UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

Director: *Dr MT Rees*
Deputy Director: *Mrs D Pritchard*
Operations Manager: *Miss A De'Ath*

Tel: *+44 (0) 1443 622185*
Email: *ukneqashandi@wales.nhs.uk*
Web: *www.ukneqashandi.org.uk*

Correspondence to:
UK NEQAS for H&I
Welsh Blood Service
Ely Valley Road
Talbot Green
Pontyclun
CF72 9WB

Interpretive Educational Scheme (iED)
Clinical Scenario 3/2021 – Transfusion/Platelet Immunology Case

Dispatched on 11th January 2022

Summary of Submitted Responses

A total of 36 responses were received, 15 from UK & Ireland (UK&I) based laboratories and 21 from Rest of the World (RoW) based laboratories.

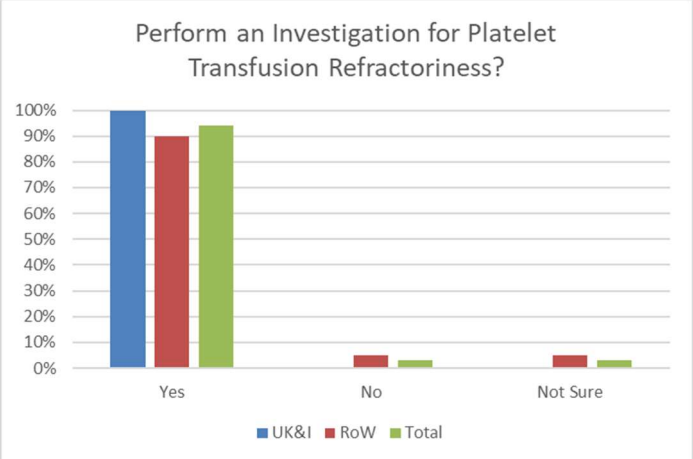
Background:

A 59-year old female with a history of pregnancy was previously diagnosed in May 2017 with Myelodysplastic Syndrome (MDS) which transformed to Acute Myeloid Leukaemia (AML) in November 2019. The patient was started on induction chemotherapy using a combination of Daunorubicin and Ara-C.

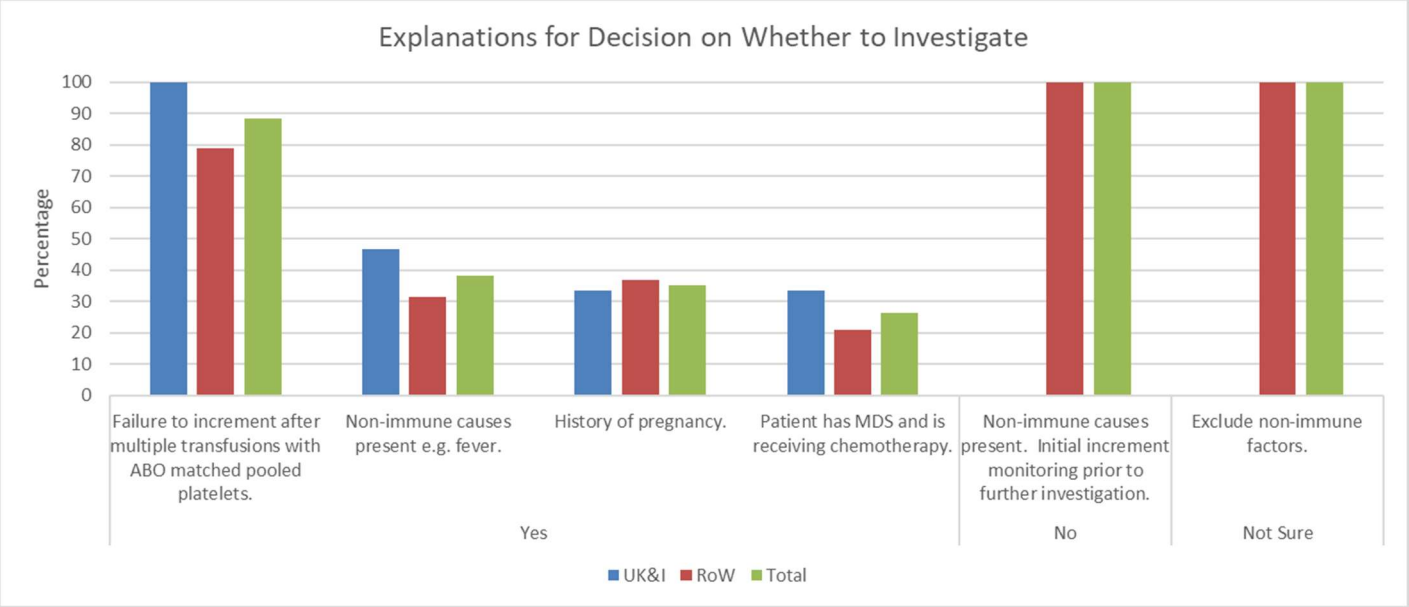
The patient was admitted to hospital with a fever and beginning on the 13/06/2020, the patient received three fresh ABO compatible random pooled platelets, but the platelet count failed to increment above $10x10^9$/L.

Question 1 – Would you investigate this patient for platelet transfusion refractoriness? Explain your answer.

	UK&I		RoW		Total	
	Number	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Yes	15	**100%**	19	**90%**	34	**94%**
No	0	**0%**	1	**5%**	1	**3%**
Not Sure	0	**0%**	1	**5%**	1	**3%**



		UK&I		RoW		Total	
Investigate	**Explanation**	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Yes	Failure to increment after multiple transfusions with ABO matched pooled platelets.	15	**100%**	15	**79%**	30	**88%**
	Non-immune causes present e.g. fever.	7	**47%**	6	**32%**	13	**38%**
	History of pregnancy.	5	**33%**	7	**37%**	12	**35%**
	Patient has MDS and is receiving chemotherapy.	5	**33%**	4	**21%**	9	**26%**
No	Non-immune causes present. Initial increment monitoring prior to further investigation.	0	**0%**	1	**100%**	1	**100%**
Not Sure	Exclude non-immune factors.	0	**0%**	1	**100%**	1	**100%**



The patient was HLA typed by PCR-SSP and screened for Class I HLA antibodies using LabScreen single antigen bead kit:

Antigen Specificity	**MFI Value**
A3	2686
A31	1272
A34	1147-1275
A74	1082
A68	1075-1352
A30	1060-1232
A11	5000-5356

Note: All other class I beads negative (<1000 MFI). Where multiple beads are present for an antigen an MFI range has been given.

HLA class I type: A*01, -; B*08, B*41; C*07, C*17
Blood Group: O Rh positive
CMV Status: Negative

Question 2 - From the list of platelet donations in Table 2, which two units would you preferentially select for the patient and why?

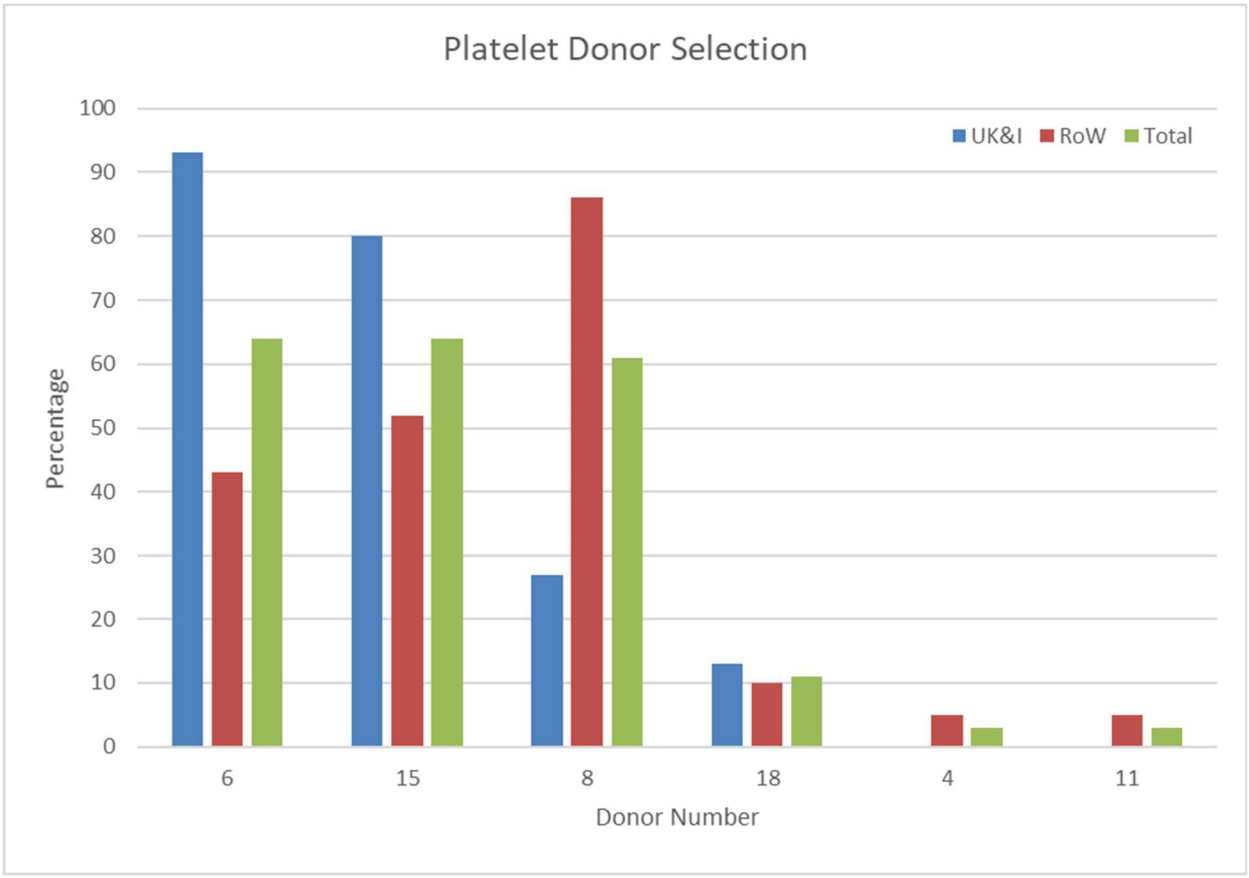
*Table 2 – List of Potential Platelet Donors**

Donor	HLA Type						ABO	Rh	CMV
	A	A	B	B	C	C			
1	1	2	7		7		B	Positive	Positive
2	2	25	18	62	7	9	O	Positive	Positive
3	2	31	7	60	7	10	O	Negative	Positive
4	1	2	8	44	7	5	O	Positive	Positive
5	1	3	7		7		A	Positive	Positive
6	1		8		7		A	Negative	Negative
7	1	11	35	55	4	9	A	Positive	Positive
8	1	2	8		7		O	Positive	Negative
9	1	31	8	60	7	10	B	Negative	Positive
10	24		7		7		A	Negative	Positive
11	24	26	38	55	12	9	O	Positive	Positive
12	2	3	7	27	2	7	O	Positive	Positive
13	3	30	7	13	6	7	A	Positive	Positive
14	30	32	50	62	6	9	A	Positive	Positive
15	1	23	8		7		O	Positive	Positive
16	2	68	7	60	7	10	O	Negative	Positive
17	1	11	8	35	4	7	O	Positive	Positive
18	1	30	8		8		O	Positive	Negative
19	2	29	44		5		A	Positive	Positive
20	3		7		7		O	Positive	Positive

**Mismatches have been highlighted in red italic. Antigens to which the patient has antibodies have been highlighted in yellow.*

	UK&I		RoW		Total		Reasons for Selection
Donor ID	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**	
6	14	**93%**	9	**43%**	23	**64%**	No donor specific antibodies. HLA matched at A and B. CMV match. ABO incompatible.
15	12	**80%**	11	**52%**	23	**64%**	No donor specific antibodies. Single antigen mismatch. ABO match. CMV mismatch. A23 mm low frequency.

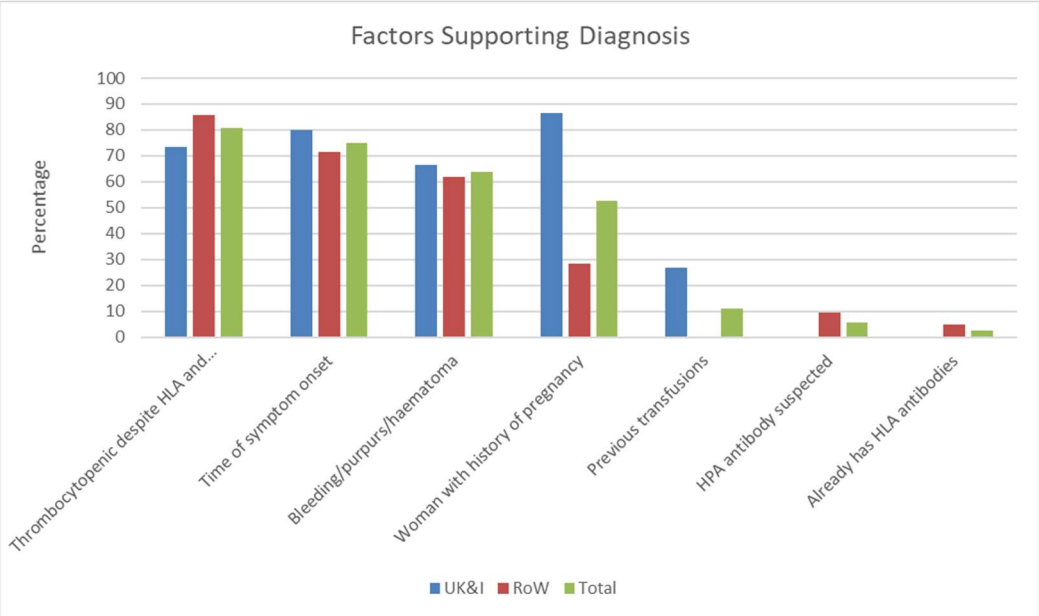
8	4	**27%**	18	**86%**	22	**61%**	No donor specific antibodies. Single antigen mismatch. ABO match. CMV match. A2 mm may cause sensitisation and limit further matches.
18	2	**13%**	2	**10%**	4	**11%**	Low level donor specific antibody to A30. Single antigen mismatch. ABO match. CMV match. A30 mm low frequency.
4	0	**0%**	1	**5%**	1	**3%**	ABO compatible. HLA antigen donor to avoid patient antibody.
11	0	**0%**	1	**5%**	1	**3%**	ABO match. CMV status is not taken into account. Avoidance of antigens with positive MFI and A2 typing in the platelet unit.



Following the transfusion of two ABO compatible HLA matched (no HLA-A, B or Cw mismatches) platelets 7 days earlier, and with a platelet count of $2x10^9$/L, the patient presented with a retinal haematoma, bleeding in the lungs with haemoptysis, bleeding round the knee joint and purpura.

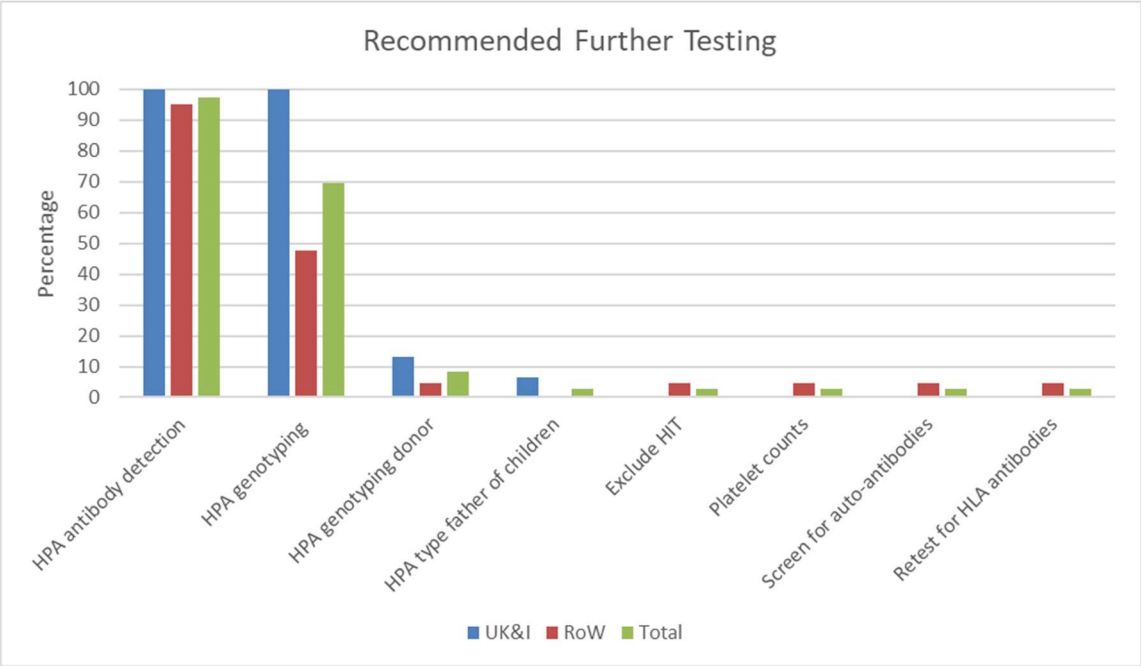
Question 3 - These symptoms could indicate PTP, what factors would support this diagnosis?

	UK&I		RoW		Total	
Factors Supporting Diagnosis	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Thrombocytopenic despite HLA and ABO matched platelets	11	**73%**	18	**86%**	29	**81%**
Time of symptom onset	12	**80%**	15	**71%**	27	**75%**
Bleeding/purpura/haematoma	10	**67%**	13	**62%**	23	**64%**
Woman with history of pregnancy	13	**87%**	6	**29%**	19	**53%**
Previous transfusions	4	**27%**	0	**0%**	4	**11%**
HPA antibody suspected	0	**0%**	2	**10%**	2	**6%**
Already has HLA antibodies	0	**0%**	1	**5%**	1	**3%**



What further testing would you recommend?

	UK&I		RoW		Total	
Further Testing	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
HPA antibody detection in patient sera	15	**100%**	20	**95%**	35	**97%**
HPA genotyping of patient	15	**100%**	10	**48%**	25	**69%**
HPA genotyping of platelet donor	2	**13%**	1	**5%**	3	**8%**
HPA type father of children	1	**7%**	0	**0%**	1	**3%**
Exclude HIT	0	**0%**	1	**5%**	1	**3%**
Platelet counts	0	**0%**	1	**5%**	1	**3%**
Screen patient for auto-antibodies	0	**0%**	1	**5%**	1	**3%**
Retest Patient for HLA antibodies	0	**0%**	1	**5%**	1	**3%**



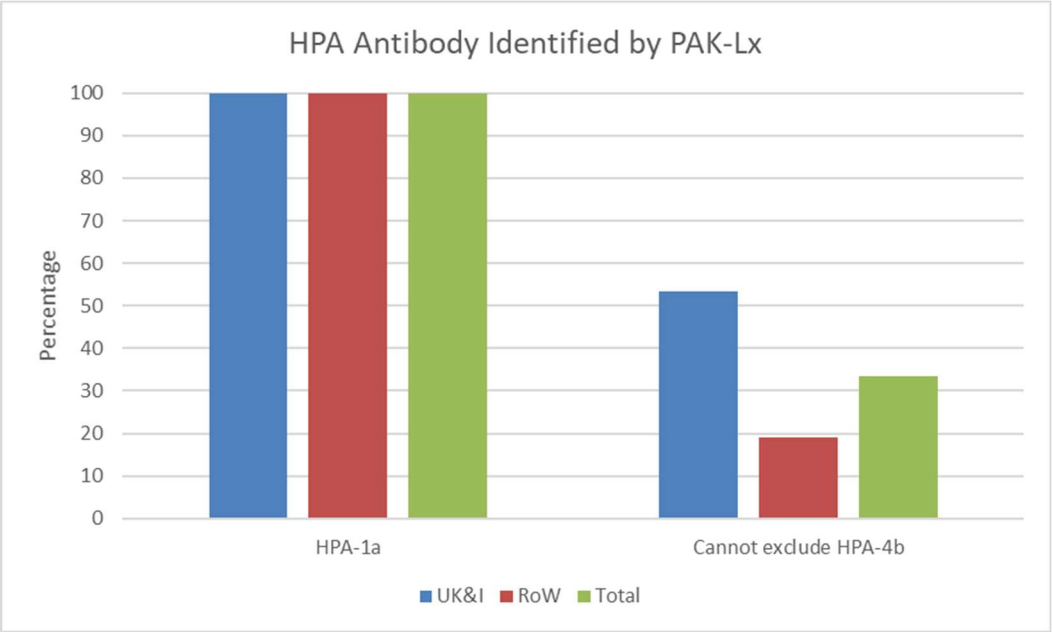
Results from PAK-Lx testing have been provided in Table 3. From the antibody reactivity pattern what HPA antibody would be defined as present?

Table 3 – Results of Immucor Pak-Lx Testing

Bead Region	Glycoprotein Group	Antigen	MFI	Bead Reactivity	Adjusted Ratio 1	Adjusted Ratio 2	Adjusted Ratio 3
13	Con1	Con1	29				
14	Con2	Con2	12				
18	Con3	Con3	40				
11	POS	POS	15766				
6	GPIV	GPIV	34	Negative	-3.06	-3.77	-2.66
10	HLA Class I	HLA Class I	20	Negative	-3.24	-4.15	-2.99
21	GPIIb-IIIa	HPA - 1a 3a 4a	5745	Positive	54.52	53.52	55.08
22	GPIIb-IIIa	HPA - 1a 3b 4a	5048	Positive	47.12	45.75	47.46
23	GPIIb-IIIa	HPA - 1b 3a 4a	97	Negative	-3.21	-4.01	-2.94
24	GPIIb-IIIa	HPA - 1b 3b 4a	89	Negative	-3.32	-4.19	-2.97
25	GPIIb-IIIa	HPA - 1a 3ab 4a	3727	Positive	34.33	33.29	34.72
26	GPIIb-IIIa	HPA - 1a 3ab 4b	6953	Positive	66.55	64.97	67.17
27	GPIb/IX	HPA - 2a	59	Negative	-3.75	-4.38	-3.1
28	GPIb/IX	HPA - 2a	44	Negative	-3.56	-4.27	-2.98
29	GPIb/IX	HPA - 2ab	30	Negative	-3.42	-3.92	-2.74
30	GPIb/IX	HPA - 2b	28	Negative	-3.43	-4.05	-2.8
32	GPIb/IX	HPA - 2b	31	Negative	-3.09	-3.59	-2.58

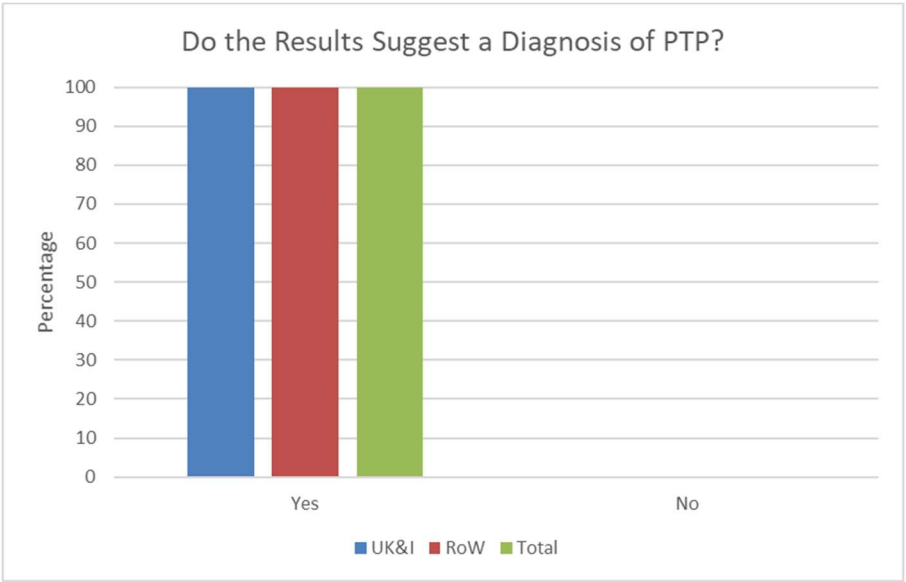
The patient's HPA type has been defined as HPA 1b/b, 2a/a, 3a/a, 4a/a, 5a/a, 15a/b

HPA Antibody Detected in PAK-Lx	UK&I		RoW		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
HPA-1a	15	**100%**	21	**100%**	36	**100%**
Cannot exclude HPA-4b	8	**53%**	4	**19%**	12	**33%**

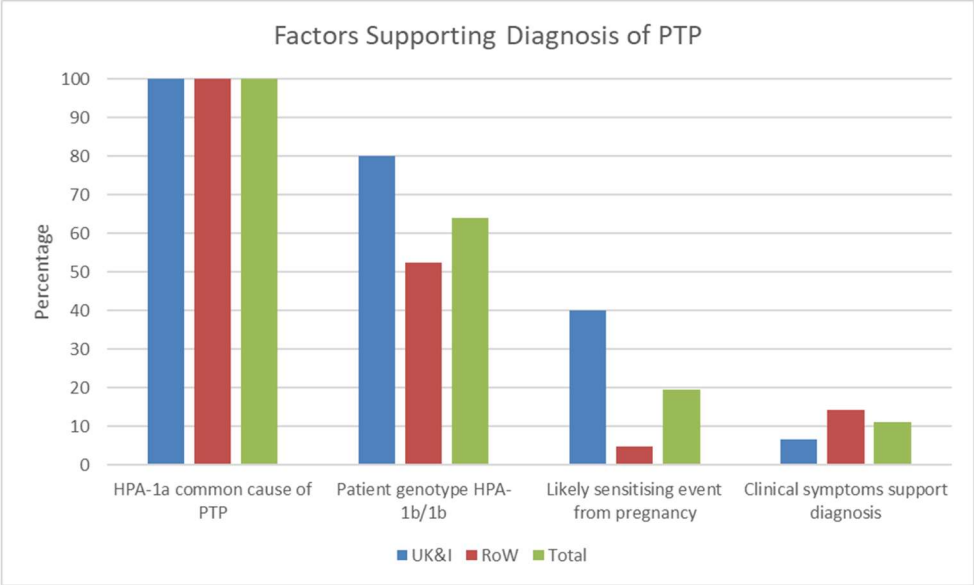


Are these results consistent with a diagnosis of PTP? Give an explanation for you answer.

	UK&I		RoW		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Yes	15	**100%**	21	**100%**	36	**100%**
No	0	**0%**	0	**0%**	0	**0%**



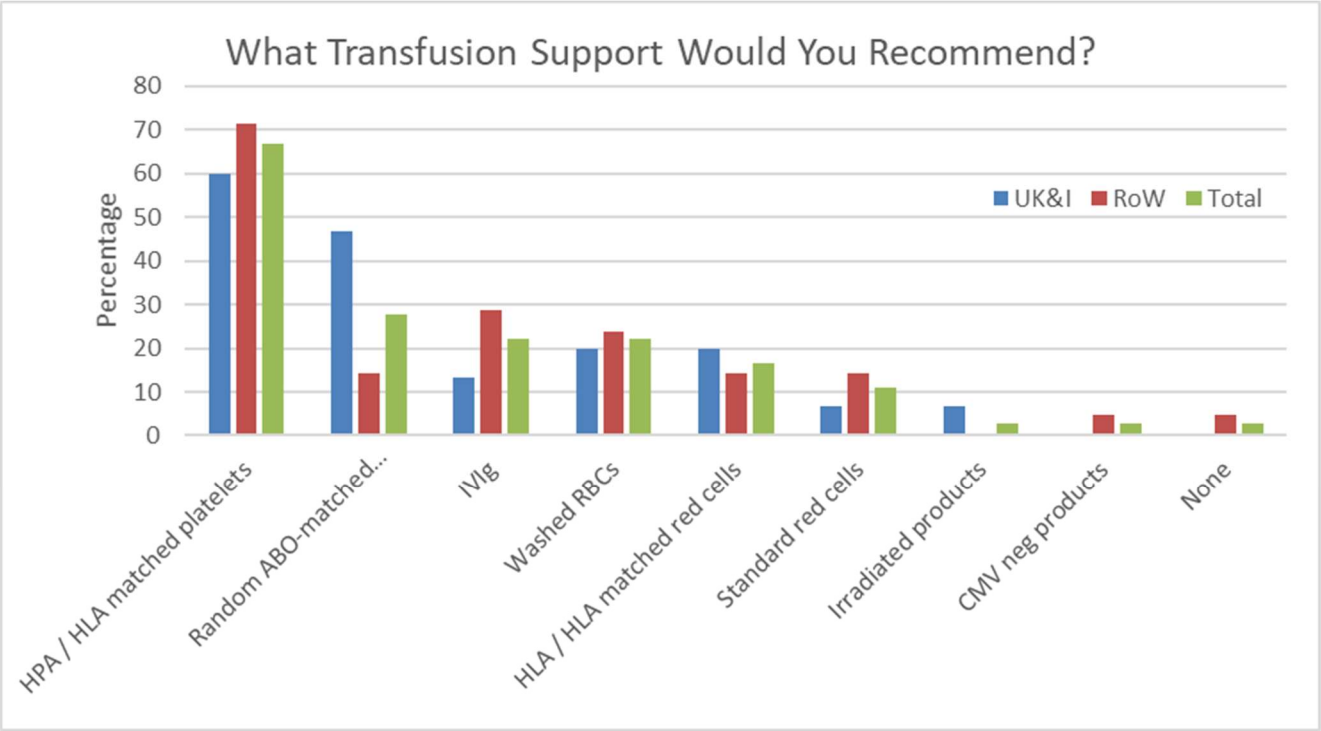
	UK&I		RoW		Total	
Factors supporting diagnosis	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
HPA-1a common cause of PTP	15	**100%**	21	**100%**	36	**100%**
Patient genotype HPA-1b/1b	12	**80%**	11	**52%**	23	**64%**
Likely sensitising event from pregnancy	6	**40%**	1	**5%**	7	**19%**
Clinical symptoms support diagnosis	1	**7%**	3	**14%**	4	**11%**



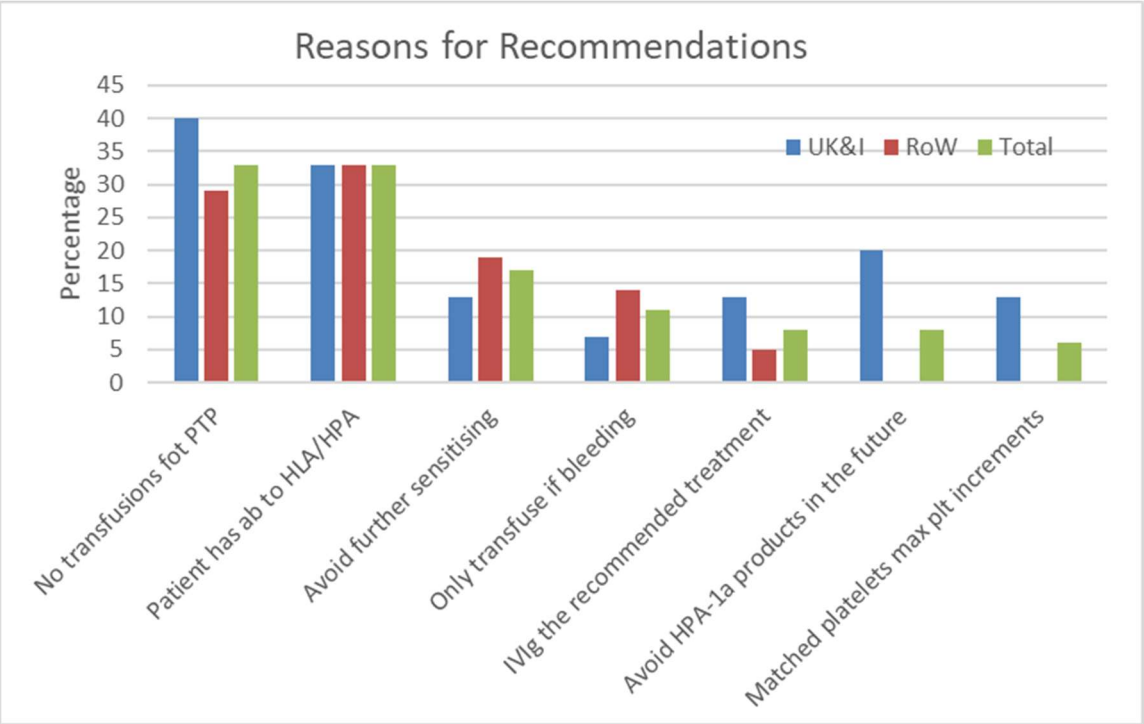
The clinician informs you the patient requires platelet and red cell transfusion support through the acute phase of this particular condition.

What products would you provide? Give an explanation for you answer.

	UK&I		RoW		Total	
Transfusion Support	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
HPA and/or HLA matched platelets	9	**60%**	15	**71%**	24	**67%**
Random ABO matched platelets	7	**47%**	3	**14%**	10	**28%**
IVIg	2	**13%**	6	**29%**	8	**22%**
Washed RBCs	3	**20%**	5	**24%**	8	**22%**
HLA and/or HLA matched red cells	3	**20%**	3	**14%**	6	**17%**
Standard red cells	1	**7%**	3	**14%**	4	**11%**
Irradiated products if patient immunocompromised	1	**7%**	0	**0%**	1	**3%**
CMV neg products	0	**0%**	1	**5%**	1	**3%**
None	0	**0%**	1	**5%**	1	**3%**



	UK&I		RoW		Total	
Reasons for Recommendations	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Platelet transfusion not recommended to treat PTP	6	**40%**	6	**29%**	12	**33%**
Patient is refractory and has HLA and HPA antibodies so needs matched products	5	**33%**	7	**33%**	12	**33%**
Avoid further sensitising patient	2	**13%**	4	**19%**	6	**17%**
Avoid transfusion in PTP unless bleeding	1	**7%**	3	**14%**	4	**11%**
IVIg the recommended treatment	2	**13%**	1	**5%**	3	**8%**
Avoid HPA-1a products in the future to prevent re-occurrence of PTP	3	**20%**	0	**0%**	3	**8%**
Matched products will maximise platelet increment	2	**13%**	0	**0%**	2	**6%**

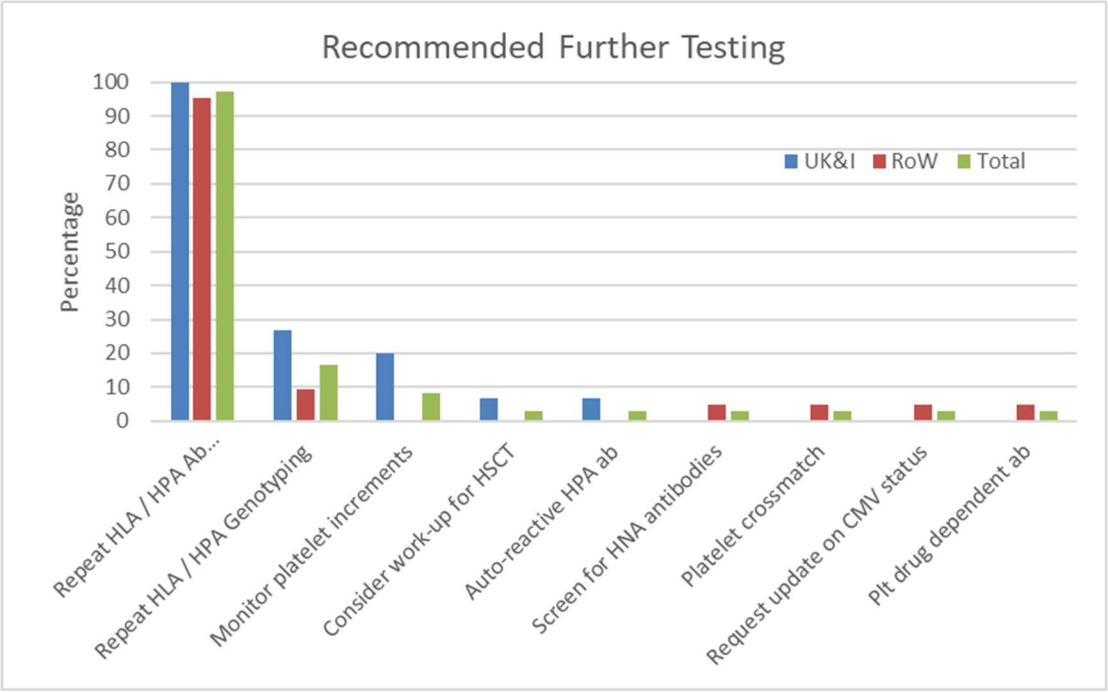


In May 2021 the patient relapsed and was given FLAG-IDA and G-CSF, an effective remission-induction therapy for poor prognosis AML. During the treatment the patient required platelet support.

Question 4 - What further testing would you recommend? Give an explanation for you answer.

	UK&I		RoW		Total		Common Reasons Given
Further Testing	**Number**	**%**	**Number**	**%**	**Number**	**%**	
Repeat HLA / HPA Antibody Monitoring	15	**100%**	20	**95%**	35	**97%**	HLA/HPA-specific antibodies may change with time and impact efficacy of platelet transfusions. Select matched units to prevent further sensitisation. Recent sensitising events. Potential changes in immunosuppression levels. To inform platelet provision. Treat with matched products if require future transfusions. Patient is homozygous at HLA-B locus and HPA 5b so could form additional anti HLA and HPA 5b antibodies. PTP is self-limiting but there is a recurrence potential so suggest receive a HPA compatible products
Repeat HLA / HPA Genotyping	4	**27%**	2	**10%**	6	**17%**	If stem cell transplant is being considered, donors should be HPA typed also. Verification HLA types are essential for confirming patient types are correct.

Monitor platelet increments	3	**20%**	0	**0%**	3	**8%**	To ensure successful increments achieved. Review which units produced best increments.
Consider work-up for HSCT if requested	1	**7%**	0	**0%**	1	**3%**	
Test for auto-reactive HPA antibodies	1	**7%**	0	**0%**	1	**3%**	Development of autoreactive anti-platelet antibodies can continue to affect platelet increments.
Screen for HNA antibodies	0	**0%**	1	**5%**	1	**3%**	The patient may develop new anti-HLA antibodies and anti-HNA after receiving blood products.
Platelet crossmatch	0	**0%**	1	**5%**	1	**3%**	These findings will help to ensure no new allo-immunisation occurs and help ensure a compatible platelet transfusion
Request update on CMV status	0	**0%**	1	**5%**	1	**3%**	The patient's CMV status may have changed, and could potentially increase the pool of donors available.
Screen for platelet drug dependent antibodies	0	**0%**	1	**5%**	1	**3%**	The induction therapy may induce antibodies that cause thrombocytopenia



The patient urgently required platelet support, with insufficient time to wait for additional testing.

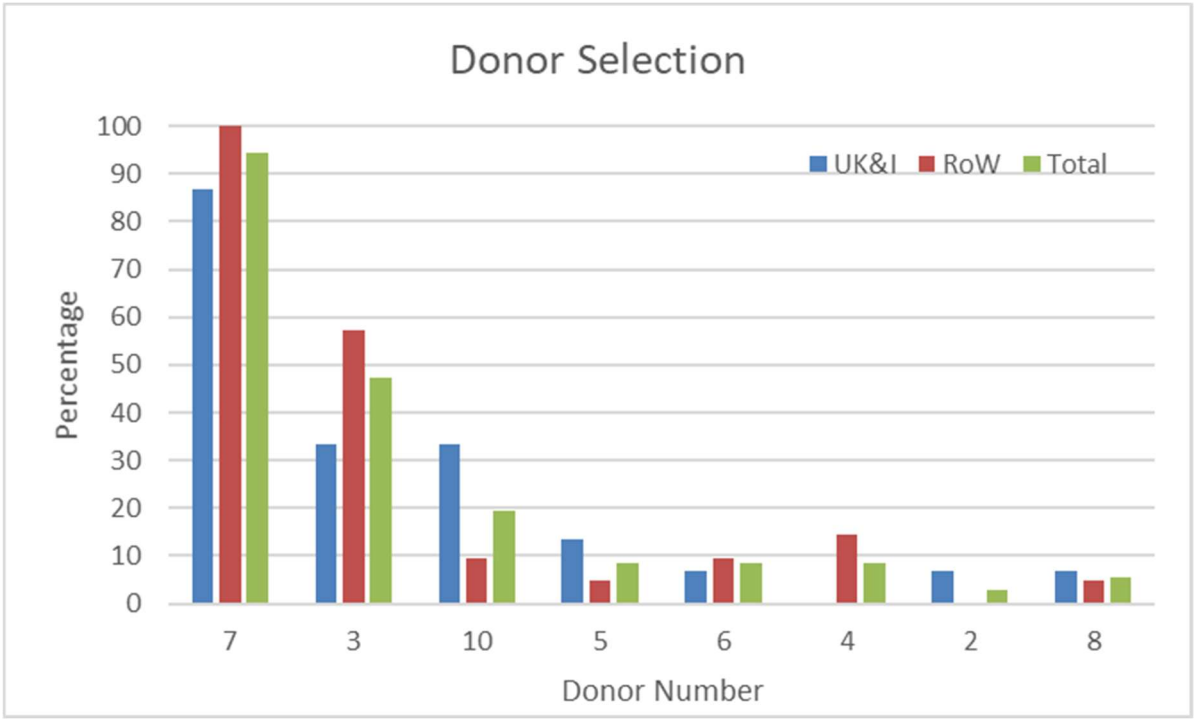
Which two of the following donations, listed in Table 4, would you preferentially select for the patient and comment on their suitability?

*Table 4 – List of Potential Platelet Donors**

Donation	HLA Type						HPA Type						ABO	Rh	CMV
	A	A	B	B	C	C	1	2	3	4	5	15			
1	1	*11*	8	*35*	*4*	7	*a*/b	a/a	a/*b*	a/a	a/a	a/b	O	Neg	Pos
2	1	*11*	8		7		b/b	a/a	a/a	a/a	a/a	a/b	A	Pos	Pos
3	1	*29*	8	*44*	7	*5*	b/b	a/a	a/a	a/a	a/a	a/b	O	Pos	Pos
4	1		8		7		*a*/b	a/a	a/a	a/a	a/a	a/b	O	Pos	Neg
5	1		*52*	*57*	*6*	*12*	b/b	a/*b*	a/*b*	a/a	a/*b*	a/b	A	Pos	Pos
6	1	*3*	8		7		b/b	a/a	a/a	a/a	a/a	a/b	O	Pos	Pos
7	1		*7*	8	7		b/b	a/a	a/*b*	a/a	a/a	a/b	O	Pos	Neg
8	1	*29*	8	*44*	7	*16*	b/b	a/a	a/a	a/a	a/a	a/b	A	Neg	Pos
9	*32*	*34*	8	*64*	7	*8*	b/b	a/a	a/*b*	a/a	a/a	a/b	A	Pos	Pos
10	1	*26*	8	*38*	7	*12*	b/b	a/a	a/*b*	a/a	a/a	a/b	O	Neg	Pos

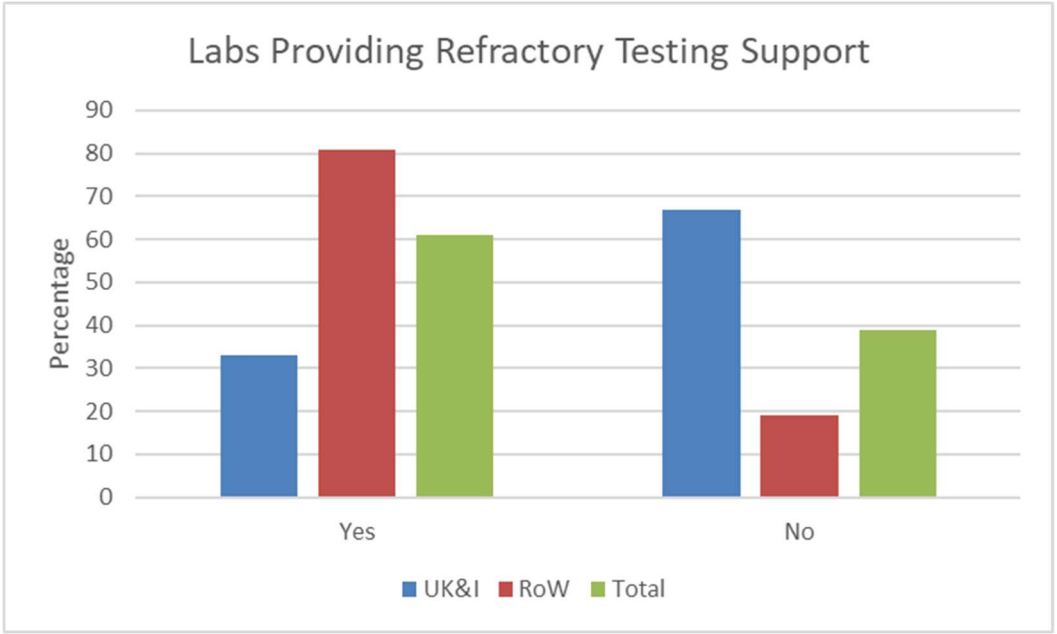
**Mismatches have been highlighted in red italic. Antigens to which the patient has antibodies have been highlighted in yellow.*

	UK&I		RoW		Total		Reason for Selection
Donor	**Number**	**%**	**Number**	**%**	**Number**	**%**	
7	13	**87%**	21	**100%**	34	**94%**	Single antigen HLA mismatch (B7 could cross-react with A2); HPA matched; ABO matched; CMV match; HPA-1a negative. No donor-directed HLA/HPA alloantibodies. Least immunogenic donor.
3	5	**33%**	12	**57%**	17	**47%**	HPA matched; ABO matched, 2 antigen mismatched (A29 low frequency and B44 high frequency but low expression).
10	5	**33%**	2	**10%**	7	**19%**	2 antigen HLA mismatches (A26 and B38 lower frequency rather other mm units); HPA matched; ABO matched; CMV mismatch (not relevant is unit is leucodepleted). Mismatch at HPA-3. No donor-directed HLA/HPA alloantibodies.
5	2	**13%**	1	**5%**	3	**8%**	2 antigen HLA mismatch. No donor-directed HLA/HPA alloantibodies. Avoids HLA-A alleles in the A10 and A19 CREG to which the patient may have cross reactive HLA antibodies
6	1	**7%**	2	**10%**	3	**8%**	HPA-1a negative, 1 antigen HLA mismatch; ABO matched; CMV mismatched
4	0	**0%**	3	**14%**	3	**8%**	ABO match, no anti-HLA-DSA found in recipient; CMV match; HPA1a so there is a chance for reaction with patients HPA 1a Ab but all the other donors who are ABO compatible are CMV positive.
2	1	**7%**	0	**0%**	1	**3%**	HPA matched.
8	1	**7%**	1	**5%**	2	**6%**	No comments received.

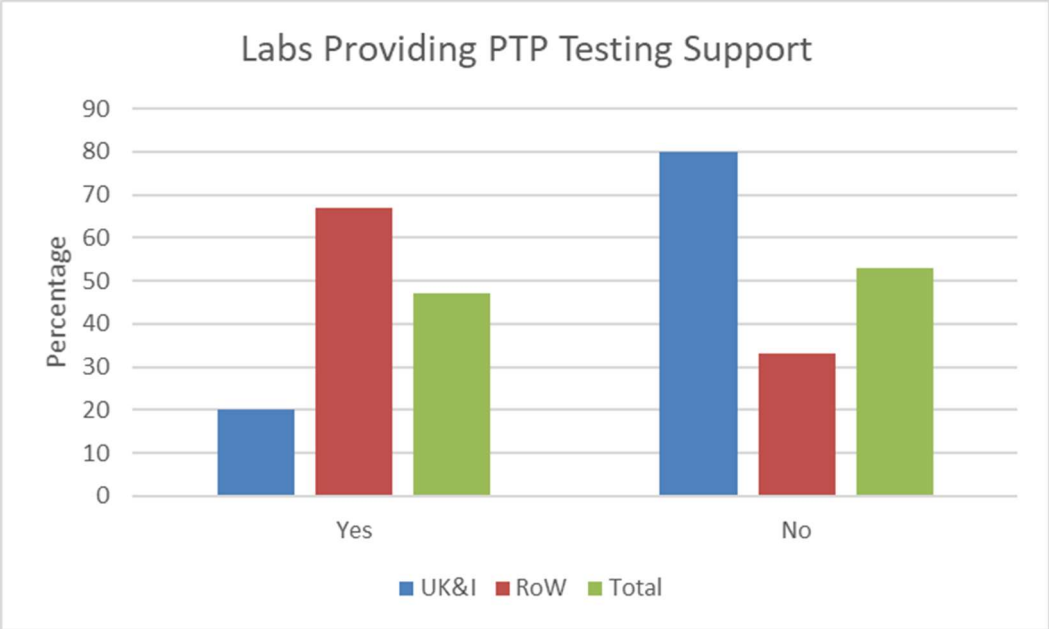


Question 5 - Do you provide a clinical testing service for Platelet Refractoriness or PTP?

	UK&I		RoW		Total	
Clinical Service for Refractoriness	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Yes	5	**33%**	17	**81%**	22	**61%**
No	10	**67%**	4	**19%**	14	**39%**



	UK&I		RoW		Total	
Clinical Service for PTP	**Number**	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Yes	3	**20%**	14	**67%**	17	**47%**
No	12	**80%**	7	**33%**	19	**53%**



Comments and suggested responses from the UK H&I experts providing this scenario*

Question 1

We would suggest an investigation for platelet transfusion refractoriness would be warranted as the patient has failed to increment when ABO compatible random pooled platelets were transfused on more than two occasions. The patient also has a history of pregnancy.

Question 2

The patient has a number of HLA specific antibodies defined. Taking these and the patient's HLA type and ABO group into consideration Donor 6 (no donor-directed antibodies, no HLA mismatches) and 15 (no donor-directed antibodies, one low frequency HLA antigen mismatch, ABO match) would be preferentially selected. CMV negative products are not indicated for this patient and have not been requested.

Question 3

The factors that support a diagnosis of PTP are the fact the patient is a female with a history of pregnancy, the patient has been transfused within the past 5-10 days and is refractory despite two HLA matched platelet transfusions.

Screening the patient for the presence of HPA antibodies and performing HPA genotyping of the patient would be recommended. The results presented from the PAK-Lx kit suggest the presence of a HPA-1a antibody, although an antibody to HPA-4b cannot be excluded. The patient's type has also been confirmed as HPA-1b1b. These results and the clinical presentation of the patient would support a diagnosis of PTP.

In terms of transfusion support for the patient, we would suggest discussing this requirement with the clinician. The BJH Guidelines (Estcourt *et al.*, 2017), recommend treatment with IVIg and random donor platelets reserved to control severe bleeding if required.

Question 4

We would suggest repeating the testing for the presence of HLA and HPA antibodies in the patient prior to receiving platelet support. It would also be prudent to provide HLA/HPA matched platelets for this patient to prevent reoccurrence of PTP. Post transfusion platelet increment data should also be monitored to ensure the effectiveness of products provided and guide the future selection of therapeutic units.

We would suggest selecting Donor 7 (single HLA antigen mismatch, HPA matched, ABO matched, no donor-directed antibodies) and Donor 10 (two low frequency HLA antigen mismatches, HPA matched, ABO matched, no donor-directed antibodies) for this patient.

Patient Update

During her second cycle of chemotherapy, the patient was admitted to the intensive care unit with respiratory difficulty, no formal diagnosis was made. At day 31 and showing signs of neutrophil recovery and a general improvement in health, the patient was extubated. Three weeks later, a bone marrow aspiration showed the patient to be in complete remission. The patient was considered for a sibling allogeneic haematopoietic stem cell transplant but sadly died due to complications of AML before this could be realised.

Suggested reading

Hawkins J, Aster RH, Curtis BR. Post-Transfusion Purpura: Current Perspectives. J Blood Med. 2019 Dec 9;10:405-415. doi: 10.2147/JBM.S189176. PMID: 31849555; PMCID: PMC6910090.

Estcourt LJ, Birchall J, Allard S, Bassey SJ, Hersey P, Kerr JP, Mumford AD, Stanworth SJ, Tinegate H; British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. Br J Haematol. 2017 Feb;176(3):365-394. doi: 10.1111/bjh.14423. Epub 2016 Dec 23. Erratum in: Br J Haematol. 2017 Apr;177(1):157. PMID: 28009056.

Please note:

These comments have been have been compiled by subject matter experts from the NEQAS Steering Committee in accordance with current guidelines. We accept that guidelines are not always explicit for every situation and therefore the responses may be aligned with the clinical practices of an individual transplant centre and may not be directly applicable across all settings. NEQAS are not necessarily endorsing these responses as the only correct action, just one possible view which, we acknowledge, may be biased towards UK practice.