American Red Cross

DAT Negative Autoimmune Hemolytic Anemia

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Autoimmune Disease

Autoimmune Disease	Antigen
Antibody-induced hemolytic anemia	Red Blood Cells
Rheumatoid arthritis	Immunoglobulin (IgG)
Pernicious anemia	Intrinsic factor
Type 1 diabetes mellitus	Pancreatic islet cells
Multiple sclerosis	CNS myelin cells
Idiopathic thrombocytopenia purpura	Platelets
Systemic lupus erythematosus	Nucleii (DNA)

Types of Hemolytic Anemia

- Non-Immune Intravascular Hemolysis
- Autoimmune Hemolytic Anemia
  - "Warm" Autoimmune Hemolytic Anemia (WAIHA)
  - Cold Hemagglutinin Disease (CHD)
  - Paroxismal Cold Hemoglobinuria (PCH)
- Congenital Hemolytic Anemia
  - Sickle cell anemia
  - Hereditary spherocytosis
  - Rh_{null}, Rh_{mod}, McLeod phenotype
- Drug-Induced Immune Hemolytic Anemia

Diagnosing the Cause of Anemia

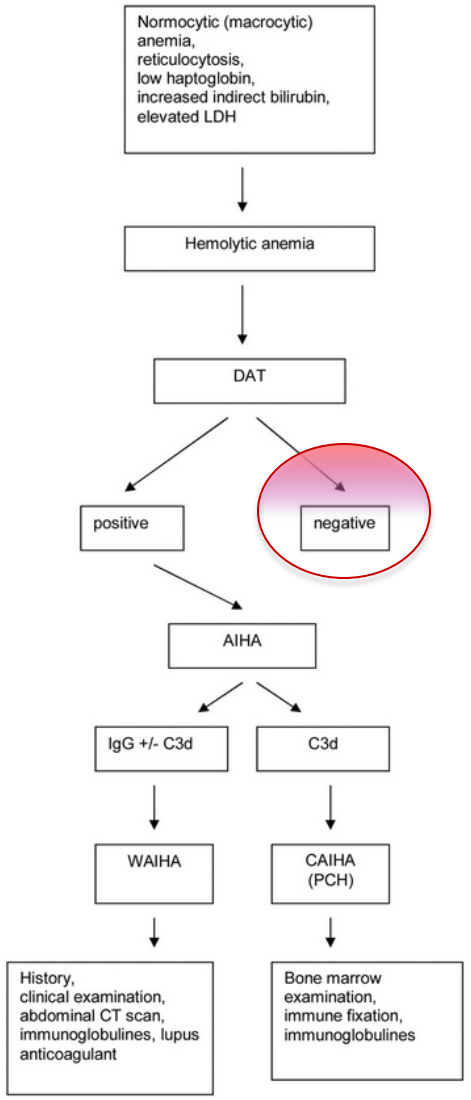
- Non-Immune Intravascular Hemolysis and Congenital Hemolytic Anemia
  - Look at patient's medical history
- Drug-Induced Immune Hemolytic Anemia
  - Clinical symptoms of Immune Hemolysis, may be acute.
  - Look for a relationship between the administration of a drug and the presence of hemolysis
- Autoimmune Hemolytic Anemia
  - Blood Bank serology combined with other laboratory data is key when AIHA is suspected

Characteristics of Autoimmune Hemolytic Anemia

	WAIHA	CHD	PCH
Usual Immunoglobulin type	IgG	IgM	IgG
Optimal in vitro reaction temperature	37^{o}C	0-4^{o}C	0-4^{o}C Antibody binding 37^{o}C Hemolysis
Usual serological presentation	IAT reactive	Direct agglutinin, sometimes a hemolysin	Biphasic hemolysin
Antigen specificity	Rh system, Kell system, LW, U, En^{a}, Wr^{b}	I system, Pr series	P

Diagnosis of AIHA

- Normocytic or macrocytic anemia
- Reticulocytosis
- Low serum haptoglobin levels
- Elevated lactate dehydrogenase (LDH) level
- Increased bilirubin
- Positive direct antiglobulin test (DAT)



Autoimmune Hemolytic Anemia (AIHA)

Typical serology of a “Warm” Autoantibody:

- Positive DAT with IgG or both IgG and C3
- Eluate demonstrates a panagglutinin
- Serum is broadly reactive at IAT
- Reactivity is enhanced with PEG, in Gel, and Solid Phase
- Adsorption of the serum at 37^{o}C removes the autoantibody allowing antibody confirmation and exclusion

Autoimmune Hemolytic Anemia (AIHA)

- Summary of three studies examining the type of Immunoglobulin causing a positive DAT in Patients with AIHA:

Immunoglobulin Associated with a Positive DAT			
DAT Positive due to:	Worliedge and Blajchman	Issitt et al.	Petz and Garratty
	121 Patients	87 Patients	104 Patients
IgG only	35.5	43.8	18.1
IgA only	2.5	0	1.7
C3 only	10.7	0	10.4
IgG & C3	43.0	47.1	46.0
IgM & C3	0	0	1.7
IgA & C3	0	0	1.7
IgG & IgA	3.3	1.1	2.7
IgG, IgA,& C3	0	1.1	12.3
IgG, IgM, & C3	2.0	4.6	3.7
IgG, IgA, IgM, & C3	0	2.3	1.7

DAT Negative AIHA

- The number of IgG molecules on the red cells is less than the number needed to cause a positive DAT, but may still be enough to cause in vivo RBC destruction
- In some cases, cross linking does not occur when anti-IgG is bound to the Fc portion of cell-bound IgG molecules
- DAT due to IgA or IgM is not detected with anti-IgG reagents
- Low affinity IgG may dissociate during the testing process

Special Laboratory Investigation of Suspected DAT-Negative AIHA

- Perform DAT panel with anti-IgG, anti-C3, anti-IgM, and anti-IgA
- Perform a Direct Polybrene test
- Perform DAT using cold saline/LISS wash and anti-IgG
- Perform DAT in Gel

In a study of 800 patients with suspected DAT-Negative AIHA, a positive result was obtained in 54% of the samples using one or more of these techniques

Case Report

The IRL was contacted regarding a patient with anemia. Patient JPI was a 57 year old male with no prior history of anemia or other acute or chronic illness. Initial laboratory values:

Hgb/Hct	Reticulocytes	LDH	Total Bilirubin
6.6 g/dL 17.8%	>17.0% (reference 0.5-1.5%)	1694 IU/L (reference 150-300IU/L)	5.1 mg/dL (0.3-1.9mg/dL)

The clinician suspected autoimmune hemolytic anemia, however when the hospital tested the sample they found a negative DAT and anti-E in the serum. The sample was referred to the IRL for additional testing.

Case Report

JPI Initial IRL testing:

ABO/Rh:

Anti-A	Anti-B	Anti-A,B	Anti-D	Rh Cont	A1 cells	A2 cells	B cells	Interp
0	4+	4+	3+	0	3+	2+	0	B Pos

DAT:

Poly	Anti-IgG	Anti-C3	Control	Gel IgG	Interp
0✓	0✓	0✓	0	0	Negative

Case Report

JPI Initial Antibody Panel:

	Supplier Lot #	Donor/ Vial#	RhHr								Kell						Duf		Kid		Lew		P	MN				Lut		X	Additional Antigens	Patient's Plasma Test Results	
			D	C	c	E	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga		IS	PEG 1:64
1	Ortho A RA054	316522 3	+	0	+	+	0	0	0	0	0	+	0	+		+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	HLA+ Bg+	0	3+
2	Ortho B RB468	309461 15	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+		0	3+
3	Ortho B RB468	308173 16	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+		0	3+
4	Ortho A RA054	310290 1	+	+	0	0	+	0	0	+	0	+	0	+		+	+	0	+	0	0	+	+	+	0	+	+	0	+	+		0	0 ✓
5	Ortho B RB468	317722 22	+	+	0	0	+	0	0	0	+	0	0	+		+	0	+	0	+	0	+	+	+	+	0	+	0	+	+		0	0 ✓
6	Ortho A RA054	116883 8	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	s	+	0	+	0	0	+	+		0	0 ✓
7	Ortho A RA054	317678 4	+	0	+	0	+	+	+	0	0	+	0	+		+	0	+	+	0	0	0	+	+	+	0	+	0	+	+		0	0 ✓
8	Ortho A RA054	319365 5	0	+	+	0	+	+	0	0	0	+	0	+		+	0	+	+	+	+	0	+	0	+	0	+	0	+	+		0	0 ✓
																															auto Control	0	2+

Case Report

JPI Red Cell Phenotyping:

Anti-C	Anti-E	Anti-c	Anti-e
0	3+	4+	3+

How do we confirm a suspected autoantibody with defined specificity in the neat serum?

1. Patient cells are antigen positive for the antibody specificity.
2. Test the plasma containing the suspected autoantibody with DAT-Negative autologous RBCs.
3. Perform adsorption to determine if the suspected autoantibody can be removed from the plasma using autologous RBC adsorption.

Case Report

Plasma vs. DAT-Negative autologous RBC and autologous adsorption:

Patient's Name JPI
Patient's Number 32-17
Date: Collected 01182017 Date: Tested 01182017

SELECTED PANEL 1:56 pm, 3/27/2017
American Red Cross, Missouri-Illinois Region
4050 Lindell, St. Louis, MO 63108
Technologist: CB

Auto ads
1x ads

	Supplier Lot #	Donor/ Vial#	RhHr								Kell					Duf		Kid		Lew		P	MN				Lut		X	Additional Antigens	37° c ZZAP tt Patient's Plasma Test Results		
			D	C	c	E	e	f	V	C w	K	k	K pa	K pb	J sa	J sb	F ya	P yb	J ka	J kb	L ea	L eb	P 1	M	N	S	s	L ua	L ub	X ga		IS	PEG IgG	PEG IgG
1	Ortho A RA054	316522 3	+	0	+	+	0	0	0	0	0	+	0	+		+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	HLA+ Bg+	0	3+	0 ✓
2	Ortho B RB468	309461 15	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+		0	3+	0 ✓
3	Ortho B RB468	308173 16	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+		0	3+	0 ✓
4	Ortho A RA054	310290 1	+	+	0	0	+	0	0	+	0	+	0	+		+	+	0	+	0	0	+	+	+	0	+	+	0	+	+		0	0 ✓	
5	Ortho B RB468	317722 22	+	+	0	0	+	0	0	0	+	0	0	+		+	0	+	0	+	0	+	+	+	+	0	+	0	+	+		0	0 ✓	
6	Ortho A RA054	116883 8	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	s	+	0	+	0	0	+	+		0	0 ✓	
7	Ortho A RA054	317678 4	+	0	+	0	+	+	+	0	0	+	0	+		+	0	+	+	0	0	0	+	+	+	0	+	0	+	+		0	0 ✓	
8	Ortho A RA054	319365 5	0	+	+	0	+	+	0	0	0	+	0	+		+	0	+	+	+	+	0	+	0	+	0	+	0	+	+		0	0 ✓	
																															Auto Control	0	2+	

Case Report

Elution Testing:

Patient's Name JP1
Patient's Number 37-17
Date: Collected 01182017 Date: Tested 01182017

SELECTED PANEL 2:52 pm, 3/27/2017
American Red Cross, Missouri-Illinois Region
4050 Lindell, St. Louis, MO 63108
Technologist: CB

	Supplier Lot #	Donor/ Vial#	RhHr								Kell						Duf		Kid		Lew		P	MN				Lut		X	Additional Antigens	Patient's Plasma Test Results			
			D	C	c	E	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga		Undate 1g6	Undate PEG 1g6	Lastwash 1g6	Lastwash PEG 1g6
1	Imm-Pano 07951	B8834 1	+	+	0	0	+		0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0		M+	2+	0	0
2	Imm-Pano 07951	C712 2	+	0	+	+	0		0	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	0	+	0	0	+	+		M+	2+	0	0
3	Imm-Pano 07951	H941 3	0	0	+	0	+		0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	+	0	0	+	0		M+	2+	0	0

Case Report

Ficin Testing:

Patient's Name JP1
Patient's Number 32-17
Date: Collected 01182017 Date: Tested 01182017

SELECTED PANEL
American Red Cross, Missouri-Illinois Region
4050 Lindell, St. Louis, MO 63108

1:56 pm, 3/27/2017
Auto ads
1x ads
Technologist: CB

	Supplier Lot #	Donor/ Vial#	RhHr								Kell					Duf		Kid		Lew		P	MN				Lut		X	Additional Antigens	37° c 2 ZAP 4+ Patient's Plasma Test Results				
			D	C	c	E	e	f	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga		IS	PEG IgG	PEG ISA	Ficin 37°	Ficin IgG
1	Ortho A RA054	316522 3	+	0	+	+	0	0	0	0	0	+	0	+		+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	HLA+ Bg+	0	3+	0	4+	4+
2	Ortho B RB468	309461 15	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+		0	3+	0	4+	4+
3	Ortho B RB468	308173 16	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+		0	3+	0	4+	4+
4	Ortho A RA054	310290 1	+	+	0	0	+	0	0	+	0	+	0	+		+	+	0	+	0	0	+	+	+	0	+	+	0	+	+		0	0		2+	mf
5	Ortho B RB468	317722 22	+	+	0	0	+	0	0	0	+	0	0	+		+	0	+	0	+	0	+	+	+	+	0	+	0	+	+		0	0		2+	mf
6	Ortho A RA054	116883 8	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	s	+	0	+	0	0	+	+		0	0		2+	mf
7	Ortho A RA054	317678 4	+	0	+	0	+	+	+	0	0	+	0	+		+	0	+	+	0	0	0	+	+	+	0	+	0	+	+		0	0		2+	mf
8	Ortho A RA054	319365 5	0	+	+	0	+	+	0	0	0	+	0	+		+	0	+	+	+	+	0	+	0	+	0	+	0	+	+		0	0		2+	mf
																														Auto Control	0	2+		4+	4+

Case Report

American Red Cross
Missouri-Illinois Region

Immunohematology Reference Laboratory
8053 Bond Street
Lenexa, KS 66214
Phone: 913-242-3557
Fax: 913-310-0354
CLIA # 17D2106199

FINAL REPORT

Patient Name: JPI **Patient ID** D01335122 **Ref Lab #** 37-17

Red Cell Studies:

ABO/Rh:	DAT Polyspecific:	DAT Anti-IgG:	DAT Anti-C3:	DAT Gel IgG:	Super DAT:
B Positive	Negative	Negative	Negative	Negative	Negative

Red Blood Cell Phenotype:

C	E	c	e	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P_1
0	+	+	+	0	0	+	+	0	+	0	0	+	+	+	+

Serum/Plasma (S/P) and Eluate Studies:

Antibody:	Phase(s) of Reactivity:	S/P	Eluate
Warm Autoantibody	Elu Kit II	☐	☒
Warm Autoantibody	FICIN/IAT	☒	☐
Auto-anti-E	PEG/IAT	☒	☐

Transfusion Recommendations and Remarks:

Select units negative for:	E
Percentage of Antigen Negative Donors:	68%

Remarks:

The eluate reacts with all cells tested.

The patient's plasma was reactive at FICIN/IAT with all cells tested. Autoanti-E was identified at PEG/IAT. All other common blood alloantibodies were ruled out at PEG/IAT and GEL/IAT. The plasma was adsorbed x1 with ZZAP treated autologous red blood cells. No reactivity was detected following adsorption, confirming that the anti-E specificity is a component of the patient's autoantibody.

If an autoantibody has clear-cut specificity for a single antigen and there is active ongoing hemolysis, there is evidence that blood lacking that antigen may survive better than the patient's own red cells. In the absence of hemolysis, autoantibody specificity is not significant. Select E- units for transfusion as indicated.

Treatment of WAIHA

- Corticosteroids
- Rituximab (anti-CD20)
- IVIg
- Splenectomy

- Transfusion is contraindicated because of
  - Formation of alloantibodies
  - Exacerbation of the autoantibody
  - Autoantibody-induced red cell destruction

Case Report

JPI laboratory results:

Day	Hgb (g/dL)	HCT	Reticulocytes (0.5-1.5%)	LDH (150-300IU/L)	Total Bilirubin (0.3-1.9mg/dL)
1	6.6	17.8%	>17.0%	1694	5.1
2	6.3	18.2%	>17.0%	1832	
3	6.3	17.2%		1862	
4	6.1	16.5%	>17.0%	1755	
5	6.0	16.5%		2044	4.9
6	5.0	14.0%		2425	5.5
7	4.9	13.3%		2475	
8	4.2	13.8%		2118	2.9
9	3.6	12.0%		2397	3.0
10	6.0	19.2%		2623	5.1

American Red Cross

Case Report

JPI laboratory results:

Day	Hgb (g/dL)	HCT	Reticulocytes (0.5-1.5%)	LDH (150-300IU/L)	Total Bilirubin (0.3-1.9mg/dL)
11	6.3	20.4%			
12	6.4	19.4%		2544	2.8
13	6.0	16.5%		2816	3.3
14	7.0	23.5%	>17.0%	3019	3.5
15	6.7	19.8%		2951	3.0
→ 16	8.2	25.9%		2914	3.3
17	7.2	19.6%		2777	

The patient was discharged on day 17 and continues to improve with no relapse of the AIHA.

References

- Garratty, George. "Immune Hemolytic Anemia Associated With Negative Routine Serology". *Seminars in Hematology* 42.3 (2005): 156-164
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- Leger, R. M., A. Co, P. Hunt, and G. Garratty. "Attempts to Support an Immune Etiology in 800 Patients with Direct Antiglobulin Test-negative Hemolytic Anemia."*Immunohematology* 26.4 (2010): 156-60
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