Antibodies to Co-trimoxazole interfering with routine antibody screening: Case

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In-vitro reactions not due to blood group antibodies are encountered when typing red cells / performing compatibility testing / antibody screen.

Patient has an antibody that reacts with a chemical present in –

- The commercial RBC suspension
- Commercial Anti-sera
- Commercial Antibody Potentiators (Albumin or LISS)

These reactions can lead to serious errors or incompatible crossmatches or delay in supplying blood for transfusion.
Table: Reported antibodies to chemicals added to reagents

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Bacteriostatic /antifungal reagents</th>
<th>Sugars</th>
<th>Dyes</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>neomycin</td>
<td>Paraben®</td>
<td>glucose</td>
<td>acriflavine</td>
<td>EDTA</td>
</tr>
<tr>
<td>chloramphenicol</td>
<td>thimerosal</td>
<td></td>
<td>yellow #5 tartrazine</td>
<td>inosine citrate</td>
</tr>
<tr>
<td>gentamycin</td>
<td>sodium azide</td>
<td></td>
<td></td>
<td>sodium caprylate</td>
</tr>
</tbody>
</table>

G. Garratty. IMMUNOHEMATOLOGY, Vol 14, 1998
Chemical Present in Commercial RBC suspending Media

Many companies
adenine
citramphenicol
glucose
inosine
neomycin sulfate
sodium chloride
sodium citrate

different companies
adenosine
citric acid
gentamicin
guanosine
hydrocortisone
magnesium sulfate
potassium chloride
potassium phosphate
sodium acetate
sodium bicarbonate
sodium gluconate
sodium phosphate
sodium pyruvate
sucrose

G. Garratty. IMMUNOHEMATOLOGY, Vol 14, 1998
Case

- 36 year old female,
- Admitted with complaints of Generalized weakness and headache for 3 days
- a known case of hydrocephalus, Aseptic meningitis
- On regular antiepileptic, antidepressant, anti-osteoporotic medications
- VP shunt was done in 2009 and admitted for surgery for revision of V-P shunt
Request for Type and screen

- Hemoglobin - 11.5

- Blood group – ABO Discrepancy
- Forward Grouping – A positive
- Reverse grouping – Mixed field reactions with A cells and O cells
- ? Cold Agglutinins

- Tube method confirmed BGP– A Positive
Antibody screen

- Using Gel method (Biorad ID-Diacell I-II-III),
- 4+ Panreactive antibody detected in AHG phase,
- Direct Coombs’ Test – Negative
- Auto control - Negative

1. Autoimmune etiology was ruled out
   Negative DCT (anti-IgG and anti-C3d) and autocontrol
Antibody Identification

2. Antibody against High frequency antigen
   - (anti-k, anti-kpb, anti-Lub) was suspected.

3. Panreactive antibody in saline phase
   - Anti-IH antibody was also kept as a possibility.

The Neurosurgery Team was informed for Positive antibody screen and possibility of Anti-HFA
Conflicting Crossmatch results

- To rule out Anti-IH, crossmatching was performed with both A and O positive units. It showed compatible results with both A/O units.

- Indirect Coombs Test by Tube method - Negative
Sample were tested with commercial screen cells of different manufacturers

- **Surgiscreen cells** using Glass bead method, Orthoclinical diagnostics
- **B. Matrix ERYGEN AS reagent red cells**; Tulip diagnostics
- The results with other commercial red cells were nonreactive.

? Antibody to reagent red cell preservative
Manufacturing Inserts

All test cell reagents are of human origin, in a buffered suspension medium at 0.9% (± 0.1%). Preservatives: the antibiotics trimethoprim and sulamethoxazole.

For antibody screening, single donors, blood group O:

ID-DiaCell I-II
ID-DiaCell I-III
ID-DiaCell IP-IIP-IIIP
ID-DiaCell Pool
ID-DiaCell I-II-III Asia

\[ \text{R}_1 \text{R}_1 + \text{R}_2 \text{R}_2 \text{ for IAT and NaCl test} \]
\[ \text{R}_1 \text{R}_1 + \text{R}_2 \text{R}_2 + \text{r} \text{r for IAT and NaCl test} \]
\[ \text{papainized, for enzyme technique} \]
\[ \text{R}_1 \text{R}_1 + \text{R}_2 \text{R}_2 \text{ (2 pooled cells for donor screening)} \]
\[ \text{R}_1 \text{R}_1 + \text{R}_2 \text{R}_2 + \text{cell of the GP.MUR phenotype, for IAT and NaCl test} \]

Ortho Clinical Diagnostics

Surgiscreen Reagent Red cells, Phosphate citrate buffered diluent, Steroid, Chloramphenicol, neomycin sulphate, Gentamycin

Special preservative media
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Bio-Rad Laboratories</th>
<th>Ortho clinical Diagnostics</th>
<th>Tulip diagnostics Matrix ERYGEN AS reagent red cells</th>
<th>Tube method Using in-house Pooled O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative</td>
<td>Trimethoprim and sulphamethoxazole</td>
<td>Chloramphenicol, neomycin sulphate, Gentamycin, Steroid</td>
<td>Contents not disclosed</td>
<td>None</td>
</tr>
<tr>
<td>Antibody screen results</td>
<td>Panreactive 4+ in AHG as well as phase</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
To confirm the nature of drug induced antibodies, reagent red cells were washed and ICT was found negative with washed reagent red cells.

- On incubating the reagent red cells in BLISS, ICT was reactive.
- Also, on incubating the donor red cells with BLISS, the crossmatch was showing reactive due to presence of same preservative drug in BLISS as Co-trimoxazole.
Red cell diluents

- **ID –Diluent 2**: Modified LISS for red cell Suspensions:
  - Preservative: the antibiotics trimethoprim and sulphamethoxazole

- **Ortho BLISS**
  - Preservative: Chloramphenicol, Trimethoprim and sulphamethoxazole
Washed reagent red cells incubated with LISS for More than 1 hour RT

Donor cells + LISS 1 hr Incubation + Pt serum

Titration 1:16
Final Diagnosis

- On review of past history, Tab Bactrim was administered for UTI two month before
- It was confirmed to be an anti- Co-trimoxazole drug induced antibody against preservative media in reagent red cells.
- Co-trimoxazole is an antibacterial drug, a combination of two drugs: Trimethoprim (TMP) and Sulphamethoxazole (SMX)
Patient evaluation

- There were no clinical features of hemolysis.
- Blood transfusion was not required during surgery.
- The patient and the physician were explained regarding potential risk of hemolysis if the same drug is taken.
- Patient was discharged with stable status with proper advice.
- The possibility of Co-trimoxazole Drug induced aseptic meningitis was also considered.
Chloramphenicol antibody causing interference in antibody detection and identification tests.

Thimerosal-dependent agglutination complicating the serologic evaluation for unexpected antibodies.

Antibodies to hydrocortisone in reagent red cells causing positive antibody screening tests.

Antibodies to co-trimoxazole (trimethoprim and/or sulfamethoxazole) related to the presence of the drug in a commercial low-ionic-strength solution.
These in-vitro reaction due to preservative antibiotic drugs are similar to reactions of DIHA

Follow “Immune complex mechanism”

Reaction is seen only when patient serum containing antibody is added to RBCs in the presence of antibiotic (i.e. the commercial RBC suspension media)

Certain drug antibodies reported has shown Antibody specificity

- Paraben – Anti-Jka
Discussion

- Co-trimoxazole is an antibacterial drug, a combination of two drugs: Trimethoprim (TMP) and Sulphamethoxazole (SMX).
- These antibodies have shown to be associated with Hemolytic anemia, renal failure, aseptic meningitis.
- Antibodies against Co-trimoxazole shows three Patterns
  - Anti-TMP + Anti-SMX
  - Anti-TMP alone (anti-Ku specificity)
  - Anti – SMX (Anti-H specificity)

- These anti-preservative antibodies should be assessed carefully, and despite absence of clinical events, the avoidance of drug should be recommended.
To summarise ..

- These anti preservative antibodies should be suspected and given attention whenever
  - Conflicting results are noted in antibody screening with different techniques of similar sensitivities,
  - Panreactive results and absence of clinical and biochemical data matching with results.
Thank you

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