



Immunohematology Case Studies 2016 - 8

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Clinical History



- 46 y/o Caucasian male
- Diagnosis of HCV & HIV
- 3 days ago received 2 Leukocyte-reduced RBCs (LRBC)
 - No antibodies detected at that time
- Hgb 5.6 g/dl, additional LRBCs are ordered for transfusion
- Sent to IRL for work-up because...
 - All RBCs tested in gel & DAT are positive!

IRL Results

Antibody Detection Test



		Gel IAT
I	R_1R_1	2+
II	R_2R_2	w+
III	rr	2+



Serum/plasma was “icteric”

Antibody Identification Panel Gel



	D	C	c	E	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	Gel IAT
1	+	+	0	0	+	0	+	0	+	0	0	+	2+
2	+	+	0	0	+	+	0	+	+	+	+	+	2+
3	+	0	+	+	0	0	0	+	+	0	+	+	W+
4	+	0	+	0	+	0	0	+	0	+	0	+	2+
5	0	+	+	0	+	0	+	+	+	0	+	0	2+
6	0	0	+	0	+	+	0	+	0	+	+	+	2+
7	0	0	+	+	0	0	+	0	0	+	0	+	W+
8	0	0	+	0	+	0	0	+	+	0	+	0	2+
Auto													3+

The autocontrol is positive

Antibody Identification Panel Gel



	D	C	c	E	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	Gel IAT
1	+	+	0	0	+	0	+	0	+	0	0	+	2+
2	+	+	0	0	+	+	0	+	+	+	+	+	2+
3	+	0	+	+	0	0	0	+	+	0	+	+	W+
4	+	0	+	0	+	0	0	+	0	+	0	+	2+
5	0	+	+	0	+	0	+	+	+	0	+	0	2+
6	0	0	+	0	+	+	0	+	0	+	+	+	2+
7	0	0	+	+	0	0	+	0	0	+	0	+	W+
8	0	0	+	0	+	0	0	+	+	0	+	0	2+
Auto													3+

The antibody detection and panel results show weaker reactivity with e-negative RBCs

Antibody Identification Panel

Test Tube Methods



PEG Saline

	D	C	c	E	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	IAT	IAT
1	+	+	0	0	+	0	+	0	+	0	0	+	1 ^s	1
2	+	+	0	0	+	+	0	+	+	+	+	+	1	1
3	+	0	+	+	0	0	0	+	+	0	+	+	w	0 ^v
4	+	0	+	0	+	0	0	+	0	+	0	+	1s	1
5	0	+	+	0	+	0	+	+	+	0	+	0	1	w
6	0	0	+	0	+	+	0	+	0	+	+	+	1	w
7	0	0	+	+	0	0	+	0	0	+	0	+	W	0 ^v
8	0	0	+	0	+	0	0	+	+	0	+	0	1	w
Auto													3	2

Weaker or no reactivity is observed with e-negative RBCs as the method sensitivity decreases, while the auto remains positive

Direct Antiglobulin Test



Polyspecific AHG	4+
Anti-IgG	3+
Anti-C3	4+
Control	0

Consistent with the positive autocontrol observed with gel and tube methods, the DAT is strongly positive due to IgG and C3.

Antibody Identification Panel

Ficin & DTT Treated RBCs



DTT
Ficin PEG

	D	C	c	E	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	IAT	IAT
1	+	+	0	0	+	0	+	0	+	0	0	+	3	1
2	+	+	0	0	+	+	0	+	+	+	+	+	3	1
3	+	0	+	+	0	0	0	+	+	0	+	+	3	1
4	+	0	+	0	+	0	0	+	0	+	0	+	3	1
5	0	+	+	0	+	0	+	+	+	0	+	0	3	1
6	0	0	+	0	+	+	0	+	0	+	+	+	3	1
7	0	0	+	+	0	0	+	0	0	+	0	+	3	1
8	0	0	+	0	+	0	0	+	+	0	+	0	3	1
Auto													3	3

The antibody specificity broadens when testing with enzyme (ficin) treated RBCs, consistent with warm autoantibody.

Correlating Clinical History with Serologic Results



- This patient has HCV and HIV
- He was transfused 3 days ago with 2 units of RBCs
 - Pretransfusion testing showed a negative antibody detection test (screen)
- His Hgb is 5.6 g/dl with no signs of bleeding

Correlating Clinical History with Serologic Results



- Now there is an antibody reactive with all panel cells tested showing relative anti-e specificity (weaker or negative with e-negative RBCs depending on the method used)
- His DAT is 4+ with IgG and C3
- Initial review of results would suggest warm autoantibody is coating the patient's RBCs and is spilling over into the patient's plasma.
- However, when correlating this with the patient's history it would be very unusual for a warm autoantibody to develop in only 3 days!

Correlating Clinical History with Serologic Results



- An alternative explanation is the patient is experiencing drug-induced immune hemolytic anemia.
- Rarely, the antibody detection test (screen) may be positive without the addition of drug to the test because the putative drug is circulating in the patient's plasma.
- The following slide lists reports of drug-dependent antibodies showing blood group specificity without addition of drug to the test.
- Note: anti-e is quite “common” on this list.

Drug-Dependent Antibodies* & Blood Group Specificity



Drug	Antibody Specificity	Reference
Chorpropamide	Anti-Jk ^a	Sosler, 1984
Rifampicin	Anti-C, Lutheran, I	Ahrens et al, 2002, others
Latanoxef	Anti-e	Habibi, 1985
Glafenine	Neg with Ko cells	Habibi, 1985
Teniposide	Anti-e	Habibi, 1985
Piperacillin	Anti-e	Johnson, 2007, Gala, 2009, Arndt, 2010
Tolmetin	Anti-e	van Dijk, 1989
Diclofenac	Relative Anti-e	Ahrens, 2004
Sulindac	Rh (Nonreactive with D--), Anti-f	DeCoteau, 1993 Johnson, 2007
Nomifensine	Ant-E	Salama, 1986

*Antibodies reactive without drug added

Drug-Dependent Antibodies & Blood Group Specificity



Antibody Specificity	Drug
Anti-e	Piperacillin, Tolmetin, Teniposide, Latanoxef, Tolmetin, Nabumetone, Sulindac, Teicoplanin
Relative Anti-e	Diclofenac
Anti-C	Rifampicin
Anti-f	Cefotetan
Ant-E	Nomifensine
Rh (Neg with D--)	Sulindac
Kell (Neg with Ko cells)	Glafenine, Trimethoprim
Anti-Jk ^a	Chorpropamide
Lutheran, I	Rifampicin
H	Sulfamethoxazole

Differentiating DIIHA from AIHA



DAT

- DDA causes strong positive reactivity that decreases when drug is stopped
 - Strength of DAT increases within hours to days and decreases in reactivity when the drug is stopped.
- Warm autoantibody strong positive reactivity persists
 - Strength of DAT increases within days to weeks

Differentiating DIIHA from AIHA



Eluate

- DDA is negative or weak
- WAA strongly positive

Serum/Plasma

- DDA disappears within days if drug is discontinued
- WAA persists

Rapid Acid Eluate



	Eluate												Last Wash	
	D	C	c	E	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	IAT	IAT
1	+	+	0	0	+	0	+	0	0	+	0	+	0√	0√
2	0	0	+	0	+	+	0	+	+	+	+	+	0√	0√
3	+	0	+	+	0	0	0	+	+	0	+	0	0√	0√
4	+	0	+	0	+	0	0	+	0	+	0	+	0√	
5	0	+	+	0	+	0	+	+	+	0	+	0	0√	
6	0	0	+	0	+	+	0	+	0	+	+	+	0√	
7	0	0	+	0	+	0	+	0	0	+	0	+	0√	
8	0	0	+	0	+	0	0	+	+	0	+	0	0√	
Auto													NT	

A negative eluate with a strong positive DAT is consistent with drug-induced immune hemolytic anemia

Patient's Medication History



- Lorazepam
- Midazolam
- Morphine
- Norepinephrine
Bitartrate
- Esomeprazole
- Fentanyl citrate
- Levoflox
- Phytonadione
- Hydrocortisone
- Vancomycin
- Rocuronium
- Zosyn™

Review Drug List



- When a patient presents with acute hemolytic anemia and a long list of medications the first step is to review the literature to determine if one of the drugs on the list has been reported to cause DIIHA.
- There are 2 drugs on this patient's drug list that are suspicious!

Patient's Medication History



- Lorazepam
- Midazolam
- Morphine
- Norepinephrine Bitartrate
- Esomeprazole
- Fentanyl citrate
- Levoflox
- Phytonadione
- Hydrocortisone
- Vancomycin**
- Rocuronium
- ZosynTM**

**Vancomycin & Zosyn (piperacillin) have been reported to cause DIIHA

Review Timing & Dosage of Drugs

- Most drug-dependent antibodies develop after previous exposure, much like an antibody to a RBC antigen
- These cases often present after a patient has had periodic, repeated exposure to said drug.
 - DIIHA does not occur in individuals who have been on a medication for years.

Timing & Dosage of Suspected Drugs



Vancomycin

- 1,000 mg for 8 days

Vancomycin

- 1,000 mg for another 8 days

ZosynTM

- 2.25 gm 8 days prior to sample

ZosynTM

- 2.25 gm 7 days prior to sample

ZosynTM

- 3.375 gm 6 days prior to sample

ZosynTM

- 3.375 gm 12 hours prior to sample

- An antibiotic that is a combination of piperacillin and tazobactam
- Most reported cases of DIIHA in patients on Zosyn™ are due to piperacillin-dependent antibodies
- Piperacillin is in the top 3 of dda's identified today (see next slide)
 - At least 1 fatality has been reported

Cases/Fatalities of DIIHA over 10 years

Blood Reviews 24 (2010) 143–150.

Drug	Number*	Percentage
Cefotetan	36 (4)	43
Ceftriaxone	17 (5)	21
Piperacillin	14(1)	17
β -lactamase inhibitors	6	7
Other Cephalosporins	11	
Others	9 [#]	11
Total	83 (10)	100

* Columns contain number (fatalities) of cases associated with each drug.
 # Oxaliplatin (3), carboplatin (1), rifampin (1), diclofenac (1), cimetidine (1), sulfamethoxazole (1), and trimethoprim (1).

Testing in Presence of Drug



<u>e+ RBCs</u>	<u>30' RT</u>	<u>60' 37C</u>	<u>IAT</u>
Patient Serum + Tazobactam	0	0	0
Patient Serum + Piperacillin	0	1	3
Patient Serum + Diluent	0	0	0
Normal Serum + Tazobactam	0	0	00
Diluent + Drug	0	0	0
Eluate + Piperacillin	0	0	3
Eluate + Diluent	0	0	0
Eluate + Tazobactam	0	0	0
Positive Control + Piperacillin	1	w	2

Piperacillin-dependent antibody is detected.
Patient Serum + Diluent is negative even though initial testing in gel and tubes was positive because the amount of circulating drug is low. Adding diluent creates a 1:2 dilution decreasing both the drug concentration present in the test and the DDA.

Conclusions



- Piperacillin-dependent antibody was detected when testing the patients serum in the presence of drug
- The physician was asked to take the patient off Zosyn™
- After 48 hours, there was no evidence of on-going hemolysis.
- Within 3 days of the cessation of drug therapy the antibody detection test was negative and the DAT decreased in reactivity (2+).

Case Challenges



- In 3 days this patient's pretransfusion antibody detection testing went from negative to positive with everything tested including the autocontrol.
- When faced with a patient whose plasma is positive in the IAT with all cells tested including a strong positive ($>3+$) autocontrol the initial interpretation is usually warm autoantibody.
- Given the recent transfusion of 2 units, one should consider a delayed hemolytic transfusion reaction but generally the DAT would be weaker positive ($\leq 2+$). In this case the DAT was 4+.

Case Challenges



- Additionally, the antibody was showing relative anti-e specificity, also consistent with autoantibody vs. alloantibody, especially in an individual of European ancestry. If this patient was of African background one might consider a newly developed partial e antibody although the strong positive DAT would be unusual for an alloantibody.
- The challenge... why was the antibody screen negative 3 days ago and why was the patient showing signs of hemolysis (serum color & drop in Hgb)?

Lessons Learned



- Drug-dependent antibodies can be detected in routine antibody detection testing if the putative drug is present in the patient's circulation.
- The autocontrol and DAT will be strongly positive when the drug is present and will gradually decrease in strength as the drug clears from the patient's circulation.
- Initial serologic results can appear as a warm autoantibody when in fact it is drug-dependent antibody

Lessons Learned



- A negative eluate is key in pointing us toward DIIHA.
- A thorough review of the patient's medication history is important in deciding on what drugs to test.
 - Timing and dosage of drugs must be assessed to determine which drug is most likely causing the problem.
- Do not forget the patient's clinical history in interpreting initial serologic results!

References



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