

## Is it Auto-, Alloantibody(ies) or Both? Tools to Resolve

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## Patient History

- Diagnosis, Race and/or Nationality
- Hemoglobin/Hematocrit
- Date of Recent Transfusion, if any
- How many, why
- Past Transfusions and/or Pregnancies
- Previous Antibody(ies)
- Drugs/medications

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## Diagnosis

- Autoimmune Hemolytic Anemia (AIHA)
- Sickle Cell Disease
- G I Bleeder
- Leukemia
- Chronic Lymphocytic Leukemia (CLL)
- Kidney Disease

3



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## Red Blood Cells (RBCs)

- Evaluate DAT
  - IgG, C3, Both, Mixed field?
  - Strength of reactivity for each component
  - Negative Control for DAT
- Patient Not Recently Transfused
  - Rh and K Phenotype?
  - EGA-treat RBCs to type for other antigens?

4



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## RBCs (cont'd)

### Recently Transfused Patient

Perform a Cell Separation?

- Purpose
  - Determine if patient's own RBCs have positive DAT
  - Phenotype patient's RBCs
- Results
  - If DAT is negative on separated cells, possible alloantibody coating donor cells (phenotype patient)
  - If DAT is positive on separated cells, autoantibody on patient's cells, possible alloantibody(ies) as well

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## Elution Studies

- Patient not recently transfused-Is elution necessary? Hospital Policy and /or SOP
- Patient recently transfused-Eluate may be informative if evaluated with select cells, patient's "separated cells", etc. Not necessary if patient has not been transfused in past 3 weeks (whatever is in eluate should be in serum).

6



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## Serum/Plasma

- Autoantibody
- Alloantibodies (mixture)
- Alloantibody directed at high-incidence antigen
- Auto- and alloantibodies (mixture)
- Preservative antibody
- Antibody to reagent additive solution

7



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## Evaluation of Serum/Plasma

- Same strength/variability
- Phenotypically similar RBC (if phenotype of patient determined or known)
- Comparison of untreated vs enzyme-treated RBCs
- Separated patient RBCs (free of IgG) with serum/plasma

8



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## Adsorption of Serum/Plasma

- Autoadsorption-patient NOT recently transfused
- Phenotypically similar RBC
- Heterologous (allogenic) adsorption  $R_1R_1$ ,  $R_2R_2$ , rr
- Enzyme/Enzyme-DTT (ZZAP) – treated RBCs
- Number of adsorptions – strength of antibody reactivity (1-2+ ads 2X), (3-4+ ads 4X)
- Test original adsorbing RBCs with adsorbed serum to determine if reactivity is removed

9



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**Autoantibody:  
Adsorbed Serum/Plasma Results**

- Adsorbing RBCs nonreactive
- Screening RBCs and/or selected RBCs nonreactive
- Alloantibodies ruled out
- Test at 37C using a LISS without potentiators or using 2-4 drops adsorbed serum without potentiators and extended incubation

10



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**Alloantibodies (mixture):  
Unadsorbed Serum/Plasma Results**

- Phenotypically similar RBCs nonreactive
- Reactivity with screening RBCs and/or selected RBCs demonstrates variation
- Tube testing can be beneficial to see different antibodies at various phases

11



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**Alloantibody Directed at High-Incidence Antigen: Adsorbed Serum/Plasma**

- Nonreactive or still reactive with adsorbing RBCs and/or phenotypically similar RBCs
- Prepare an eluate from RBCs used for first adsorption
- Separated RBCs of patient would be nonreactive with unadsorbed serum/plasma

12



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### Auto- and Alloantibody(ies): Adsorbed Serum/Plasma

- Phenotypically similar RBC nonreactive
- Reactivity with screening RBCs and/or selected cells shows antibody specificity(ies)
- Phenotypically similar cell still reactive (possible antibody directed at high-incidence antigen)

13



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### Case #1 Auto- or Alloantibody?

- 55 y.o. white male
- Diagnosis anemia
- Request for 3 u RBCs
- Initial screening and panel cells all reactive in IAT
- Autologous control reactive
- What to do next?

14



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### Case #1 (cont.)

- Obtain patient's history-no previous transfusions
- Characterize DAT (IgG 3+, C3 0)
- Perform Rh, K1, Jk<sup>a</sup>, Jk<sup>b</sup> phenotype? (use monoclonal reagents)
- EGA-treat RBCs to obtain negative DAT and type for other antigens?

15



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### Case #1 Adsorption Studies

- Perform a warm autoadsorption (3-4 X at 37C), using enzyme- (ficin) treated RBCs of patient, or ZZAP- or WARM-treated RBCs
- Results: Screening cells nonreactive (no alloantibodies present)
- Conclusion: Warm-reactive autoantibody
- Transfuse Rh and K1 specific RBCs?

16



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### Case #2 Auto- and Alloantibody?

- History:
- 45 y.o. white female
- G3P3
- Diagnosis: GI Bleeder
- Transfusions: multiple, last one 3 weeks ago
- Request for 3 RBCs

17



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### Case #2 Serology Results

- All screening cells and panel cells react to varying degrees (1+ to 3+)
- Autologous control reactive (2-3+ with some mixed field)
- Crossmatches all incompatible
- What to do next?

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## Case #2 Approach to Resolving

- Send to a Reference Laboratory?
- Characterize DAT (IgG 3+ C3 0)
- Cell separation-DAT still positive (but weaker and only 1+)
- Phenotype separated cells for Rh, KI and Kidd
- EGA-treat to obtain negative DAT to type for other antigens

19



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## Case #2 Heterologous Adsorption

- Neat serum of patient reactive with EGA-treated cells of patient (negative DAT) and a phenotypically similar cell
- Adsorbed patient's serum with a E- K- S- Jk(a-) phenotypically similar cell
- Adsorbed serum demonstrates presence of anti-E, anti-K and anti-Jk<sup>a</sup>

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## Case #2 Conclusion

- Patient's serum contains a warm-reactive autoantibody
- Alloanti-E, -K and anti-Jk<sup>a</sup> were demonstrated in the adsorbed serum
- Limitation to adsorption: have not ruled out alloantibody directed at high-incidence antigen

21



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## Points to Consider

- Always obtain a good patient history when performing adsorption studies
- Know the limitations of cell separation and adsorption techniques
- Become proficient in performing special techniques, especially when investigating warm-reactive autoantibodies

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## AIHA Case Study

- Jan 1996: DAT and antibody screen ordered on 62 year old Caucasian female
- DAT 3+ with PS AHG and Anti-IgG
  - Anti-C3 is negative
- Antibody screen is negative
- Eluate reacts with all cells tested
- Units compatible with serum

23



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## May 1996

- Request for 2 units RBCs
- Antibody screen now positive
- Panel study: All RBCs (including autologous control) are positive
- What to do next?

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## Questions

- Patient history?
- Remove apparent autoantibody?
- Type patient's RBCs for common blood group antigens?
- Eluate?
- Select donor units?

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## Patient History

- Patient never transfused, but had 3 pregnancies
- Has received steroids since Jan 1996
- HgB is 6.2 g/dL
- Autoadsorption is method of choice for removing serum reactivity

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## Methods of AutoAdsorption

- Untreated RBCs
- Remove IgG:
  - Chloroquine diphosphate
  - Glycine acid/EDTA: Kell destroyed
- Enzyme-treated RBCs: Rh enhanced, En<sup>a</sup>FS and En<sup>a</sup>TS destroyed
- DTT-treated RBCs: Kell, LW, etc. destroyed
- ZZAP-treated RBCs: DTT and Enzyme

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## PEG Autoadsorption

- Yew Wah Liew, Polyethylene glycol in autoadsorption of serum for detection of alloantibodies. Transfusion. Letter to the editor. 1995;35:713.
- Transf. 1996;36:384 Champagne/Moulds
- Supplement p20S Abstracts S78 and S79
  - Rapid adsorption of warm autoantibodies PEG
  - Differential adsorption using PEG

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## PEG Autoadsorption (cont'd)

- Combs, et al. Transfusion 2001;41:30S (Abstract)
- The use of Polyethylene Glycol in Adsorption: More Evidence that Antibodies May be Missed
- Conclusion: Overall, 2/3 of the antibodies removed by PEG were clinically significant

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## Typing Patient's RBCs for Common Antigens

- Can perform Rh, Kell and Kidd typings with antisera that do not require IAT
  - Can also do Lewis, MN and P<sub>1</sub> typings
  - Important if there is any RT reactivity
- EGA-treat patient's RBCs to perform Ss and Duffy typings
- Chloroquine-treat RBCs to perform any typings for antigens destroyed by EGA
- Genotype by DNA

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## June 1996

- Request for 2 units RBCs
- We could have drawn extra tubes in May, saved for another autoadsorption (DARN!)
- Example: triple adsorption
- What if we had typed the patient in May?
- Adsorption with a phenotypically similar cell, instead of triple adsorption

31



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## Recently Transfused Patient

- Adsorption studies to remove autoantibody and rule out or demonstrate alloantibody
- If patient has been transfused within the past 3 weeks, perform an elution if there has been a change in strength of DAT
- Do a cell separation to check for strength of DAT on patient's reticulocytes
- If DAT is positive, EGA-treat RBCs and test with serum and eluate to show autoantibody

32



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## Summary

- Patient care is the primary concern
- Patient Information-Key element for resolving serological problems
- Necessary serological tools
- Expertise-training, practice makes perfect, the more you do the better you become and confidence is gained
- Ask advice from coworkers and mentors
- Education leads to knowledge

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