Immunohematology Case Studies 2020 - 6

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Clinical History

Medical History

A 17 year old female, presented with vaginal bleeding during pregnancy. Her haemoglobin was 49g/L and blood for transfusion was requested.

Transfusion History

Multiple transfusion received in Papua New Guinea

Pregnancy History

Unknown

Serological History

- No history of antibodies
- Routine blood group and antibody screen performed
- The referring laboratory observed that the plasma was positive with all cells, except the patient's own cells
- The Direct Antiglobulin Test (DAT) was positive with IgG and all cross matched units were incompatible by IAT (CAT)
- The sample was referred to the Red Cell Reference Laboratory at the Australian Red Cross Blood Service for further testing

Current Serology

Blood Group & Phenotype:

O positive, C+ E- c- e+, K-, Fy(a+b-), Jk(a-b+), M- N+ S- s+

DAT:

Weakly positive

1+ with anti-IgG

1+ with anti-C3d

Antibody Identification Preliminary Result:

Pan reactive antibody with a possible auto antibody reactive in tube IAT. Papain treated cells by tube IAT were positive showing no variance in reaction strength

Initial Panel

	D	C	E	c	e	C^w	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	P1	Le^a	Le^b	Lu^a	Co^b	Sal RT	Sal 37	IAT	Pap IAT
1	+	+	0	0	+	+	0	+	0	0	+	0	+	+	+	+	+	0	0	+	0	0	0	0	2+	3+
2	+	+	0	0	+	0	+	+	0	+	0	+	+	0	+	0	+	$+^w$	0	+	0	0	0	0	2+	3+
3	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	3+	3+
4	+	0	+	+	0	0	0	+	0	0	+	+	0	0	+	0	+	$+^s$	0	+	0	+	0	0	3+	3+
5	+	0	+	+	0	0	0	+	0	+	0	0	+	+	0	0	+	+	0	+	0	0	0	0	3+	3+
6	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	+	0	+	0	0	0	0	0	3+	3+
7	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	3+	3+
8	0	0	0	+	+	0	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	0	0	0	3+	3+
9	0	0	0	+	+	0	0	+	0	0	+	0	+	+	0	+	+	$+^s$	0	0	0	0	0	0	3+	3+
10	0	0	0	+	+	0	0	+	0	0	+	+	0	+	0	0	+	0	0	0	0	0	0	0	3+	3+
11	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	0	0	0	3+	3+
AC																							0	0	w	0

Challenges

- No compatible donor units
- Possibly an antibody to a high frequency antigen
- Possible auto antibody
- Antibody to a high frequency antigen is more likely given the strong reactions with the panel cells
- Slightly weaker reactions with 2 of the 3 c- cells along with the patients c- phenotype may indicate the possibility of an allo anti-c

Additional Serology

- Allogenic homologous adsorption with R_1R_1, R_2R_2 and rr cells
- All antibody reactivity was removed by adsorption and underlying antibodies were excluded including anti-c
- The sample was tested against a panel of cells negative for a range of high frequency antigens – all cells were positive.
    - Ge:-2-3, Jk(a-b-), Lu(b-), Yt(a-), Vel-, Lan-, Jr(a-), Co(a-), Kp(b-), K_o, Rh_{null}, Lu(a-b-) and Hy-Jo(a-) cells

- Enzyme and chemical treatment of the cells

DTT	Trypsin	α-Chymotrypsin
Resistant	Resistant	Sensitive

    - Possible Cromer, Scianna or Diego (3rd loop)

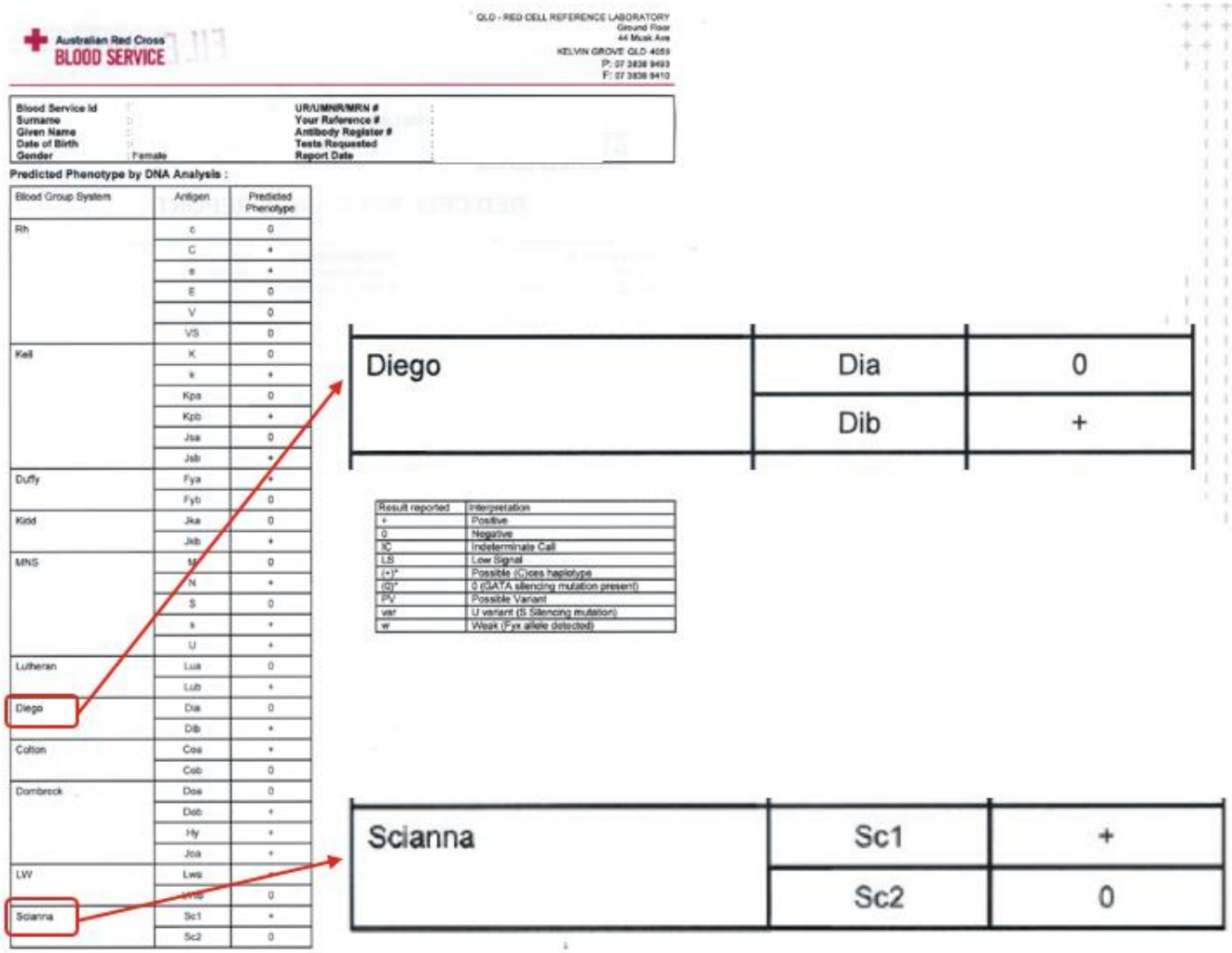
Process of Elimination

- Diego was possible but considered unlikely, genotyping predicted the phenotype to be Di(a-b+).
- Inhibition studies with recombinant proteins for Cromer (Human CD55 / DAF Protein (Fc Tag), Cat# 10101-H02H, Sino Biological Inc)
  - Inhibition of the antibody with CD55 was unsuccessful, a Cromer related antibody was now unlikely
- Further phenotyping - Patient was positive with
  - anti-Ge2, -UMC, -Wes^{b} , -IFC, -CD59, -Wb, -Yt^{b}, -Wr^{a}, -K17, -Ny^{a}, -Ri^{a}, -Ul^{a}, -Wd^{a} and –Rd
  - Patient was negative with anti-Sc1 and anti-Sc2
- The antibody was positive with Sc:-1,2 cells and negative with Sc:-3 cell
- The antibody is most likely Scianna related

Next Steps

Genotyping

The predicted phenotype from the genotyping for Sc1 did not match the serology.



Australian Red Cross BLOOD SERVICE

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Ground Floor
44 Musk Ave
KELVIN GROVE QLD 4059
P: 07 3838 9493
F: 07 3838 9410

Blood Service Id		UR/UMNR/MRN #	
Surname		Your Reference #	
Given Name		Antibody Register #	
Date of Birth		Tests Requested	
Gender	Female	Report Date	

Predicted Phenotype by DNA Analysis :

Blood Group System	Antigen	Predicted Phenotype
Rh	c	0
	C	+
	e	+
	E	0
	V	0
	VS	0
Kell	K	0
	k	+
	Kpa	0
	Kpb	+
	Jsa	0
	Jsb	+
Duffy	Fya	+
	Fyb	0
Kidd	Jka	0
	Jkb	+
MNS	M	0
	N	+
	S	0
	s	+
	U	+
Lutheran	Lua	0
	Lub	+
Diego	Dia	0
	Dib	+
Colton	Coa	+
	Cob	0
Dombrock	Doa	0
	Dob	+
	Hy	+
	Joa	+
LW	Lwa	+
	Lwb	0
Scianna	Sc1	+
	Sc2	0

Diego	Dia	0
	Dib	+

Result reported	Interpretation
+	Positive
0	Negative
IC	Indeterminate Call
LS	Low Signal
(+)*	Possible (C)oes haplotype
(0)*	0 (DATA silencing mutation present)
PV	Possible Variant
var	U variant (S Silencing mutation)
w	Weak (Fy# allele detected)

Scianna	Sc1	+
	Sc2	0

Preliminary Report

- The negative patient phenotype, compatibility with Sc:-3 cells and the enzyme and chemical modification results supported a preliminary result of anti-Sc3
- The patient's Melanesian ethnicity supports this result.
- The genotype reports the common Sc:1,-2 probable genotype which was inconsistent with serology and the Sc:-3 phenotype required for allo anti-Sc3
    - This could be an incorrect predicted phenotype due to a change not detected on this array
    - Or it could still be a case of an auto-anti-Sc3
- An interim report was issued indicating the antibody is likely in the Scianna system and further investigation is required
- Follow-up samples post delivery were not available to determine if this was a pregnancy related suppression of the Sc1 antigen.

About the blood group

Scianna Blood Group System – SC (013)

- Established in 1974
- Consists of 7 antigens
  - Low prevalence Sc2, Rd
  - High prevalence Sc1, Sc3, STAR, SCER,SCAN.
- Sc1 and Sc2 discovered in 1962 by Schmidt et al.
  - Originally called Sm and Bua
- Sc:-1,-2 phenotype found by McCreary et al.
  - 1986 – 4yo girl in PNG is Sc:-1,-2
- Most people will type as Sc:1,-2
- Sc3 is expressed on all red cells except Sc:-1,-2
- Sc:-1,-2,-3 phenotype more common in Pacific Islanders

Clinical Significance

- In general anti-Sc1, anti-Sc2 and anti-Sc3 are considered to be unlikely to cause transfusion reaction
- This suggests that where antigen negative units are not available the least reactive units by crossmatch may be transfused

	Transfusion reaction	HDFN
Anti-Sc1	Not reported	Pos Dat / no HDFN
Anti-Sc2	No	No clinical HDFN to mild
Anti-Sc3	No to mild/delayed	Mild
Anti-Sc4 (Rd)	No	Mild to severe

Reference: Reid M, Lomas-Francis C, Olsson M. The Blood Group Antigen Factsbook. 3rd ed. Oxford: Elsevier Ltd; 2012

NexGen Sequencing

Sequencing was performed using the Illumina TruSight™ One Sequencing panel (TSO) which enables targeted DNA sequencing of exonic and 3'untranslated regions of 39 genes related to blood group systems

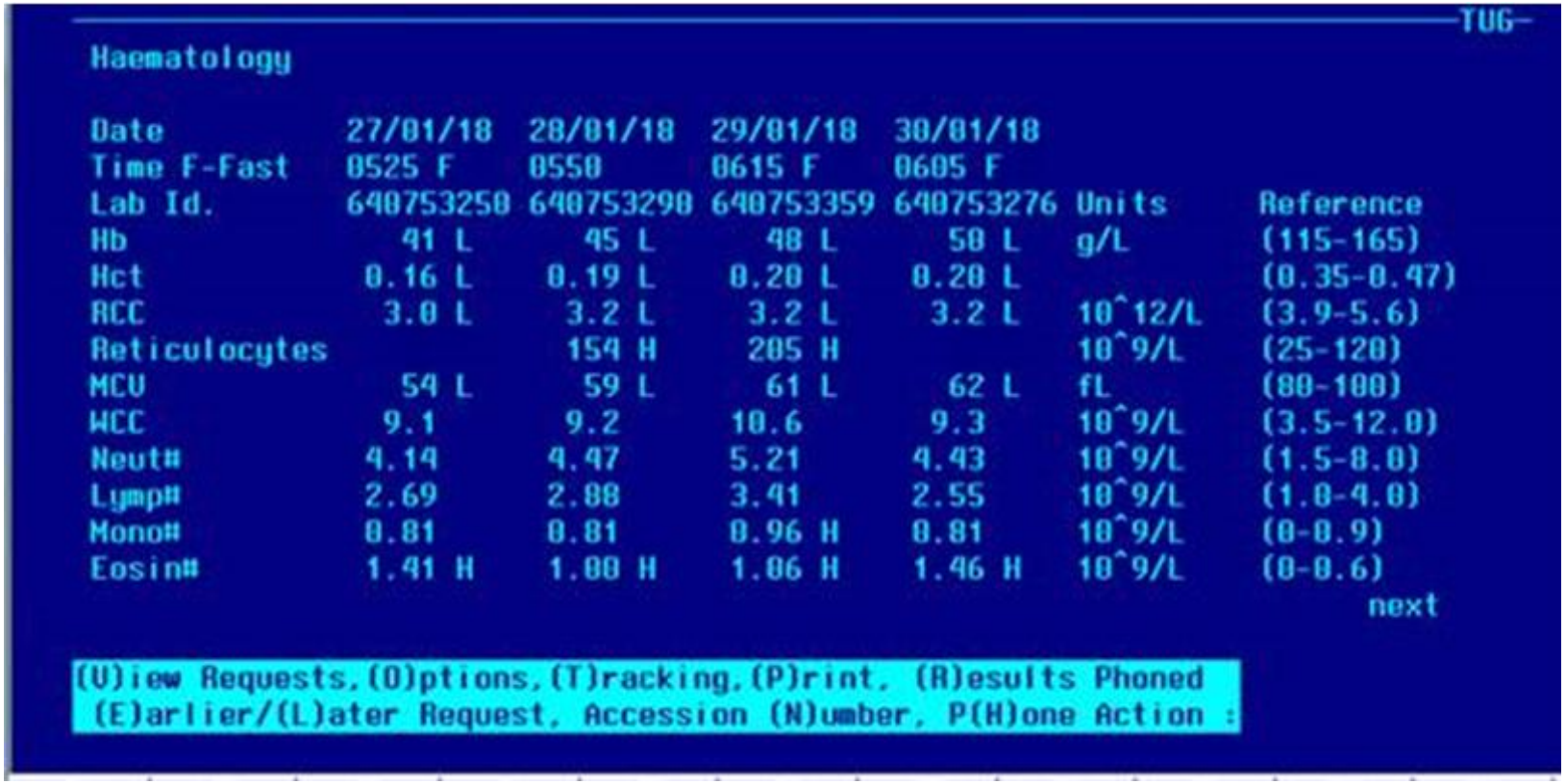
- There was insufficient sequencing depth to reliably call the Scianna genotype or predict a phenotype.
- The TruSight™ One Sequencing Panel has been shown to give suboptimal sequencing depth for the ERMAP (SC) gene

Sequencing using the custom designed panel revealed the patient is homozygous for allele *SC*01N.02* which arises from the presence of a nonsense mutation c.994C>T causing a premature stop codon (p.Arg332Ter). The SNP was detected in 557 out of 569 reads, consistent with homozygosity for c.994C>T

- Predicted phenotype Sc:-1,-2,-3

Outcome

- Transfusion support was not required for this patient
- The patient was treated with Haematinics for iron deficient anemia.



TUG

Haematology

Date	27/01/18	28/01/18	29/01/18	30/01/18		
Time F-Fast	0525 F	0550	0615 F	0605 F		
Lab Id.	640753250	640753290	640753359	640753276	Units	Reference
Hb	41 L	45 L	48 L	50 L	g/L	(115-165)
Hct	0.16 L	0.19 L	0.20 L	0.20 L		(0.35-0.47)
RCC	3.0 L	3.2 L	3.2 L	3.2 L	10^12/L	(3.9-5.6)
Reticulocytes		154 H	285 H		10^9/L	(25-120)
MCV	54 L	59 L	61 L	62 L	fL	(80-100)
WCC	9.1	9.2	10.6	9.3	10^9/L	(3.5-12.0)
Neut#	4.14	4.47	5.21	4.43	10^9/L	(1.5-8.0)
Lymp#	2.69	2.88	3.41	2.55	10^9/L	(1.0-4.0)
Mono#	0.81	0.81	0.96 H	0.81	10^9/L	(0-0.9)
Eosin#	1.41 H	1.00 H	1.06 H	1.46 H	10^9/L	(0-0.6)

next

(V)iew Requests,(O)ptions,(T)racking,(P)rint, (R)esults Phoned
(E)arlier/(L)ater Request, Accession (N)umber, P(H)one Action :

Conclusion

- We had no suitable donors available in the country
- There was one known Sc:-3 donor listed on the International Rare Donor Panel
- Thankfully our patient responded well to treatment avoiding the need for transfusion

Lessons Learnt

If this patient had required blood for transfusion there is very limited availability.

Patient Blood Management strategies were effective in avoiding transfusion with incompatible donor units.

Unfortunately, we were unable to test family members and the patient was not interested in autologous donations.

The current commercially available genotyping platforms do not screen for Sc:-3.

The Sc1 and Sc2 genotyping showed discrepant results with Scianna phenotyping in this phenotypically Sc:-3 individual

References

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5. McDowell MA, Stocker I, Nance S, Garratty G. Auto anti-Sc1 associated with autoimmune hemolytic anemia [abstract]. Transfusion 1986;26:578.
6. Peloquin P, Moulds M, Keenan J, Kennedy M. Anti-Sc3 as an apparent autoantibody in two patients [abstract]. Transfusion 1989;29(Suppl):49S.