Rare Case of AIHA

Nour Alhomsi, BSc, MLT, MBA

Technical Specialist, Transfusion Medicine Hamilton Health Sciences, Hamilton, ON



St. Joseph's Healthcare & Hamilton



Background

A 19-year-old Male

 Post-renal transplant (2006) was admitted with severe progressive hemolytic anemia (HB 59g/L)

G&S sent to TM and request for 2 RCCs STAT







Transfusion Medicine

St. Joseph's Healthcare Hamilton



Patient History Check

<u>2012:</u>

Blood Group: A Positive

Antibody Screen: Negative





ABO & Rh

ECHO-Solid Phase Capture

Mono Ctrl	Anti-A	Anti-B	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
С	С	С	С	С	С	С	Ctrl fail

Manual Tube

Anti-A	Anti-B	Rh C	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
4+	3+	2+	4+	4+	0	2+	?

*Prewarm did not resolve ABO discrepancy



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Antibody Screen & DAT

ECHO-Solid Phase Capture Screen

SIAT

Screen 1	Screen 2	Screen 3	Pos Ctrl	Interp.	Screen 1	Screen 2	Screen 3	Interp.
2+	2+	2+	4+	Positive	2+	2+	2+	Positive

ECHO-Antibody Panel

Few weak reactions

All common clinically significant antibodies excluded

ECHO-DAT

DAT Rxn	Ind Ctrl	Interp.
M*		Invalid

*M: Monolayer verification error

Manual-DAT

AHG	6%Alb Ctrl	Interp.
3+	2+	Invalid
		_

*Prewarm did not resolve Positive Alb Ctrl



What explains these reactions?

- ? Cold Autoantibody
- **?** Warm Autoantibody
- ? Strong anti-C3d
- ? IgG Alloantibody
- ? Sample is contaminated



Cold Agglutinin Screen

1:64

Patient Cells	Cord Cells	Adult Cells
0	0	0

Thermal Amplitude

1:4

37°C	30°C	22°C	4°C
+	+	+	0



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- CBC invalid, manual HCT
- Free Hb 🗖
- Haptoglobin
- Unconjugated Bilirubin
- LDH Normal
- Retic 33

Provide O negative Rh & Kell matched Least Incompatible

National Immunology Reference Laboratory Canadian Blood Services





Initial Blood Group & DAT

ABO/Rh

Anti-A	Anti-B	Rh C	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
4+	1+	2+	3+	4+	0	4+	NTD

DAT

PS-AHG	Anti-IgG	Anti-C3d	PBS Control
2+	2+	2+	2+



2nd Source of Anti-A/Anti-B

ABO/Rh

Anti-A	Anti-B	Anti-AB	Rh C	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
4+	2+	4+	3+	3+	4+	0	4+	NTD

Prewarm

Anti-A	Anti-B	Anti-AB	Rh C	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
4+	2+	4+	2+	3+	4+	0	4+	NTD



Both Failed to resolve ABO Discrepancy



DTT Treated Patient RBC

ABO/Rh

Anti-A	Anti-B	Anti-AB	Rh C	Anti-D1	Anti-D2	A1 Cells	B cells	Interp.
4+	0	4	0	3+	4+	0	4+	A Pos

DAT

PS-AHG	Anti-IgG	Anti-C3d	PBS Control
Wk+	0	Wk+	0

Canadian Blood Services BLOOD PLASMA STEM CELLS ORGANS & TISSUES



Weak with AHG and C3d but Negative with Anti-IgG after DTT treatment





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DTT (Dithiothreitol)

As a blood bank reagent, it dissolves IgM antibody disulfide bonds and eliminates activity of the antibody.

When used to treat RBC it will remove the reactivity of any bound IgM antibodies as well as destroying any antigens with disulfide bonds including those in the Kell system.



Initial Panel

	Supplier /	Donor /				- -	-Hr					1	ĸe	en l		_	Du	πу	KI	aa	Lev	NIS	٢		м	N	-	LU	In	X		Gel			
	Lot	RhHr - Vial	D	с	E	c	e	c*	f	v	к	k	Кр ^а	K₽®	Js ^a	Jsb	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Leª	Leb	P1	м	N	s	s	Lua	Lub	Xg ^a		IAT	1.5.	370	SIAI
ł	Immucor 38637	B10113 R1R1 #1	÷	+	0	0	+	0			0	+	0	+	0	+	0	+	+	0	0	+	÷	+	0	+	+	0	+	+		2	0	2	1
ł	Immucor 38637	C6453 R2R2 #2	+	0	+	+	0	0			+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0		2	0	2	1
ł	Immucor 38637	H1722 rr #3	0	0	0	+	+	0			0	+	0	+	0	+	+	0	0	+	+	0	0	+	0	+	0	0	+	+		2	0	2	1
ţ	Immucor 38294	C6232 R2R2 #2	+	0	+	+	0	0			+	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+		2	0	2	1
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Panreactive by Gel IAT and SIAT ; Strong reaction at 37, but negative at I.S.



RESt (Rabbit Erythrocyte Stroma)

	Quantiza /	Deres (лh	-Hr						Ke	ell			Du	iffy	Ki	dd	Le	wis	Р		М	N		Lu	th	X	Additional	Tes	t Results
	Lot	RhHr - Vial	D	с	E	c	e	c*	f	v	к	k	Kpª	Kp ^b	Jsa	Jsb	Fv ^a	Fv ^b	Jka	Jkb	Leª	Leb	P1	м	N	s	s	Lu	Lub	Xaª	Antigens	SIAT	
1	Immucor 38637	B10113 R1R1 #1	+	+	0	0	+	0			0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	+		wk	
2	Immucor 38637	C6453 R2R2 #2	+	0	+	+	0	0			+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0		wk	
3	Immucor 38637	H1722 rr #3	0	0	0	+	+	0			0	+	0	+	0	+	+	0	0	+	+	0	0	+	0	+	0	0	+	+		wk	
4	Immucor 38294	C6232 R2R2 #2	+	0	+	+	0	0			+	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+	r Jam HD	wk	
																															Auto		

F

Fails to clear reactivity at IAT (meant to remove cold reactive antibodies)







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RESt (Rabbit Erythrocyte Stroma)

- Is a commercial product used by blood bankers to remove (or "adsorb") cold antibodies from a patient's blood sample.
- These antibodies are usually autoantibodies that have specificity to the "I" or "IH" antigens.



Cold Panel

			Rh-Hr	Kell	Duf Kid Lew P MNS Lut X		Test Results
-	Supplier / Lot	Donor / RhHr - Vial	DCECewfV	K K J J p p s s K k a b a b	JFFJJLL syykkeeP bababab1MNSsaba	Additional Antigens	4°C
1 tunn	immunoR 111432					A.	wk
2	WMMULLO PC 1129153					B2	1
3	LMAULOR 113432					В	4
Ч	Emmuwik 114454					0 -	1
5	Ennuel 42319	D450 # 4				O, PHM-	1
6	Ennuur 42319	NU973 #16				0. Pi-mi	1
[m	0155 1064322.000821	60006046169				Cord O	1
-						AWD	2



To look for antibody specificity: No specificity determined



Eluate

	Supplier	De				'n	(h-H	Ir					Ке	11			uffy	/K	idd	Le	wis	P		M	N		Lut	h	хI		Те	st Results	i	
	Lot	RhHr	r - Viai	D	с	ε	c	c*	f	v	к	k P	<p<sup>a k</p<sup>	(p ^b J	s ^a J	s ^b F	a Fv	ð Jk	a Jk	Le ^a	Leb	P1	м	N	s	s	a	, b	xaa	Antigens	EL MTS	LW MTS		_
1	Immucor 38637	B10 R1R	0113 R1 #1	+	+	0	0 +	• 0			0	+	0	+ () +	+ 0) +	+	0	0	+	+	+	0	+	+	0	+	+		0	0		-
2	Immucor 38637	C6 R2R	453 82 #2	+	0	+	+ (0			+	+	0	+ () +	+ .0) +	+	0	0	+	+	0	+	0	+	0	+	0		0	0		_
3	Immucor 38637	H1 rr	722 #3	0	0	0	+ +	• 0			0	+	0	+ () +	+ +	0	0	+	+	0	0	+	0	+	0	0	+	+		0	0		
4	Immucor 38204	C6 P2P	232	+	0	+	+ (0 0			+	+	0	+ () -	+ 0	} +	+	+	0	+	+	+	0	+	+	0	+	+		0	0		-
1			-¦-			Rh	– H :	c			K	e11	-	Γ	uf	Ki	dI	Lew	Р	1	MNS		Lu	t	ĸ						Tes	t Resul	ts	_
	Supplier /	Donor BhHr - 1	/ Vial	D	С	Ec	0	C	= v	к	k F	K	J s	JE	FF	Jka	J] k (LL	P 1	м	NIS		L u a	L	X g						FI	IW/	 	
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4.00	TUNNULOR 111432																											A		cells	0	0		
2	113432																											в		cells	0	0		
													Π	Τ	T	Π	Т				Τ			Т	Τ									



Negative using Gel IAT testing (also negative with A and B cells so not anti-H)



Cell for Alloadsorption: Phenotype similar cell to Patient with a matching Rh phenotype

	Supplier /	Dener				Rh	-Hr						ŀ	Kell				Duff	fy	Kid	d	Lew	vis	P		М	N	Lu	ith	X	Additional		Tes	t Re	esu	ılts	
	Lot	RhHr - Vial	D	с	E	с	e	c*	f	v	к	k	Kp	aKp	, J	s ^a J	s ^b F	y ^a F	y ^b	Jk ^a J	lk ^b l	Leal	e	P1	м	N	s	\$ Lu	Lu⊳	Xg ^a	Antigens	SIA'	Т				
1	CBS 20221121	ON6089855 R1R1 #TC	+	+	0	0	+		0		0							+			0						0				Pheno	1					
-																																					



Antibody is reactive with adsorbing cell



Alloadsorption

Advanced blood banking technique designed to bind antibodies to red blood cells in order to remove them from a particular patient's plasma and better analyze the antibodies that might remain behind.



Follow 37°C Adsorption using ZZAP

	Cumpling /	Damas (r\ir	-			1			۲.e	11		ľ	Dui	y	r\iu	u li	-ew	/IS	-		IVI	N		Lu	ui	^	Additional	1	เธอเท	\ธวนแจ	
	Lot	RhHr - Vial	γ D	ير د) E	à	% e	c ^w	f	v	,× K)0 k	<p<sup>a</p<sup>	č,	saJ	і́с Is Б	ار Fy ^a l	г [№] ь Fy	[∵] a Jk	» Jk ^b L	× e ^a l	ю е	َرُہ P1	р М	х° N	s	ж s	Lu ^a	ار Lu	y Xgª	Additional Antigens	I.S.	37C	SIAT	сс
1	Immucor 38637	B10113 R1R1 #1	+	Ð	0	0	÷	0			0	Ð	0	Ð	0	÷	0	Ð	Ð	0	0	Ð	Ð	Ð	0	+	+	0	Ð	Ð		0	0	0	2
2	Immucor 38637	C6453 R2R2 #2	÷	0	÷	Ð	0	0		ł	•	+	0	+	0	+	0	+	+	0	0	+	+	0	÷	0	Ð	0	+	0		0	0	0	2
3	Immucor 38637	H1722 rr #3	0	0	0	+	+	0			0	+	0	+	0	+ (Ð	0	0	Ð	Ð	0	0	+	0	÷	0	0	+	+		0	0	0	2
4	Immucor 38294	C6232 R2R2 #2	+	0	+	+	0	0			÷	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+		0	0	0	2
5	CBS 20221121	ON6089855 R1R1 #TC	+	+	0	0	+		0		0						+			0						0					Adsorbing	0	0	0	2



No reactivity of plasma at IS, 37 or IAT







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ZZAP

- Is a reagent composed of a mixture of a proteolytic enzyme (papain) and a sulfhydryl reagent (dithiothreitol, or "DTT").
- Removes immunoglobulins and complement from the surface of DAT-positive red blood cells.
- Antigens damaged/destroyed by ZZAP include all Kell antigens, M, N, Fy^a and Fy^b
- Following treatment with ZZAP, red cells can be used for a variety of special techniques, such as adsorption.



DTT Treatment of Control Plasma containing IgM anti-P1 antibody. Following DTT treatment

	Supplier /	Depor (RII	-רו			1			r.t	:11			ЫП	пу	NIC		Lev	vis	۲		M	N		Luti	1	•	Additional		16	est r	esu	IS	1
	Lot	RhHr - Vial	D	с	E	с	e	c*	f	v	к	k	Kp ^a	Кр	Js ^a	Jsb	Fy ^a	Fy ^b	Jk ^a	Jk ^b l	Lea	Leb	P1	M	N	s	s L	u ^a L	u ^b Xa	æ	Antigens	Ne R	eat T	C	OTT RT		
1	Immucor 42325	B5185 R1R1 #2	+	+	0	0	+	0			0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0 -	+ 0			2	2		0		
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DTT Treatment of Patient Plasma (Patient Test)

	Cumpling /	Dener				Rh	-Hr	-					Ke	ell			Du	ffy	Ki	dd	Le	wis	Ρ		M	N		Lu	th	х	Additional		est Res	sults	
	Lot	RhHr - Vial	D	с	E	c	e	cw	f	v	ĸ	k	Kp ^a	Кр⁵	Js ^a	Jsb	Fy ^a	F۷ ^b	Jk ^a	Jk	Leª	Leb	P1	м	N	s	s	Lua	Lub	Xaª	Antigens	<mark>Patient</mark> Test	сс	Patient	
1	Immucor 38637	B10113 R1R1 #1	+	+	0	0	+	0			0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	+		0	2	2	
2	Immucor 38637	C6453 R2R2 #2	+	0	+	+	0	0			+	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0		0	2	2	



non-reactive at SIAT; patient plasma untreated is reactive at SIAT



Conclusion and Report Out National Immunology Reference Laboratory

Canadian Blood Services





IgM antibodies present (forward typing discrepancy and invalid DAT corrected with DTT treatment of cells which destroys bound IgM antibodies); DAT following DTT treatment is positive for AHG and C3d but not IgG (again suggesting the autoantibody is IgM in nature)

Initial testing is positive at 37 (IgM); weaker at IAT and negative at IS suggesting a warm reactive IgM antibody Cold panel is positive but non specific

RESt adsorption fails to remove antibody 37°C alloadsorption with pheno similar cell treated with ZZAP removes reactivity and allows confirmation of no underlying alloantibodies

DTT treatment of plasma also clears reactivity confirming IgM nature of autoantibody

Canadian Blood Services Break

Overall Conclusion



Α	DAT	Warm	No
Rh Positive	Positive	Reacting IgM	Alloantibody
	with C3d	Autoantibody	Detected

Summary of the Patient's Clinical Course Over 3-month Period



Hb: Hemoglobin, LDH: Lactate Dehydrogenase, RBC: Red Blood Cells

Poor RBC transfusion increments led to initiation of daily plasma exchange with excellent response (Hb > 130g/L)

Secondary workup revealed underlying post-transplant lymphoproliferative disorder, for which Rituximab 375mg/m2 weekly x 4 was administered

Lessons Learned

This patient had a warm reacting IgM autoantibody which is rare but often associated with life threatening. ⁽¹⁾

It can be difficult to diagnose IgM warm AIHA because routine serologic data are not always informative (e.g., routinely used antiglobulin sera do not contain anti-IgM); the diagnosis is sometimes confused with CAS. ⁽¹⁾



Our Patient

PLEX was an effective treatment to temporarily reduce IgM antibody levels and hemolysis, improving the patient's clinical condition until a final diagnosis could be established and a treatment strategy determined (Rituximab)

August 30th, 2023: HB 122g/L, DAT: Negative, no signs of hemolysis!! ^(C)



Specialized serological tests performed by NIRL (DTT treatment, RESt and ZZAP) are based on common serological principles used routinely in testing. Is it time for larger academic centers to consider implementation of these test as an alternative to sending samples to a reference laboratory?



THANK YOU

Special thanks to NIRL for the comprehensive testing that they performed to help understand the complex serological findings associated with this case. Many thanks to the Transfusion Medicine staff at Hamilton Health Sciences and St. Joseph's Healthcare in Hamilton for their commitment to understand the serology leading to hemolysis in this patient which was critical for appropriate management of this case.