

More twists on antibodies with e specificity

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Anti-e

- Normal alloimmune anti-e in e-negative individuals
- Alloimmune anti-e in e-positive individuals with variant e antigen
- Autoimmune anti-e in e-positive individuals (Warm autoantibodies with e-specificity)
- And now.... something different!

Case Study

- Sample referred to IRL 1/31/10 for ABID and compatibility testing. Previous history of probable WAA with e-specificity (2008).
- 59 y/o female
- Dx: pancreatitis and ESRD
- Facility reported that antibody screen was negative in December, 2009.

Initial results

- O Rh positive
- DAT 3+ PS and -IgG, weakly positive with -C3
- Antibody screen and selected cells show antibody with relative e specificity
 - e negative cells weakly positive
 - e positive cells 2-3+

Fly in the Ointment

- Eluate results: Non-reactive
- When the DAT is strongly positive due to WAA, strongly positive reactions (2+ - 4+) are expected when testing the eluate
- A negative eluate (or unexpectedly weak eluate) is highly suggestive of a drug-dependent antibody

Other considerations

- Positive DAT with non-reactive eluate may also be due to:
 - Passive ABO antibodies from out of group trx, IVIG (Would be detected when reverse grouping cells are included in eluate testing.)
 - Cytophilic IgG (hypergammaglobulinemia)

Importance of Drug History

- Referring facility faxed over medication history
 - IV Zosyn was recently administered (Piperacillin-Tazobactam)
 - When looking at patient's drug history, may also be important to look at what drugs pt might have received 1 to 2 weeks ago
- Drug studies with piperacillin were initiated and hospital alerted to possible drug-induced immune hemolytic anemia

Drugs associated with DIIHA

- Too numerous to mention! In 2007, Garratty and Arndt had data that supported 125 drugs causing DIIHA.
- In 2009, Garratty reported the 3 drugs most frequently associated with DIIHA are cefotetan (Cefotan), ceftriaxone (Rocephin), and piperacillin.

Classifications for detecting Antibodies to Drugs



- **Drug-independent autoantibody:** Drugs are not necessary to detect the autoantibody. The serologic picture can not be distinguished from classic WAIHA with strong DAT and eluate reactive with all reagent cells. Examples: α -methyldopa (Aldomet), mefenamic acid (Ponstel), procainamide (Pronestyl).

Classifications for detecting Antibodies to Drugs



- **Drugs that bind tightly to rbc membrane:**
Drugs bind effectively to the membrane. Antibodies directed to the drug attach to the rbc-bound drug and cause positive DAT. Drug-treated rbc's are required to demonstrate the antibody. Examples: penicillins, 3rd generation cephalosporins (cefotetan)

Classifications for detecting Antibodies to Drugs



- **Drugs that do not bind to rbc membrane:**
Drugs bind to proteins to form immunogens that evoke antibody response. IgG, in addition to C3, may be detected on rbc. Eluates usually nonreactive and serum tests react only in presence of drug. Examples: quinine, piperacillin, many NSAIDs

Classifications for detecting Antibodies to Drugs



- **Nonimmunologic adsorption of protein:**
RBC membranes are modified by the drug allowing nonimmunologic adsorption of all proteins (albumin, immunoglobulins, etc.).
Examples: cephalosporins, β -lactamase inhibitors (sulbactam, tazobactam)

Piperacillin

- Semi-synthetic penicillin
- Is commonly used in combination with tazobactam for treating serious infections. The combined antibiotic (Zosyn) is active against many Gram-pos and Gram-neg bacteria

Anti-piperacillin

- Does not react like anti-penicillin
 - Testing with drug-coated cells is unreliable
 - High percentage of normal donor sera react with piperacillin-coated rbc's.
 - Most likely due to exposure from environment, e.g. antibiotics added to livestock feed, antibiotics present in milk
 - Best detected by testing patient's serum and eluate in the presence of drug (previously referred to as "immune-complex method")

Other considerations

- The drug must be put into solution at the proper concentration. Best to get drug in powder form if possible - drugs pre-diluted by the pharmacy may not provide optimum drug concentration for study.
- Zosyn-coated cells can be reactive due to non-immunologic protein adsorption due to tazobactam, resulting in a positive DAT.
- Drug studies performed in the presence of pure tazobactam have not yielded any cases of anti-tazobactam to date.
- Testing done using piperacillin solution of 1 mg/mL in PBS

Testing for Drug-dependent antibodies in presence of drug



- May use enzyme-treated and/or untreated red cells
- Serum should be used; EDTA plasma cannot be used for detection of hemolysis
- RBCs should not react with the patient's neat serum or eluate
 - May require titration of patient's serum
- Antibodies may be IgM or IgG
- Controls, controls, controls



Testing for Drug-dependent antibodies in presence of drug (unttd e- rbc)

	30" RT	60" 37C	60" AHG
Pt serum (PS) + drug	0	0	2+
PS + drug+ complement (normal serum)	0	1+	2+
PS + saline	0	0	0 ✓
PS + complement + saline	0	0	0 ✓
Complement + drug	0	0	0 ✓
Complement + saline	0	0	0 ✓
Saline + drug	0	0	0 ✓

Testing for Drug-dependent antibodies in presence of drug (untd e- rbc)



	60" 37C	60" AHG
Eluate + drug	0	2+
Eluate + saline	0	0 ✓

Conclusions

- Piperacillin-dependent antibodies were detected in the patient's serum
- Initial serologic results were identical to those seen in cases of WAA with relative e-specificity
- The negative eluate was critical in differentiating DIIHA from WAIHA
- What about patient's history of probable WAA from 2008?

A step back in time....

- Dx: Anorexia, weight loss, ESRD, failure to thrive, ? Obstruction w/ very high bilirubin
- Received sample dated 03-10-2008
- Group O positive
- DAT 3+ IgG, 1+ C3
- Phenotype: C+, E-, c+, e+

2008 results (cont)

- Serum results: Reactive with e+ cells in PeG only. Reactive with patient's EGA-treated, DAT-neg pre-transfusion cells. Consistent with WAA demonstrating e-specificity.
- Eluate results: Non-reactive
- Medication history: Piperacillin-Tazobactam (Zosyn) administered IV starting on 03-06-2008 per referring facility.

2008 results (cont)

- Reported probable WAA with e-specificity, with notation “The finding of a strong positive DAT with a non-reactive eluate is suggestive of drug-induced hemolytic anemia. Piperacillin-Tazobactam has been implicated as a causative agent. Drug studies are recommended.”

Why accurate interpretation is important



- DIIHA typically occurs when the patient is taking the drug and subsides when the drug is stopped. In cases of DIIHA, discontinuing the drug will lead to remission of HA. Steroids are usually not required.
 - Depends on half-life of implicated drug; some drugs may persist longer than others
 - Also, avoidance of drug in future is important, as additional exposure to the drug may lead to more severe episodes of HA

Summary

- When a positive DAT is detected, a thorough medication history is important
 - Prescription drugs
 - Over the counter drugs
 - Alternative (herbal) medications
 - History of any surgical procedures in which antibiotics might have been administered by surgeon or anesthesiologist

Summary

- An eluate should be performed on initial workup of apparent cases of WAIHA
- When an antibody to a drug is detected, the patient and the patient's physician should be informed to avoid that drug in the future to prevent another hemolytic event.

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