NHS
Blood and Transplant

Serological problem solving with case studies

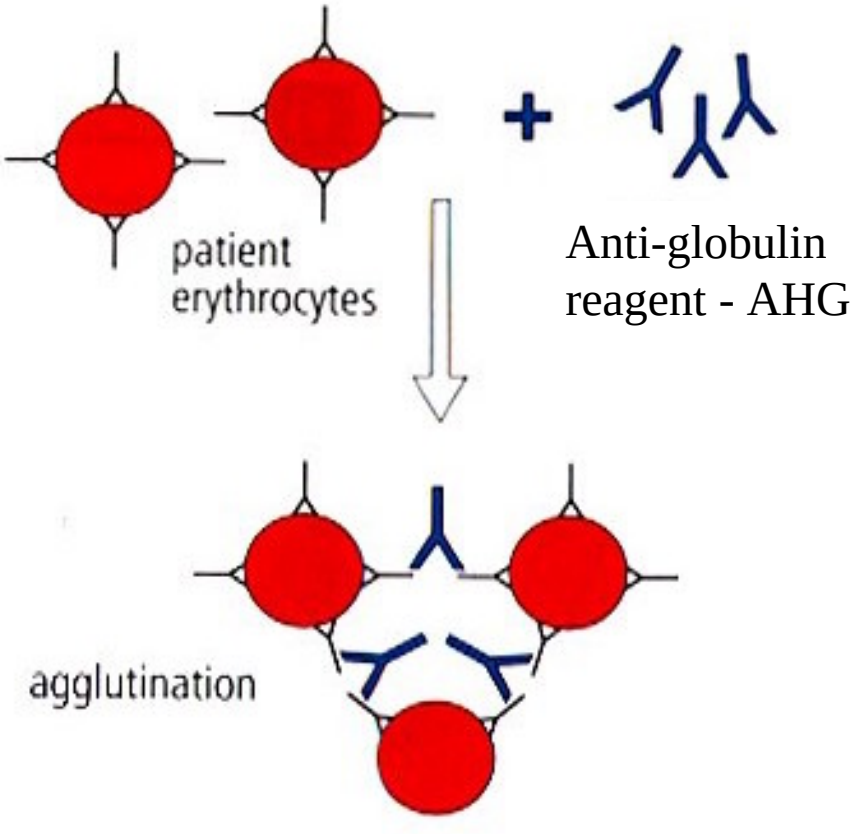
Robert Stamps, RSM, RCI Sheffield

NHS
Blood and Transplant

Serological advances

- ABO blood groups - Landsteiner 1900
- Rh blood groups – Levine and Stetson 1939 Landsteiner and Weiner 1940
- AHG – anti-human globulin – Coombs 1945

  LISS – Low ionic strength solution – 1970's
- Monoclonal blood typing sera – 1980's
- Gel column technology - Invented1985 – DiaMed introduced test system in 1988


patient
erythrocytes
Anti-globulin
reagent - AHG
agglutination

NHS
Blood and Transplant

Rates of alloimmunisation

- Higher risk of antibody production in multi-transfused patients
- Ethnic minority patients at greater risk
- Up to 30% become alloimmunised
- Most produce Rh (anti-D, C, E, c, e) and K antibodies

NHS Blood and Transplant

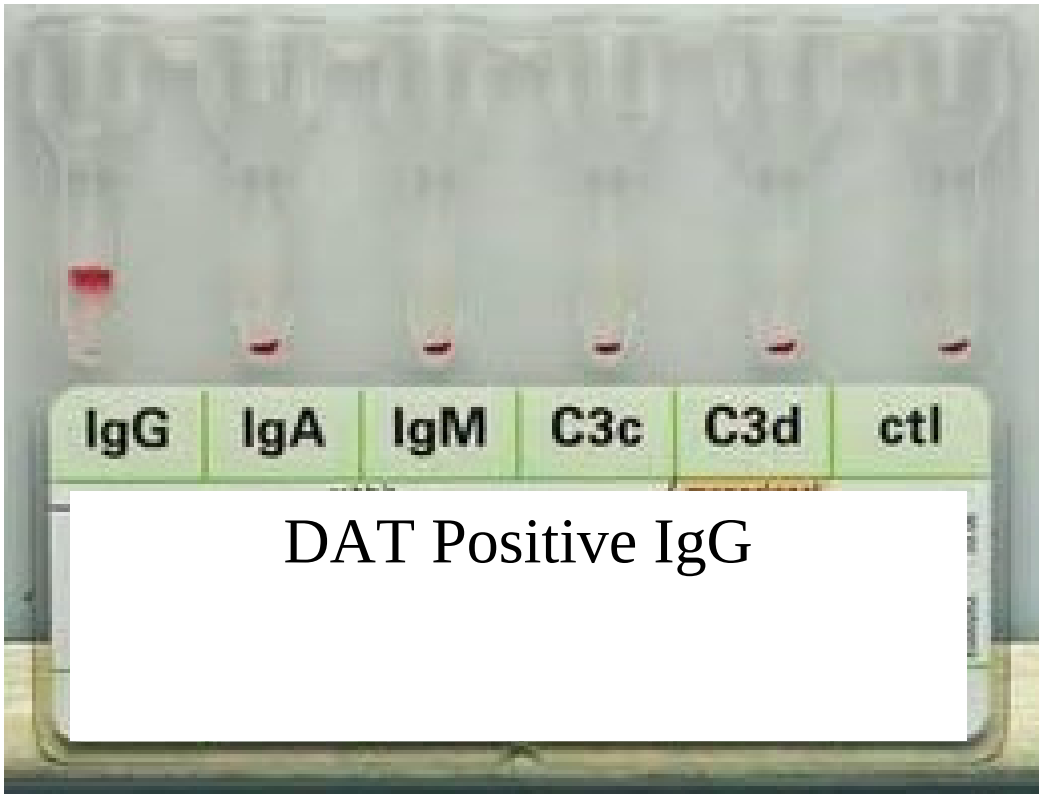
- Spanos et al, Red cell alloantibodies in patients with thalassemia, *Vox Sang 1990; 58*
- Rh – 34%, K – 30%, MSs – 8%, Jk – 8%, Fy – 4%
- Ameen et al, RBC allo and auto immunisation among transfusion-dependent Arab thalassemia patients, *Transfusion 2003;43*
- Rh – 49%, K – 31%, Jk – 5%, Fy – 3%
- 11% developed autoantibodies - immunomodulation
- Anti-E was most commonly detected antibody in both studies

NHS Blood and Transplant

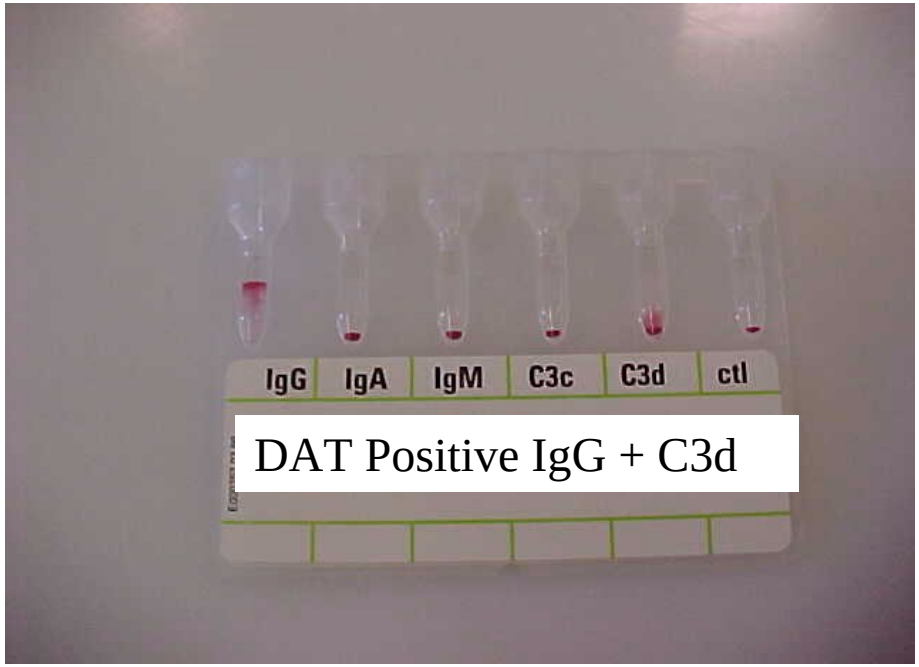
Delayed haemolytic transfusion reaction

- 45 year old female, hysterectomy – O Pos
- Transfused 2 units
- 3 days later jaundice, raised LDH, Hb 8.5
- DAT – Pre transfusion – Negative
- DAT – Post transfusion – Positive – C3d and weak IgG
- Antibody screen – Pre repeat neg, post -Pos

NHS
Blood and Transplant



DAT Positive IgG



DAT Positive IgG + C3d

Panel results

NHS Blood and Transplant

ID Panel **NBS REAGENTS**

Lot R1412016	NAME	NUT Mary	DATE OF BIRTH	25/12/1964	GROUP	O Pos R1r	
Exp. 31/12/2501	HOSPITAL	NCH	HOSPITAL No.		SAMPLE No.	1	

	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT			
1	$R_1{}^WR_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		2	4			
2	$R_1\,R_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		0	0			
3	R_2R_2	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		0	0			
4	r ` r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	0	0			
5	r`` r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		2	4			
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		0	0			
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		2	4			
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		0	0			
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		2	4			
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		0	0			
Auto																								2				

Robert Stamps, RSM, RCI Sheffield

NHS Blood and Transplant

Delayed Haemolytic Reaction

- Antibody level may be too low to detect
- After transfusion rapid increase in antibody concentration after 3-7 days
- May be rapid destruction of transfused cells
- Anti-Jk^{a} most commonly described
- Can occasionally be seen after ABO incompatible transfusion in elderly or immune suppressed

NHS Blood and Transplant

HTR after computer cross-match

- Female patient reacted to one of three units (rigors and vomiting)
- Standard panel and repeat antibody screen negative
- Unit incompatible by IAT
- Antibody to low incidence antigen
- Identified as anti-Vw – MNS system
- Antigen very rare in UK (0.06%)
- Antibody present in about 1%

NHS Blood and Transplant

Serological Investigation of Transfusion Reaction

- Pre-transfusion sample - ABO and Rh phenotype - repeat red cell antibody screen and crossmatch. DAT
- Post transfusion sample - ABO Rh D type - antibody investigation and repeat crossmatch. DAT
- Eluate on post transfusion sample – Can be useful even when DAT negative

NHS Blood and Transplant

Eluates - elution

- Removal of immunoglobulin (usually IgG) from patient/donor red cells
- Allows serological investigation of bound antibody to determine nature and specificity
- Many methods available –
- Heat – Landsteiner-Miller
- Solvents – Rubin's – Ether
- acid solution – pH 3.0

NHS
Blood and Transplant

Jaundiced baby with Positive DAT

- 2 day old baby with low Hb and jaundice
- Maternal antibody screen – Negative
- DAT on Baby Positive IgG
- Mother group O baby group A

NHS Blood and Transplant

- Maternal serum contains IgG anti-A as well as IgM anti-A
- IgG anti-A can cross placenta
- Babies group A antigens strengthen after birth
- If exchange transfusion required – Group O blood cross-matched x maternal plasma

Antenatal patient - Panel results

ID Panel **NBS REAGENTS**

Lot R1412016	NAME	Sickely Pat	DATE OF BIRTH	18/12/1984	GROUP	B Neg rr
Exp. 31/12/2501	HOSPITAL	NCH	HOSPITAL No.		SAMPLE No.	1

	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz			
1	$R_1^WR_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		3	5			
2	R_1R_1	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		3	5			
3	R_2R_2	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		3	5			
4	r ` r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	0	0			
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		0	0			
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		0	0			
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		0	0			
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		0	0			
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		0	0			
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		0	0			
Auto																								0				

NHS Blood and Transplant

Is it immune? or is it prophylactic?

- Patient 32 weeks pregnant
- Attended A/E with PV bleed
- Panel revealed anti-D
- Is anti-D due to 28 week prophylactic anti-D injection?
- Quantitate anti-D and check history – if injected and level < 0.15iu likely to be remains of injection
- Important not to assume it is prophylactic

NHS Blood and Transplant

Patients requiring long term support

- Myelodysplastic syndromes
- Thalassaemias
- Sickle cell disease
- Severe aplastic anaemias
- Warm/cold autoimmune haemolytic anaemias
- Other congenital or acquired chronic anaemias

NHS Blood and Transplant

Sickle patients

- Most common phenotype is Ror (cDe/cde) - 56%
- 68% Fya-b-
- Only rarely form Fy antibodies
- Usually anti-Fy^a - although Fy^b not present on red cells it is present on other tissues
- May eventually form anti-Fy3
- Supply of blood more difficult if patient not Ror ie R1R1 or RhD-

NHS Blood and Transplant

Sickle case study 1

- RhD- (Cde/cde) female
- Multitransfused D- blood
- Alloantibodies present – Anti-E, K, M, S, Fy^a, Jk^b Le^{a+b}, Knops
- Hyperhaemolysis event after ignoring anti-M
- Eventually formed anti-Fy3
- Very few O D-, M-, S-, K-, Fya-b-, Jkb- donors

NHS Blood and Transplant

Sickle case study 2

- Group O R1R1 patient with anti-c
- Developed anti-Fy3
- Only TWO suitable donors in UK
- Blood sourced from Amsterdam

NHS Blood and Transplant

Pan reactive antibodies

- HTLA – High titre low avidity
- Common in multi-transfused patients
- Many specificities – Ch, Rg, Yk^a, Yt^a, Sl^a, Kn^a etc
- All clinically insignificant but may mask significant alloantibodies
- Phenotyped matched blood

NHS Blood and Transplant

Blood grouping												
Anti-A	Anti-B	Anti-A,B	Anti-D (1)	Anti-D (2)	Reagent Control	Reverse Group (cells)				Antibody Screen (IAT)		
						A_1 rr	A_2 rr	B rr	O R_1r	Cell 1	Cell 2	Cell 3
0	0	0	5	5	0	5	5	5	5	5	5	5

Antibody identification panel

	Rh	M	N	S	s	P1	Lu^a	K	k	Kp	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	SAL	Enz R / T	IAT
1	$R_1^wR_1$	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	5	5	4
2	R_1R_1	+	0	+	0	4	0	+	+	0	0	+	+	0	+	0	5	5	4
3	R_2R_2	0	+	0	+	3	0	0	+	0	0	+	0	+	+	0	5	5	4
4	r'r	0	+	0	+	0	0	0	+	0	0	+	0	+	0	+	5	5	4
5	r''r	+	0	+	+	3	0	0	+	0	0	+	+	0	0	+	5	5	4
6	rr	0	+	+	0	5	0	0	+	0	0	+	0	+	+	0	5	5	4
7	rr	+	+	0	+	3	0	+	+	0	+	0	0	+	0	+	5	5	4
8	rr	+	0	+	0	3	+	0	+	0	+	0	+	0	+	+	5	5	4
9	rr	+	+	0	+	4	0	0	+	+	0	+	0	+	0	+	5	5	4
10	rr	0	+	0	+	5	0	+	0	0	0	+	+	0	+	0	5	5	4
Auto																	0	0	0

NHS Blood and Transplant

Autoantibodies

- Over 50% samples referred to reference lab have autoantibodies
- Many require regular transfusions
- Most are warm type IgG autoantibodies
- Adsorption of autoantibody required to detect underlying alloantibodies – Autoadsorption or allogeneic adsorption
- Crossmatch is positive and blood issued as ‘suitable’

NHS Blood and Transplant

Autoadsorbed patient –typical serology

ID Panel **NBS REAGENTS**

Lot R1412016	NAME	Hill Harry	DATE OF BIRTH	25/01/1926	GROUP	O Pos R2r
Exp. 31/12/2501	HOSPITAL	NCH	HOSPITAL No.		SAMPLE No.	1

	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT	Auto abs xIAT		
1	$R_1{}^WR_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		4	5	3		
2	R_1 R_1	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		4	5	0		
3	R_2R_2	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		4	5	0		
4	r ` r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	4	5	0		
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		4	5	0		
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		4	5	2		
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		4	5	3		
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		4	5	3		
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		4	5	0		
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		4	5	0		
Auto																								5				

Adsorption reveals anti-Fya

Allogeneic adsorption – typical results

ID Panel **NBS REAGENTS**

Lot R1412016	NAME	GOK Jane	DATE OF BIRTH	18/12/1929	GROUP	O Pos R1R1
Exp. 31/12/2501	HOSPITAL	NCH	HOSPITAL No.		SAMPLE No.	7

	Rh	C^W	C	c	D	E	e	M	N	S	s	P_1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other	IAT	Enz IAT	Ads x rr	Ads x R1R1	
1	$R_1{}^WR_1$	+	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	0		5	5	5	0	
2	$R_1\ R_1$	0	+	0	+	0	+	+	0	+	0	2+	+	+	+	0	0	0	0	+	0	+		5	5	5	0	
3	R_2R_2	0	0	+	+	+	0	+	+	0	+	0	0	0	+	0	0	+	0	+	0	+		5	5	5	4	
4	r ` r	0	+	+	0	0	+	+	+	0	+	4+	0	0	+	0	0	0	0	+	0	+	Yk (a-)	5	5	0	2	
5	r``r	0	0	+	0	+	+	+	+	+	0	5+	0	0	+	0	0	+	0	+	+	0		5	5	0	4	
6	r r	0	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	+	+	+	0	+		5	5	0	4	
7	r r	0	0	+	0	0	+	+	+	+	+	4+	0	+	+	0	+	0	+	0	+	0		5	5	0	4	
8	r r	0	0	+	0	0	+	0	+	0	+	2+	+	0	+	0	0	+	+	0	0	+		5	5	0	4	
9	r r	0	0	+	0	0	+	0	+	0	+	5+	+	0	+	+	0	+	0	+	+	0		5	5	0	4	
10	r r	0	0	+	0	0	+	+	0	0	+	4+	0	+	0	0	0	+	0	+	0	+		5	5	0	4	
																								5				

Adsorption reveals allo anti-c and auto anti-D

NHS Blood and Transplant

Cold autoantibodies

- CHAD – IgM wide thermal range cold auto anti-I
- Adsorption not usually required
- Pre-warming of tests usually successful
- Rabbit stroma useful if adsorption required

NHS
Blood and Transplant

Maintenance of phenotyped bank

- All donations are tested for Rh (C,c,E,e) and K
- A number of units are typed for (related to demand and usually rr, R1R1 and R2R2) M,S,Cw,Jk^a,Jk^b,Fy^a,Fy^b and HbS
- Very rare units offered to frozen blood bank
- RCI inform Donor Testing of any problematic patients
- Testing ensure suitable units are held for named patients

NHS Blood and Transplant

The future

- Red cell genotyping using DNA
- Shirey RS et al, Prophylactic antigen matched donor blood for patients with warm autoantibodies, an algorithm for transfusion management, *Transfusion 2002, vol 42*