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Objectives

- Define the term Unidentified Antibody as used by the IRI
- Examine the techniques used by the IRL to help characterize the unidentified reactivity
- Review two case studies outlining the process for further characterization of previously unidentified antibodies
- Discuss transfusion recommendations for patients with unidentified antibodies



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Distinguishing Significant Antibodies

Trying to distinguish between significant and insignificant antibodies requires experience, persistence, and a variety of techniques. Given apparent nonspecific or broad reactivity, how do we proceed?

- Examine the patient's history!
- Is there any variation in reaction strength?
- · What is the result of the DAT?
- What is the result when testing different enhancement methods?
- What are the results when testing enzyme or chemically treated cells?



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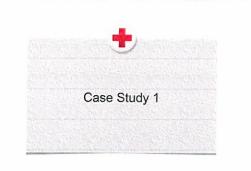
Distinguishing Autoantibodies from Alloantibodies

It can be difficult to determine whether broad specificity in a patient's serum is due to autoantibody, particularly when the DAT is positive.

- Compare the strength of the DAT to the strength of the antibody in the serum.
- · What does the eluate show?
- · What is the result of the DAT?
- If the patient has not been recently transfused, try an autoadsorption.
- Test the patient's serum against their DAT negative autologous red cells



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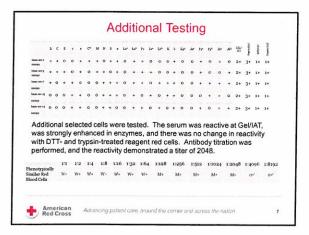
Patient History

- Our laboratory received a sample on a 70-year-old Caucasian in November of 2018. This patient was hospitalized for sepsis and was last transfused in 2016. A history of a warm autoantibody was provided to us from another facility.
- The patient is A Negative, DAT is negative, and a common red cell phenotype was performed.

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37/IAT				0~	0				RT	01



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seasy		+	+	0	0	+			0		+	0	+		13	0	0		0	0		0		0	0	0	1+	1+
seesys				0	0	+	0	10	+	0	+	0	+	0	0	,	0	+	0	0	0		+	0	0	0	1+	1+
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PEG/IAT. Reactivity was noted with all reagent red cells tested at the	Auto	0	0	0		+		+	0	0	+			0	0	+	10	+			0	+	0	*	0	0	01	04
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Conclusion of 2018 Testing

- At this time, we had no additional sample to perform further testing. The patient was discharged, and no blood was transfused.
- The specificity was reported as "Unidentified Antibody".
 - Recommendations to avoid transfusion were given.
 - If necessary, phenotypically similar red blood cells negative for D, C, E, K, Fy^a, Jk^a, S were recommended.



The Patient's Next Admission

- On August 13, 2019 the patient was hospitalized for "pancytopenia". Her Hgb at admission was 6.9 and two units of blood were ordered.
 - Washed Red Blood Cells were ordered due to "allergic" reactions to previous transfusions.
- There was no change in the reactivity of the patient's serum from the 2018 to the 2019 sample.
- Two units of D-, C-, E-, K-, Fy(a-), Jk(a-), S- units were washed and sent to the facility for transfusion.
- Additional phenotypically similar Washed RBCs were sent for transfusion until 08/24/2019, when a transfusion reaction was called.

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Transfusion Reaction Workup

- The patient experienced vigorous shaking, hypertension, chills, and nausea.
- The pre-transfusion DAT was negative and the posttransfusion DAT was 1+ on the hospital's workup.
- Samples were sent to the IRL for a transfusion reaction investigation.
- The IRL obtained a negative DAT on the pre- and posttransfusion samples (note that the post-transfusion sample was collected at a later time than the hospital's sample).
- There was no change in the reactivity of the serum.

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Characterization of the Unidentified Antibody

The patient's serum was tested with cord cells, null cells, and cells that lack high prevalence antigens which are not affected by cell treatments.

	D	C	E			C	м	H	5		Lus	Lak	PI	Les	Lab	K	A	Kp*	Fy	Fy ⁴	Jk*	Jih.	Dim	GHA
DRK-RSD 12 IRC-00518	0	0	0	0	0	0	+		0	+	0			0	+	0	+	0		0		+	Rh-17	2+
404C	+	+	0	0							0	0	0	0	+	9	+		0	+	+	0		W-
DRK-8SD-8 IRC-00528	0	13	0	+		0	0		0	+	o	0	+	0		0	+	0	+	+	0	+	AnWj-	04
RK-RSD 12 RC-00525	0	0	0	+	+	0	0	+	+	+	0		0	0	0	0	+	0		+	*	+	Jr(a-)	2+
DRK-BSD 1 IRC-oogađ	6	+	0	+	+	0	0	+	0		0	+	0	+	0	0	+	0	0	+	+	+	Se2	2+
RK-85D 11		+	0	0		0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	Vel	2+
gac	+	+	+	+	+		+	0	+	+				+		0			+		+		Ge- 2,3	W
147C	+	+	0	+	+		*	+	0	+			0	0	+	0			0	+	+	+	Ge-2-	1+
egC	+	+	+	+	+		+	+	0	+						0			+	0	+	+	CORD	01
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Characterization of the Unidentified Antibody

The negative Lu(a-b-) cell was also AnWj-. The AnWj antigen is weak on dominant Lu(a-b-) cells. Cord cells were nonreactive. Cord red blood cells do not express the AnWj antigen. At this time, additional AnWj-cells were tested to confirm the specificity.

The AnWj antigen is the receptor for Haemophilus influenzae and is carried on the CD44 proteoglycan. The AnWj- phenotype is usually the result of transient suppression of the antigen, which may be long term. The clinical significance of Anti-AnWj is variable, and it does not cause HDFN.



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Determining Clinical Significance

Because of the severe transfusion reaction, a monocyte monolayer assay (MMA) was performed.

			IAT	MMA
Patient Serum	AnWj+ Red Cells		1+	33.5%
Patient Serum	AnWj+ Red Cells	Fresh Normal Serum	1+	35.5%
Patient Serum	AnWj- Red Cells		0	1.3%
Patient Serum	AnWj- Red Cells	Fresh Normal Serum	0	1.3%

MMA results are expressed as % reactivity of monocytes. Analysis suggests that results greater than 5% can be associated with overt transfusion reactions in patients who receive incompatible RBCs.



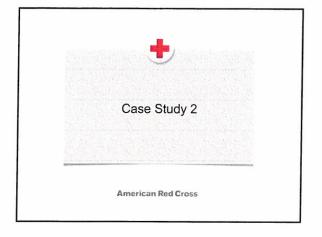
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Transfusion

The patient was transfused successfully with AnWj- red cells (not washed). Because of the extraordinary rarity of this blood type, the search also included Lu(a-b-) cells. These RBCs often possess weak expression of the AnWjantigen. An international search was conducted by the American Rare Donor Program. Products were located in England; however they were not imported.

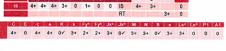


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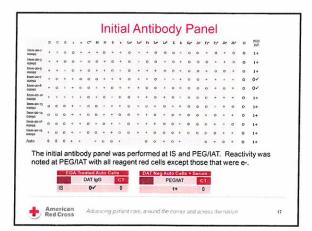


Patient History
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 Our laboratory received a sample on a 34-year-old Caucasian female in July of 2020. This patient was hospitalized with cystic fibrosis and had a hemoglobin of 5.6. They had previous transfusions but none in the prior three months. The hospital reported panreactivity, positive DAT, cold autoantibody reactivity, and nonspecific reactions.







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Imm-cH-5 manya		0	+		٠	0	0	+	+	0	+	0	+	+	+	0	0	+	0	0	+	0	0	+	0~
Imm-ee-)	*	0			٠	0	e	0		+	+	0	+	+	0	٠	+	+	0	0	+		+	+	0~
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Back to the Beginning

We are now re-evaluating what we know and our investigation. The serological picture is not clear, and we can't determine whether the apparent e specificity is something to worry about or not.

- We examined the patient history again:
 - The patient has CF and is currently in the hospital being treated for an infection.
 - The patient is demonstrating symptoms of hemolysis with increased reticulocytes, LDH, and bilirubin with a strongly positive DAT.
 - · The patient is currently taking Zosyn®
 - This drug is a mixture of Piperacillin and Tazobactam.
 - Piperacillin antibodies are known to cause drug-induced hemolytic anemia (DIHA).



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Investigating the Presence of Drug-Associated Antibodies

Complications from drug antibody formation can be mild or life-threatening. Depending upon the mechanism of reactivity, there are two ways to assess a sample:

- Test the patient's serum against a reagent red cell coated with the drug:
 - This method works well for drugs which act through the drug adsorption or membrane modification mechanisms.
 Penicillins and Cephalosporins are examples of drugs which would work well with this method of investigation.
- Test the patient's serum in the presence of drug in the test system:
 - This method works well for drugs which act through the "immune complex" method of action, such as Piperacillin.

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Drug Study Results

Drug study (Piperacillin + Tazobactam):

	60° 37°C	Saline/IAT
Patient Serum + Drug	0	2+
Patient Serum + Drug + Normal Serum	0	1+
Patient Serum - PES	0	0
Patient Serum + PBS + Normal Serum	0	0
Drug + Normal Serum	0	0
PBS + Normal Serum	0	0

The patient's serum is tested against a group O reagent cell with the addition of a solution of drug. A complement source is also added. Controls are tested to ensure that the normal serum does not react in the presence of the drug, and that no nonspecific reactivity is noted.



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Discussion

We now know our patient's serum contains an antibody reactive in the presence of the drug Zosyn®.

Piperacillin is known to cause Drug-Induced Hemolytic Anemia. The symptoms include acute intravascular hemolysis with hemoglobinuria. Once a drug-dependent antibody of this type has formed, severe hemolytic episodes may occur after exposure to a very small quantity of the drug.

The Tazobactam component of the drug is known to cause nonimmunologic protein adsorption and a positive DAT. Hemolytic anemia rarely occurs with this mechanism.

The drug must be discontinued, and the patient's medical record should be updated to include this information.



Serology Resolution

What about the apparent anti-e specificity in the serum?

In 2010 George Garretty's laboratory investigated anti-e-like reactivity in a patient sample submitted for DIHA investigation. A piperacillin antibody was identified, and the serum also reacted with all e+ cells tested.

Their conclusion was that piperacillin antibodies may show a preference to e+ red blood. In addition, drug-anti-drug complexes in the serum may mimic anti-e. It is important not to confuse piperacillin-induced hemolytic anemia with a hemolytic transfusion reaction or with autoimmune hemolytic anemia.

Bandara, Mahesh, et al. "Piperacillin-Induced Immune Hemolytic Anemia in an Adult with Cystic Fibrosis." Case Reports In Medicine, vol. 2010, 2010, doi:10.1155/2010/161454.



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Conclusions

Investigating complex antibody cases can be both frustrating and rewarding. Although on paper these cases follow a logical progression, the testing represents hours of investigation and hard work by the IRL.

It is important not to dismiss reactivity as something insignificant. Consult with your Medical Director and the patient's Physician to assess patient transfusion needs when an unidentified antibody is present.



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