>
</tbody>
</table>

- Patient forwards as A
- Results consistent with an alloantibody
- Reactivity with group O cells usually indicates ab to non-ABO ag, but could indicate anti-H
Autoantibody Resolution

- Adsorption to remove unwanted reactivity
  - Autologous
  - Allogeneic
    - Using group O cells if pt recently tx’ed
      - Do not use adsorbed serum for ab detection or crossmatches
  - RESSt
    - Can remove clinically significant antibodies
    - Anti-B, -D, -E, -Vel, others
    - Do not use RESSt adsorbed serum for serum grouping or crossmatches
- Prewarming
## Interpretation

<table>
<thead>
<tr>
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<td>Patient Serum</td>
<td>1+</td>
<td>4+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
</tr>
</tbody>
</table>

- Patient forwards as A
- If patient has not been recently tx’ed, results consistent with an autoantibody
Passively Acquired Abs

- Check patient transfusion history!
- Most common cause is transfusion of multiple components containing ABO-incompatible plasma
  - Platelets
- Other antibodies not common
  - Blood donors are screened for abs
Special Techniques to Resolve ABO Discrepancies

- When performing testing outside the norm, make sure to follow package inserts
  - Secretor studies / hemagglutination inhibition
    - Use of human polyclonal reagents is recommended
  - Adsorption / elution studies
    - Monoclonals may give false positives
  - Extended incubation / decreased temperature
    - Most monoclonals allow for extended incubation time, but not 4 C
    - If rgt used outside package insert instructions, validation and controls are necessary
Case 1

- An institution used monoclonal reagents for routine ABO and Rh typing. Patient JS is a 72-year-old female, admitted to the institution with a broken hip. She has no history of recent transfusion. She is scheduled for surgery in the morning. A type and screen were ordered; initial typing results are as follows:
## Initial Typing Results

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; cells</th>
<th>B cells</th>
<th>Anti-D</th>
<th>Rh Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
</tr>
</tbody>
</table>
Probable Causes

- Patient is group $A_2$ and her serum contains anti-$A_1$
- Patient is group A and her serum contains a cold-reactive alloantibody
- Patient is group A and her serum contains a cold reactive autoantibody
## Additional Test Results

<table>
<thead>
<tr>
<th></th>
<th>$A_1$ cells</th>
<th>$A_2$ cells</th>
<th>B cells</th>
<th>O cells</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS</td>
<td>2+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
# Additional Testing

<table>
<thead>
<tr>
<th>JS Serum</th>
<th>$A_1$ cells</th>
<th>$A_1$ cells*</th>
<th>$A_2$ cells</th>
<th>$A_2$ cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2+</strong></td>
<td><strong>2+</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Two different sources of $A_1$ and $A_2$ cells were used

<table>
<thead>
<tr>
<th>Anti-$A_1$ Lectin</th>
<th>Patient JS</th>
<th>$A_1$ cells (Positive Control)</th>
<th>$A_2$ cells (Negative Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>4+</strong></td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>
Conclusion

- Patient JS is group A₂, Rh positive
- Her serum contains anti-A₁
  - Caused initial typing discrepancy
Case 2

- An institution uses monoclonal reagents for routine ABO and Rh typing. Patient JK is a 37-year-old female blood donor. The donor states that she has never been transfused, and the institution has no previous records for JK. Initial typing results are as follows:
### Initial Typing Results

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A₁ cells</th>
<th>B cells</th>
<th>Anti-D</th>
<th>Rh Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>JK</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>4+</td>
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<td>0</td>
</tr>
</tbody>
</table>
Probable Causes

- Patient is group $A_2$ and her serum contains anti-$A_1$
- Patient is group A and her serum contains a cold-reactive alloantibody
- Patient is group A and her serum contains a cold reactive autoantibody
<table>
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<tr>
<td>JK</td>
<td>2+</td>
<td>0</td>
<td>4+</td>
<td>2+</td>
<td>0</td>
</tr>
</tbody>
</table>
Explanation

- Reactivity with group O cells but not with AC suggests presence of cold-reactive alloantibody
- $A_2$ cells are most likely negative for the offending antigen and are nonreactive
### Antibody Screen on JK’s Serum

<table>
<thead>
<tr>
<th></th>
<th>Screening Cell I</th>
<th>Screening Cell II</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>0</td>
<td>2+</td>
</tr>
<tr>
<td>37 C LISS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IAT</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

- Anti-M identified
Resolution

- Repeat serum grouping using prewarming test
- Select M-negative reverse-grouping cells
- Enzyme treat reverse-grouping
  - Check package insert
- Validate
Conclusion

- Patient JK is group A, Rh positive
- Serum contains anti-M
  - Caused initial typing discrepancy
Suggested Resolution Process for Serologic Problems

- Repeat testing on same sample
  - Wash patient cells
- Obtain patient information
  - Diagnosis
  - Historical blood group
  - History – tx, transplants, meds
- Review results with group O red cells and AC
  - Allo or autoabs
- Obtain new sample if contamination is suspected