14. DELAYED TRANSFUSION REACTIONS

Definition
Delayed transfusion reactions are defined in this report as those occurring more than 24 hours following a transfusion of blood or blood components. In practice, these are almost invariably delayed haemolytic reactions due to the development of red cell alloantibodies. Simple serological reactions (antibody development without a positive DAT or evidence of haemolysis) are excluded.

This category accounted for 12.9% of non-infectious hazards reported and 12.7% of all hazards.

40 new initial reports were received and 3 were carried forward from the previous year. Four additional reports were felt not to fit the definition of a DTR and were withdrawn. This chapter highlights the main findings from 39 completed questionnaires (36 from the current reporting year).

Gender (39 reports)
Males 6
Females 33

Age (39 reports)
Age range 18 - 88 years
Median age 69 years

Table 30
Timing of Reaction/Diagnosis in relation to previous transfusion

Days post-transfusion	No. of cases
1-5	7
6-10	18
11-15	7
16-20	1
>20	4
Not stated	2

Range 2- 30 days
Median 8 days

Reactions Reported

There were 6 deaths in this group of which 5 were reported to be unrelated to the transfusion reaction and one possibly related to the reaction. The outcome of one patient is not known. The remaining patients suffered minor, or no morbidity.

All reactions were related to the administration of allogeneic red cells.
In total 48 post-transfusion antibodies (excluding autoantibodies and enzyme-panagglutinins) were noted in the 39 patients who suffered DHTR. In five patients (cases 2, 5, 6, 27, 28) the causative antibodies were present, and should have been detectable by IAT technique but were not detected before transfusion. In two of these cases no pre-transfusion antibody screen was performed.

A further 2 patients (cases 7, 39) had known pre-transfusion antibodies with additional antibody specificities detectable post-transfusion.

Although the antibody specificities are reported below, this is not intended to imply that these antibodies have been proven to be the cause of the haemolytic reactions. Indeed, in some cases (e.g. anti-Chido, enzyme-only anti-E, cold-antibodies) it seems unlikely or impossible that these were implicated in the haemolysis. In some patients, multiple antibodies developed but it is likely that only one of these was implicated in the haemolysis. Autoimmune haemolysis or drug-induced haemolysis may have been implicated (but not recognised or investigated) in some of these cases.

Urgency of Transfusion Requirement

In 29 patients the transfusion was said to be routine and in 10 urgent. In one case (case 6) pre-transfusion testing had to be performed urgently during elective surgery, as this had not been requested pre-operatively. As a result a detectable pre-transfusion antibody was missed.

New Post-transfusion Antibodies

Table 31 shows the new post-transfusion antibodies (50 in 39 patients) according to antigen specificity and Table 32 gives details of these antibodies for individual patients.

Table 31
New* post-transfusion red cell antibodies in 39 patients: according to antigen specificity

Antibody group	Number	Sole antibody
Kidd		
Jka	15	12
Jkb	4	3
Duffy		
Fya	9	4
Kell		
K	1	
Rh		
Cw	1	
C	2	
D	2	1
E	9	2 (1 reacting only be enzyme)
e	1	1
Lutheran		
Lua	1	1
M,N,S,s		
M	1	
S	2	1
s	1	
Other		
Chido	1	1

* in 6 cases these were probably present pre-transfusion but not detected - either because no screen was done or because the techniques were insufficient or inadequately performed

Table 32
New* post-transfusion red cell antibodies in individual patients

ID	Antibody(ies)	Comment
1	Jk^b	
2	Jk^a	no antibody screen pre-transfusion
3	Jk^a+Fy^a	
4	Jk^a	
5	s	retrospective testing showed this was present but missed in pre-transfusion sample
6	Jk^a	present pre-transfusion but missed
7	Jk^a	Fy^a pre-transfusion
8	Jk^a	
9	Jk^a	
10	M	
11	Jk^a	
12	S	
13	Jk^a	
14	Fy^a	
15	E + enzyme-only C^w	
16	Jk^a	retrospective testing of pre-transfusion sample showed anti-Jk^a by enzyme technique, reacting with homozygous cells
17	E + Fy^a	
18	E + autoantibody	
19	E	
20	Jk^a	
21	Jk^b + Fy^a + E	
22	Jk^a	
23	E + Fy^a	
24	Jk^a	
25	Fy^a	
26	Jk^b	
27	C+ Fy^a	known anti-E pretransfusion, "flying squad" units used without pre-transfusion testing as life-threatening bleed
28	Jk^a	
29	e	
30	Jk^b	
31	D + E + Lu^a	
32	D	
33	Chido	clinical significance dubious
34	E	enzyme-only
35	Fy^a	
36	Jk^a	
37	Fy^a	
38	E + C, + S + enzyme-panagglutinin	M + unidentified antibody pre-transfusion, no cross-match done apparently
39	K	E + unidentified antibody pre-transfusion.

* in 6 cases these were probably present pre-transfusion but not detected -either because no screen was done or because the techniques were insufficient or inadequately performed

Clinical sequelae

Symptoms and signs could be divided into 4 categories as follows:

- Group 1 Asymptomatic (with positive DAT and/or spherocytes)
- Group 2 Falling haemoglobin (↓Hb)/positive DAT/spherocytes (2 of these parameters)
- Group 3 ↓Hb + jaundice ± positive DAT ± spherocytes
- Group 4 As group 3 + renal impairment

Group 1
There were 3 patients in this group. All survived without sequelae.

Group 2
There were 13 patients in this group of whom all survived without sequelae (other than fatigue in 1 case). The outcome for one patient (case 22) was not stated.

Group 3
There were 21 patients in this group of whom 15 survived without sequelae, 5 patients died from unrelated causes and 1 patient's death was "probably not related" to the transfusion reaction. One patient had ongoing jaundice at the time of the report, felt to be due to the transfusion reaction. No other sequelae were felt to be due to the DTR.

Group 4
There were 2 patients who developed renal impairment which was felt to be due to the transfusion reaction. These 2 patients did not require dialysis and recovered without ongoing sequelae.

The above results are detailed in Table 33. There is no clear relationship between the specificity of the antibody and the severity of the reaction.

Table 33
Grouping of cases by clinical sequelae of DHTR*

Group 1		Group 2		Group 3				Group 4	
ID	**Antibody**	**ID**	**Antibody**	**ID**	**Antibody**	**ID**	**Antibody**	**ID**	**Antibody**
6	Jk^a	1	Jk^b	2	Jk^a	29	c	13	Jk^a
8	Jk^a	7	Jk^a	3	$Jk^a + Fy^a$	31	$D+E+Lu^a$	39	K
23	$E+Fy^a$	9	Jk^a	4	Jk^a	32	D		
		14	Fy^a	5	s	33	Chido		
		17	$E + Fy^a$	10	M	35	Fy^a		
		18	E + auto	11	Jk^a	37	Fy^a		
		22	Jk^a	12	S	38	E+C+S		
		24	Jk^a	15	$E + C^w$(enzyme)				
		25	Fy^a	16	Jk^a				
		27	$C + Fy^a$	20	Jk^a				
		30	Jk^b	21	Jk^b+Fy^a+E				
		34	E(enzyme)	26	Jk^b				
		36	Jk^a	28	Jk^a				

*case 19 – severity not stated

There is no apparent relationship between the speed of onset of the reaction and the severity (data not shown). However, it should be born in mind that, in the absence of prospective monitoring, the timing of the onset of signs and symptoms is likely to be extremely inaccurate.

Analysis of serological information

Antibody screening and Cross-match techniques

Table 34 gives information on the serological methods used for antibody screening and cross-matching in the 39 reported cases.

Table 34
Summary of serological methods used for antibody screening and cross-match

Screening Method	No Serological Cross Match	Immediate Spin Cross-match	IAT cross-match	Not stated	Total
Tube LISS IAT			2		2
Column IAT (Diamed)		1	26	1	28
Column IAT (Ortho)	2[a]		4		6
Solid Phase		1	1		2
Not done	1[b]				1
Total	**3**	**2**	**33**	**1**	**39**

[a] 1 patient with anti-M + unidentified antibody in pre-transfusion had no serological cross-matching.
[b] 1 patient issued with blood in emergency without screen or serological cross-match.

Column technology was used for antibody screening +/- cross-matching in 87% of cases. Laboratories are still using IAT cross-matching even for pre-transfusion samples with a negative antibody screen yet this does not appear to be able to prevent the transfusion reactions seen.

Repeat testing of the pre-transfusion sample was performed in 24 cases and revealed the same results in 21. In the three cases in which a different result was obtained, different or additional techniques were used for the repeat testing (enzyme technique; Capture-R rather than LISS-tube; Capture-R in addition to column technology - but in this case the original technique also gave a different result).

In 7 cases the transfusion reaction occurred within 5 days of transfusion (cases 1, 7, 8, 26, 27, 36 and 39). In 5 of these cases the implicated antibody was anti-Kidd (2 $Jk^{b,}$ 3 Jk^{a}) and in one case a weak anti-Kell was detected post-transfusion, reacting only with homozygous cells (Case 39, see below). In one patient (Case 27) the clinical urgency precluded performance of a pre-transfusion antibody screen, which would have revealed the presence of anti-C and Fy^{a}, in addition to her known anti-E.

Details of some unusual serological cases are given below:

Case 2
Details on this case, initially reported in August 2000, are incomplete. However, this 87 year-old female was transfused with 6 units of red cells during elective surgery. She became extremely jaundiced 14 days later, with a positive DAT. It is reported that no pre-transfusion antibody screen was performed (no explanation given) but that the units were cross-matched by IAT techniques. It is not clear if this was an error or if clinical expediency prevented full testing. The post-transfusion sample at 14 days revealed an anti-Jka.

Case 5
This 77 year-old, previously transfused female presented with gastro-intestinal bleeding and required an urgent transfusion of 2 units of red cells. 4 days later she required a further 2 units and 3 days later was noted to have haemoglobinuria, anaemia and jaundice. An antibody screen by column technology had shown no pre-transfusion antibody. A post-transfusion sample, tested using Capture-R and column technology demonstrated anti-s. This was also detectable when these techniques were used to repeat testing on the pre-transfusion sample. The laboratory was using NBS cells for the column technology, with the dilution prepared in-house and it is suggested that the initial cell suspension used may not have been an optimal concentration for the technique used.

Case 6
This 71 year-old female with a previous transfusion history, underwent an elective mastectomy and reconstruction without prior pre-transfusion testing. Routine pre-transfusion testing would have been by use of Capture Ready Screen but as blood was required urgently during surgery a LISS-IAT tube technique was used for screen and cross-match. This revealed no incompatibility. The blood was issued and transfused before the presence of a weak anti-Jka was demonstrated by the routine techniques. Three of the four units transfused were Jk^{a} positive. The patient developed a positive DAT (IgG and C3) and a minor fall in Hb only.

Cases 31 and 32
In 2 cases (Cases 31 and 32), Rh D Negative female patients, aged 70 and 80, were electively transfused with RhD positive units and developed anti-D with the onset of haemolysis at 6 and 8 days, respectively. One had been

previously transfused and the second had had at least one pregnancy. Case 31 had also developed anti-E and anti-Lu[a]. Both recovered without ill-effects.

Case 33
This 77 year-old male was transfused with 5 units of red cells during and after surgery for a fractured neck of femur. He gradually became anaemic again and a further sample taken two weeks post-op was incompatible with all units tested. The only antibody detectable in the post-transfusion sample (at the International Blood Group Reference Laboratory) was anti-Chido. It was confirmed that the pre-transfusion samples had no antibody. Anti-Chido is not known to cause a haemolytic transfusion reaction - this antibody is generally considered to be of no clinical significance when selecting blood for transfusion. There was no evidence of auto-immune haemolysis but a drug-dependant antibody was not investigated in this patient. The patient has not been transfused again and made an uneventful recovery.

Case 34
This 56 year-old female was transfused with a single unit of red cells during elective surgery. She had no previous transfusion history and it is unknown if she has been pregnant in the past. Pre-transfusion serology (IAT by column techniques, IAT cross-match and DAT) were all negative. One month later it was noted that she was anaemic with a positive DAT (IgG). An antibody screen using enzyme techniques in addition to the above showed the presence of anti-E by enzyme techniques only. Two further units of red cells had been transfused before this enzyme-only antibody was detected (by chance these were E-negative). Retrospective testing of the pre-transfusion sample by enzyme techniques could not be done as it had been discarded. It seems unlikely that this antibody had caused the fall in Hb and positive DAT and this may be an incidental finding in a patient who had more significant intraoperative or post-operative blood loss than had been suspected.
A fatal transfusion reaction which was apparently due to an enzyme-only reactive anti-E was presented in last year's report.[9]

Case 38
This 67 year old female was transfused with 4 units of red cells for a gastrointestinal bleed. She had had two transfusions within the preceding month. Pre-transfusion testing of a fresh sample using column technology showed an apparent anti-M plus an unidentified antibody. M-negative blood was given but it is reported that no cross-match was performed (however, it is not clear if this was a mis-understanding of the question on the report form). Nine days later the patient was noted to be jaundiced, anaemic and dyspnoeic. Repeat investigations at this point showed the presence of anti-M, E, C, S and an enzyme-reactive antibody. It seems possible that at least some of these antibodies may have been detectable in the pre-transfusion serum.

Case 39
This 75 year-old female with myelodysplasia had been transfused on several previous occasions, the most recent 10 weeks before the reported incident. A pre-transfusion sample, drawn 5 days before transfusion was shown to contain anti-E and non-specific pan-agglutinins by enzyme and LISS techniques. She became unwell 2 days post-transfusion and was readmitted 10 days post-transfusion with anaemia, jaundice and renal insufficiency. A repeat sample revealed a weak anti-Kell reacting only with homozygous cells. The pre-transfusion sample was unavailable for repeat testing. The patient recovered well.

Cross-matching - timing

Interval between drawing cross-match sample and transfusion

Table 35
The interval between cross-matching and sampling is shown below for 30 reports

Interval between cross-matching and sampling (hrs)	No. of cases
0-47	31
48-71	2
72-96	
>96	4[a]
Not known	1
Not done	1

[a] including one sample sent 53 days before transfusion (pre-admission clinic) and stored frozen - no recent transfusion history

In all cases in which sufficient details were provided, the timing of pre-transfusion samples were in keeping with the national guidelines[37] It was not always possible to ascertain from the questionnaire the timing of an earlier transfusion and the implicated transfusion.

Table 36
Recommended timing of pre-transfusion sampling in relation to most recent transfusion[37]

Timing of previous transfusion	Samples to be taken
3-14 days	max 24hr pre-transfusion
14-28 days	max 72hrs pre-transfusion
28 days - 3 months	max 1 week pre-transfusion

Reporting to Blood Centres and Hospital Transfusion Committees

19 (49%) of cases were reported to the local Blood Centre and 33 (85%) were reported to the HTC. The involvement of the HTC has again increased compared to previous years and presumably reflects the more widespread availability of these committees and clarification of their role.

COMMENTARY

As in previous years, Kidd antibodies feature prominently as a cause of DHTRs in 19 of the 39 patients (49%) and 19/48 (40%) of the "new" post-transfusion antibodies.

In 5 cases it is likely that the antibodies could have been detected pre-transfusion but were missed. In 1 case (case 27) a life-threatening bleed precluded prior testing. In four cases (Cases 2, 5, 6, 38) it appears that the routine screening techniques should have revealed the antibody(ies) but may have been inadequately performed or were omitted. In one of these cases (Case 38) multiple antibodies were present but only one was fully identified before transfusion. Use of enzyme-techniques revealed a very weak antibody in 1 case (Case 16) but this technique is not now commonly used. An additional enzyme-only reactive anti-E (Case 34) seems an unlikely cause of the anaemia and positive DAT in this patient.

In general there is little evidence of inadequate performance of the laboratory techniques but available technology appears to be ineffective in detecting the risk of haemolytic transfusion reactions due to anti-Kidd.

In one case, emergency techniques had to be used because the patient had had no pre-transfusion testing before elective surgery. The techniques selected, or the inadequate performance of them, missed the presence of a clinically significant antibody (anti-s).

As noted in Mollison's text-book "Blood Transfusion in Clinical Medicine"[38], the development of DHTRs in patients with a pre-existing alloantibody which has not been detected in pre-transfusion tests is a recognised phenomenon. In addition, a patient with a relatively low-titre antibody may have an immediate haemolytic reaction which is mild, and therefore not detected, followed by a DHTR as the antibody re-appears in the circulation. The transient disappearance of the implicated antibody is common and may account for some of the failures to detect a clinically-relevant antibody in post-transfusion testing in some of these cases.

RECOMMENDATIONS

- **Attention to timely pre-transfusion testing of surgical patients is essential, especially if there is a history of previous transfusion or pregnancy. Where possible, investigations should be performed within normal working hours in order to make best use of available expertise. Laboratory staff should be given adequate notice of impending surgery and the potential role of pre-admission clinics in facilitating timely pre-transfusion testing should be assessed in each hospital.**

- **In the SHOT report from 1999-2000[9] a need for improved technologies to identify extremely weak Kidd antibodies was identified and this need persists.**

- **Hospital laboratories must take care to avoid missing antibodies which may be masked by another specific antibody or by broadly-reacting non-specific antibodies. Deficiencies in this area were highlighted in a recent "paper" exercise run by the National External Quality Assurance Scheme for Blood Transfusion Laboratory Practice (see NEQAS-BTLP exercise**

00E6).[10] There is a great deal of useful material in this exercise which should be shared with all the BMSs working in transfusion laboratories. Nevertheless, it appears that this problem is a minor contributor to the occurrence of DTRs reported to SHOT.

- **Although 2 patients developed haemolysis due to the development of anti-D post-transfusion, there were no long term sequelae. There is no indication to alter the current policy of administering RhD positive units to RhD negative patients without detectable anti-D, when RhD negative units are in short supply, unless these patients have child-bearing potential.**

- **Laboratories must be aware of the guidelines on pre-transfusion testing and ensure that these are followed by laboratory staff both within normal working hours and in the "out-of-hours" setting.[37]**

- **If a clinically significant red cell antibody is found in a recipient, it is essential that a cross-match is performed, even if phenotyped units are supplied.**