Variables determining blockage of WBC-depleting filters by Hb sickle cell trait donations

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BACKGROUND: Sickle cell trait donations can block leukodepletion (LD) filters or fail to LD, but the variables affecting blockage are unclear.

STUDY DESIGN AND METHODS: To identify critical variables for further study, the relationship was investigated between filter blockage and donor characteristics, processing conditions, PLT and coagulation system activation, and microvesicle formation in donations with (n = 63) and without (n = 40) sickle trait. With eight filter types whole blood was LD either at ambient temperature on Day 0 or after overnight 4°C hold. Markers of PLT activation (CD62P and CD63 expression and soluble CD62P) and coagulation activation (activated FXII and prothrombin fragment 1 + 2 [F1 + 2]), RBC microvesicles, blood gases, and residual WBCs were measured.

RESULTS: All Day 0 filtrations blocked (n = 7). On Day 1, no filter tested was 100 percent successful, with most achieving an approximate 50 percent success rate. Two filters blocked consistently and an additional filter did not block, but resulted in 50 percent of units with high residual WBC counts (30×10^6-394×10^6/unit). Day 1 filtration was not improved if performed at 4°C. Donor RBC variables and prefiltration measures varied little between blocked and successful filtrations except pO_2, where 9 of 17 blockages had a pO_2 of less than 5.0 kPa, compared with 0 of 13 completed filtrations. F1 + 2 levels increased after filtration in sickle trait units, a consequence of slow flow rate.

CONCLUSION: Filter blockage in sickle trait donors cannot be predicted by donor characteristics or filter type and is not related to PLT or coagulation activation, but can be reduced by storing units at 4°C before filtration.

In 1999, the UK Transfusion Services implemented universal leukodepletion (LD) of the blood supply as a precautionary measure against an unknown risk of transmission of variant CJD. LD is usually achieved by filtration of whole blood or blood components with most filters providing 4 log reduction in WBCs.[1] A number of small studies have suggested that LD of whole blood or RBCs from donors with sickle cell trait may be problematic.[2-7] A high proportion of these donations block filters and some may result in final blood components that do not meet the UK specification for a residual WBC count of less than 5×10^6 cells per unit.[8] The magnitude of the problem facing blood transfusion services is dependent on the prevalence of sickle cell trait in the donor population. In the southeast of England, approximately 0.1 percent of donations are from donors with sickle cell trait, but in some areas of the US the proportion can be as high as 1 percent and in Martinique 7 percent.[5] It is highly desirable to retain donors with sickle cell trait because these donations provide useful phenotypes for Afro-Caribbean patient populations and because there is a drive in the UK to encourage donations from ethnic minorities.

Sickle cell Hb (HbS) is a variant of adult Hb (HbA) resulting from the substitution of valine for glutamic acid at β6, leading to the polymerization and gelation of Hb during deoxygenation.[9-11] The formation of Hb polymers causes a significant reduction in RBC deformability and sometimes a distortion of cell morphology (sickling). In the homozygous condition, sickle cell disease (HbSS), the individual has a low Hb level and requires regular treatment by blood transfusion or antisickling agents to reduce

ABBREVIATIONS: CO = carbon monoxide; F1 + 2 = fragment 1 + 2; HbA = adult Hb; HbS = sickle cell Hb; LD = leukodepletion(-ed).

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the frequency of sickling crises. In the heterozygote sickle cell trait (HbAS), 25 to 45 percent of Hb in each RBC is HbS, and the cells are morphologically normal but less deformable. This does not usually result in any clinical consequences, but sickling can occur under certain extreme circumstances such as during acute infections or under low oxygen tension.[10,12]

There is increasing evidence that filter blockage in units from sickle cell trait donors is a consequence of gelation of HbS.[2,4,5,11,13,14] This is consistent with the observation that treatment of HbAS cells with carbon monoxide (CO), which prevents HbS polymerization, results in complete filtration of units,[14] but this approach cannot be applied to units intended for transfusion since CO binds irreversibly to Hb. Filter blockage might also be mediated by activation of coagulation or PLTs or by RBC microvesicle formation[15] consequent to sickling, resulting in clot formation in the filter. Sickle cell disease is known to be associated with increased coagulation[16-18] and PLT activation,[16,19] resulting in a prothrombotic state. There are limited data on coagulation activity in individuals with sickle cell trait, but enhanced activation of coagulation has been reported, as detected by raised thrombin-antithrombin (TAT) complexes, fragment 1 + 2 (F1 + 2) and D-dimers.[20]

Previous studies have not addressed systematically whether varying either the type of filter used for LD or the processing conditions can improve the results with sickle trait donations. The aim of this study was to investigate the performance of a variety of whole-blood filters with different filtration conditions to determine from these preliminary investigations whether there were any donor characteristics or processing variables that might merit large-scale investigation.

MATERIALS AND METHODS

Donor selection

Sickle trait donors eligible to donate according to UK guidelines[8] were identified from a database of Afro-Caribbean donors who had been tested in-house with a sickle screening assay and confirmed by external testing as having sickle cell trait by HPLC. Donors able to attend for study were recruited with informed written consent and according to National Blood Service procedures at the time of the study. These procedures permitted donations to be approved for in-house research without referral to an external ethics committee. On the day of donation, donors had to pass the Hb screening test to ensure that the donation was representative of clinical transfusions. No additional screening criteria for the study were applied.

Blood collection and processing

Whole blood (450 ± 45 mL) was collected into CPD (63 mL) and filtered either at ambient temperature (20-25°C) within 4 hours of collection (Day 0) or at ambient temperature or 4°C on Day 1 following an overnight hold at 4°C. Filtration in a 4°C environment was established for this study and is not routine UK practice, although Day 1 processing after 4°C overnight hold is permitted. Sickle cell trait units and controls were LD with one of several whole-blood filters (Asahi RS2000, R7455, Baxter Healthcare, Newbury, UK; Asahi RZ2000, R7490, Baxter; WBF2, Pall Europe Ltd, Portsmouth, UK; Pall WBF3, Macopharma LST1 Macopharma, Middlesex, UK; Macopharma LST2, NPBI T2926, NPBI, Oxford, UK; or WBSP, Terumo, Merseyside, UK).

The choice of filter used for each donation was initially made to generate six donations with each filter. Nevertheless, this had to be modified to take account of 1) the manufacturer producing a newer version of the same filter which superceded the original one (this occurred with WBF2 [replaced by WBF3, Pall], RS2000 [replaced by RZ2000, Baxter], LST1 [replaced by LST2, Macopharma]); 2) filters showing 100 percent blockage (LST2) where use of more donations to confirm failed results could have been considered unethical; and 3) filters whose performance seemed good (WBF2), where it was desirable to increase donation numbers to gain more information on reproducibility. Some donors donated on more than one occasion, in which case the filter type was selected to differ from that used with their previous donation.

The filtration was recorded as either successful or blocked, where blocked was defined as blood left in the prefiltration pack 2 hours after commencing filtration and successful was defined as filtration of the entire unit within 2 hours. The time taken to complete filtration (commencement to cessation of flow through the filter) or for the filter to block was recorded. For complete filtrations, the filtration rate was calculated by dividing the volume after filtration by the time taken for filtration to be completed. The postfiltration volume was calculated with the net weight of the unit and a specific gravity of 1.06. For blocked filtrations the postfiltration volume was recorded. A 20-mL sample was taken from whole blood before and after filtration with a sample pouch (R4R2004, Baxter) by sterile connection to the pack (TSCD, Terumo). An 8-mL sample was retained for immediate analysis and 12 mL was centrifuged at 3000 × *g* for 30 minutes to obtain plasma aliquots, which were stored at –80°C for later analysis.

Laboratory analysis

Residual WBCs and reticulocytes were measured with reagent kits (LeucoCOUNT, Becton Dickinson, Oxon, UK; and Coulter ReticONE, Beckman-Coulter, Bucks, UK, respectively) on a flow cytometer (Coulter Epics XL MCL, Beckman-Coulter) according to the manufacturers' instructions. Full blood count measurements were

obtained with a hematology analyzer (Model SE9000, Sysmex UK, Milton Keynes, UK). RBC microvesicles were measured by flow cytometry as previously described,[21] with antibodies to glycophorin A. Cell surface CD62P and CD63 expression was measured by flow cytometry in prefiltration samples only, owing to the low PLT count after filtration. The PLT population was defined by forward versus side scatter characteristics, and fluorescein-conjugated anti-CD42a (Immunotech, Beckman-Coulter) was used to ensure that this population contained greater than 95 percent CD42a-positive events. In separate tubes, fluorescein-conjugated anti-CD62P or PE-conjugated anti-CD63 (Immunotech, Beckman-Coulter) was used to quantify the percentage of PLT events expressing CD62P. Isotype- and fluorochrome-matched controls were used to correct for nonspecific binding, and assays were completed within 1 hour of sampling. pO_2 and pCO_2 were determined with a blood gas analyzer (Model ABL5, Radiometer, Crawley, UK) immediately after samples were taken. ELISAs were performed with commercially available kits: soluble P-Selectin (R & D Systems, Oxon, UK), prothrombin F1 + 2 (Dade-Behring, Marburg, Germany), and activated FXII (Axis-Shield, Dundee, UK). Fetal (%HbF) and sickle Hb (%HbS) levels were determined by HPLC.

Statistical analysis

Measures in whole blood before filtration in sickle trait and control units, measures in donor samples from blocked and completed filtrations, and decreases in Hb levels following filtration in blocked and completed filtrations were compared with the Mann-Whitney U test. Comparison of measures between whole blood before and after filtration was made with the Wilcoxon signed rank test. Where statistical comparison was made, the difference between medians (with 95% CI) was also calculated. All results are given as median (range).

RESULTS

Filtration conditions and filter type

In total 63 sickle cell trait donations were obtained from 38 donors and compared to 40 control donations. All control units filtered successfully with a residual WBC count of less than 5×10^6 per unit (median, <1 cell per µL). On Day 0, at ambient temperature, LD of sickle cell trait units was consistently unsuccessful with seven of seven filters blocking irrespective of the filter type used (Table 1). Four of these 7 units also failed to LD, with residual WBC in the range 118 to 1540 cells per µL. For filtration at ambient temperature on Day 1, following a 4°C overnight hold, the blockage rate was approximately 50 percent and was similar in most of the whole-blood filters investigated (Table 1). Nevertheless, two of the filters (Terumo WBSP and Macopharma LST2) had a 100 percent blockage rate. The modification of two filters by the manufacturer by adding more filter media to improve LD performance (Macopharma LST1 to LST2 and Pall WBF2 to WBF3) appeared to increase the proportion of units that blocked (Table 1).

For the majority of filters, at least 1 unit where filtration was not complete failed to meet the UK specification of less than 5×10^6 residual WBCs per unit. One filter type did not block but resulted in 3 of 6 filtered units that failed to meet the LD specification (Macopharma LST1, Table 1). All other successful filtrations were within the UK LD specification. Following an overnight hold at 4°C, filtration in a 4°C cold room did not appear to improve the filter performance of the

TABLE 1. Summary of filtration outcome in donations with sickle cell trait

Filtration conditions and filter type	Number of units assessed	Complete WBC filtration*		Blocked WBC filtration*		Number of residual† WBCs in final component (cells/µL)	Number of residual† WBCs in final component ($\times 10^6$/unit)
		<5×10^6/unit	>5×10^6/unit	<5×10^6/unit	>5×10^6/unit		
Day 0 filtration ambient temperature							
Pall WBF2	3	0	0	2 (67)	1 (33)	11 (2-118)	0.79 (0.08-28.0)
Macopharma LST1	3	0	0	0	3 (100)	456 (178-1540)	121 (30.8-541)
Terumo WBSP	1	0	0	1	0	0	0
Day 1 filtration at ambient temperature after overnight hold (4°C)							
Pall WBF2	9	7 (78)	0	2 (22)	0	1 (0-9)	0.46 (0-4.12)
Pall WBF3	7	3 (43)	0	2 (29)	2 (29)	1 (0-369)	0.27 (0-108)
Macopharma LST1	6	3 (50)	3 (50)	0	0	35 (1-907)	15.7 (0.45-394)
Macopharma LST2	5	0	0	0	5 (100)	225 (42-570)	76.8 (11.5-195)
Baxter RS2000	5	2 (40)	0	2 (40)	1 (20)	1 (0-70)	0.32 (0-14.6)
Baxter RZ2000	7	4 (57)	0	1 (14)	2 (29)	0 (0-127)	0 (0-15.3)
Terumo WBSP	6	0	0	6 (100)	0	1 (0-11)	0.20 (0-2.90)
NPBI T2926	7	2 (29)	0	5 (71)	0	1 (0-4)	0.19 (0-1.16)
Day 1 filtration at 4°C after overnight hold (4°C)							
NPBI T2926	5	1 (20)	0	3 (60)	1 (20)	1 (0-61)	0.20 (0.02-21.6)

* Data are reported as number (%).
† Residual WBCs represented by median (range).

NPBI T2926 filter compared to filtration at ambient temperature.

Seventeen donors gave more than one donation and these were all filtered on Day 1. Of these 5 of 17 blocked two or more filter types with none of their donations filtering completely. An additional 5 donors had successful filtrations with 2 or more filter types and no blockages, but in 2 of these the units failed to meet the residual WBC specification. Seven of 17 donors had a mixture of both blocked and complete filtrations, the complete filtrations being successfully LD in 6 of 7 donors. Thus only 3 of 17 donors (18%) had two or more consecutive donations that met UK specifications.

Donor-related variables

Six donors (five women) were unable to give repeat donations owing to a Hb level below the UK specification of 120 g per L (women) and 130 g per L (men).[8] An additional three female donors who consented to the study were unable to donate at all owing to low Hb levels. RBC parameters measured in sickle trait donors (RBC count, mean cell volume, total Hb, percent reticulocytes, percent HbS, and percent HbF) were not significantly different between donations that blocked LD filters and those that filtered successfully, except for MCV, which was significantly higher in those which blocked (Table 2).

Donation-related variables

Levels of Hb before filtration were significantly lower in whole-blood samples from sickle cell trait donors compared to controls ($p = 0.002$), but not for RBC microvesicles, pO_2, or pCO_2 (Table 3). Filtration outcome could not be predicted in HbAS units by prefiltration Hb, RBC microvesicle concentration, or pCO_2. Nevertheless, of sickle trait donations that blocked LD filters, 9 of 17 (52.9%) donations had a pO_2 level before filtration of less than 5.0 kPa, in contrast to 0 to 13 sickle trait units that completed filtration. In all groups, including controls, pO_2 levels increased following filtration, but to a greater extent in sickle units than controls (Table 3). Only in sickle units that blocked was there a decrease in pCO_2. In sickle units, there was a slight decrease in Hb concentration following successful filtration (median, 1 g/L), which was greater in those that blocked (median, 14 g/L; $p = 0.0003$), presumably owing to RBC trapping in the filter. There was no significant change in the levels of RBC microvesicles following filtration with the exception of sickle trait units that blocked (WBCs $< 5 \times 10^6$/unit) where levels were lower after filtration (Table 3).

Prefiltration levels of soluble CD62P as well as CD62P and CD63 expression did not differ between control units and sickle cell trait units that blocked or completed filtration (Table 4). In all groups, including controls, soluble CD62P levels were significantly lower and levels of activated FXII significantly higher after filtration (Table 4). Nevertheless, the degree of change induced by filtration was not significantly different between control and sickle units. In contrast, levels of prothrombin F1 + 2 only increased following filtration in sickle cell trait units that blocked filters or failed to LD (Table 4). To establish whether this was a primary cause of blockages, or secondary to the slower flow rate, the flow rate across the filter in 5 HbAA units was deliberately slowed from 20 mL/min to 1.5 to 5.0 mL/min by partial occlusion of the downstream filter tubing. In these occluded units, we also observed an increase in F1 + 2 levels following filtration that was not observed in nonoccluded HbAA units.

Interestingly, sickle trait units LD with the Terumo WBSP filter (the only filter used in this study that permits PLTs to pass through) appeared to contain lower levels of residual PLTs compared to controls [42 (35–68) $\times 10^9$/L vs.

TABLE 2. RBC and Hb measures in samples from donors with sickle cell trait categorized by filtration outcome*

	WBC filtration $< 5 \times 10^6$/unit			WBC filtration $> 5 \times 10^6$/unit	
Measure	Complete	Blocked	Difference between medians of complete vs. blocked	Complete	Blocked
% HbS	34.3 (29.6 to 37.7) n = 14 units, 12 donors	36.5 (31.2 to 38.4) n = 15 units, 13 donors	–1.60 (–4.80 to 0.40)	36.7 (34.6 to 37.3) n = 3 units, 3 donors	33.9 (29.8 to 33.8) n = 8 units, 6 donors
% HbF	0.8 (0.6 to 1.7) n = 13 units, 11 donors	0.8 (0.3 to 2.2) n = 15 units, 13 donors	0.1 (–0.5 to 0.4)	1.6 (0.5 to 2.6) n = 2 units, 2 donors	1.0 (0.4 to 2.1) n = 8 units, 6 donors
Hb level (g/L)	125 (100 to 141) n = 12 units, 10 donors	124 (106 to 154) n = 14 units, 12 donors	–3.0 (–13.0 to 7.0)	122 (102 to 129) n = 3 units, 3 donors	127 (117 to 138) n = 7 units, 6 donors
RBC count ($\times 10^{12}$/L)	4.45 (3.91 to 5.14) n = 12 units, 10 donors	4.43 (3.58 to 5.25) n = 14 units, 12 donors	0.18 (–0.21 to 0.54)	4.33 (3.58 to 4.44) n = 3 units, 3 donors	4.72 (4.21 to 4.87) n = 7 units, 6 donors
% Reticulocytes	1.70 (0.84 to 2.10) n = 13 units, 11 donors	1.66 (0.78 to 2.80) n = 13 units, 11 donors	0.04 (–0.47 to 0.57)	2.40 (2.30 to 2.50) n = 2 units, 2 donors	1.70 (0.90 to 2.20) n = 8 units, 6 donors
MCV (fL)	83.9 (74.1 to 89.0) n = 12 units, 10 donors	87.3 (78.2 to 90.3) n = 14 units, 12 donors	–3.25 (–8.60 to –0.20)	87.2 (80.4 to 91.2) n = 3 units, 3 donors	81.2 (78.5 to 88.1) n = 7 units, 6 donors

* Results are represented by median (range) and difference between medians (95% CI). There was no significant difference in donor samples between filtrations that completed or blocked (WBCs $< 5 \times 10^6$/unit) for all measures except MCV ($p = 0.041$).

TABLE 3. RBC and blood gas analysis before and after filtration in donations with sickle cell trait and control units*

Measure	All units	WBC filtration < 5×10^8/unit		WBC filtration > 5×10^8/unit	
		Complete	Blocked	Complete	Blocked
Hb level (g/L)					
Sickle trait					
Before filtration	113 (89 to 144) n = 49	112 (89 to 144) n = 21	119 (101 to 138) n = 17	105 (103 to 116) n = 3	115 (106 to 127) n = 8
After filtration	105 (32 to 138) n = 48[d]	108 (73 to 129) n = 21[a]	99 (32 to 138) n = 16[d]	100 (98 to 110) n = 3	105 (95 to 123) n = 8[a]
Difference between medians	8.0 (5.0 to 11.5)	1.5 (0.5 to 8.0)	16.0 (10.5 to 26.0)[e]		9.8 (2.5 to 15.0)
Controls					
Before filtration	122 (94 to 153) n = 40				
After filtration	121 (104 to 142) n = 40[d]				
Difference between medians	1.0 (0.5 to 1.5)				
RBC microvesicles					
Sickle trait					
Before filtration	804 (259 to 3335) n = 27	662 (538 to 1381) n = 11	804 (351 to 3335) n = 9	1148 n = 1	1003 (259 to 1216) n = 6
After filtration	556 (191 to 2259) n = 27	642 (371 to 2259) n = 11	352 (191 to 686) n = 9[c]	748 n = 1	1076 (240 to 2081) n = 6
Difference between medians	129 (–145 to 349)	–59 (–682 to 298)	488 (206 to 1707)		–302 (–978 to 88)
Controls					
Before filtration	834 (240 to 1781) n = 28				
After filtration	508 (249 to 3860) n = 28				
Difference between medians	98 (–19 to 233)				
pO_2 (kPa)					
Sickle trait					
Before filtration	5.5 (3.3 to 8.1) n = 30	5.8 (5.0 to 7.2) n = 11	5.0 (3.3 to 8.1) n = 10	5.2 (5.0 to 5.4) n = 2	4.7 (3.4 to 6.0) n = 7
After filtration	6.7 (4.0 to 14.7) n = 27[d]	6.2 (5.5 to 9.0) n = 11[b]	9.4 (4.0 to 14.7) n = 7[a]	7.0 (5.9 to 8.1) n = 2	6.7 (6.1 to 9.8) n = 7[a]
Difference between medians	–1.6 (–2.6 to –0.9)	–0.5 (–1.0 to –0.2)	–3.1 (–5.8 to –0.4)		–2.3 (–3.3 to –1.6)
Controls					
Before filtration	5.1 (3.6 to 9.1) n = 30				
After filtration	5.2 (3.8 to 15.5) n = 30[d]				
Difference between medians	–0.4 (–0.6 to –0.3)				
pCO_2 (kPa)					
Sickle trait					
Before filtration	12.2 (10.6 to 14.3) n = 30	11.6 (10.6 to 13.5) n = 11	13.2 (11.1 to 14.3) n = 10	12.4 (11.6 to 13.2) n = 2	12.9 (11.4 to 14.1) n = 7
After filtration	11.8 (9.8 to 14.6) n = 27	11.7 (10.7 to 13.2) n = 11	11.4 (9.8 to 12.5) n = 7[a]	12.4 (11.6 to 13.1) n = 2	12.5 (9.8 to 14.6) n = 7
Difference between medians	0.4 (–0.1 to 0.9)	–0.1 (–0.3 to 0.3)	1.5 (0.2 to 2.9)		0.1 (–0.4 to 1.0)
Controls					
Before filtration	12.5 (10.3 to 14.9) n = 30				
After filtration	12.6 (10.3 to 15.2) n = 30				
Difference between medians	–0.1 (–0.3 to 0)				

* Results represented by median (range) and difference between medians (95% CI). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.005$, and [d]$p < 0.001$ before versus after filtration; [e]$p < 0.005$ blocked versus complete. There is a significant difference between sickle trait and control units before filtration for Hb ($p = 0.002$), difference between medians –9.0 (–14.0 to –3.0); but not for RBC microvesicles, 16 (–230 to 235); pO_2, 0.2 (–0.4 to 0.8); or pCO_2, 0 (–0.6 to 0.6). The change induced by filtration was significantly different between controls and all sickle units for Hb, pO_2, and pCO_2 ($p < 0.01$) but not RBC microvesicles. Statistical analysis of data on less than 6 units was not performed.

159 (78–241) $\times 10^9$/L)]. Nevertheless, this filter consistently blocked, with only 21 to 345 mL in the final component.

DISCUSSION

This study was designed to identify donor or processing variables that may impact on outcome when filtering sickle trait donations. The LD failure rate, filter blockage, and high residual WBC counts observed here have confirmed the findings of previous investigations[2-7] that filtration of units from sickle cell trait donors is problematic. For whole-blood LD on Day 0 at ambient temperature, all the filtrations blocked, whereas filtration after a 4°C overnight hold (Day 1) resulted in approximately 50 percent of units passing through the filter. Lowering the filtration temperature on Day 1 from ambient temperature to 4°C

TABLE 4. Levels of PLT and coagulation activation markers before and after filtration*

Measure	All units	WBC filtration < 5×10^6/unit		WBC filtration > 5×10^6/unit	
		Complete	Blocked	Complete	Blocked
Cell surface CD62P (%)					
Sickle trait before filtration	25.8 (7.8 to 47.8) n = 20	24.6 (16.2 to 40.5) n = 7	27.5 (19.7 to 41.6) n = 6	7.8 n = 1	25.7 (20.3 to 47.8) n = 6
Controls before filtration	27.7 (14.2 to 49.0) n = 20				
Difference between medians	−0.7 (−8.5 to 5.5)				
Soluble CD62P (ng/mL)					
Sickle trait					
Before filtration	44.1 (25.3 to 88.3) n = 38	44.1 (26.0 to 88.3) n = 14	45.2 (25.3 to 62.1) n = 13	42.3 (31.2 to 49.4) n = 3	43.7 (28.2 to 77.1) n = 8
After filtration	36.9 (17.6 to 89.4) n = 38[c]	35.7 (23.8 to 65.0) n = 14[a]	33.9 (17.6 to 62.1) n = 13[a]	49.6 (36.5 to 55.5) n = 3	40.6 (29.2 to 89.4) n = 8
Difference between medians	5.7 (1.6 to 9.2)	10.1 (5.4 to 15.7)	7.1 (0.9 to 12.2)		−2.3 (−12.9 to 9.4)
Controls					
Before filtration	48.8 (26.1 to 144.8) n = 38				
After filtration	38.9 (16.8 to 129.8) n = 38[a]				
Difference between medians	8.6 (6.1 to 11.0)		9.3 (3.7 to 16.2) n = 6	5.7 n = 1	9.7 (3.9 to 10.2) n = 6
Cell surface CD63 (%)					
Sickle trait before filtration	8.2 (3.7 to 16.2) n = 20	7.2 (4.8 to 13.0) n = 7			
Controls before filtration	6.1 (2.7 to 12.3) n = 20				
Difference between medians	1.4 (−0.4 to 3.7)				
FXIIa (ng/mL)					
Sickle trait					
Before filtration	1.16 (0.32 to 2.36) n = 38	1.17 (0.66 to 2.36) n = 14	1.07 (0.63 to 1.63) n = 13	1.40 (0.92 to 1.52) n = 3	1.20 (0.32 to 1.45) n = 8
After filtration	1.45 (0.51 to 2.75) n = 38[a]	1.41 (0.52 to 2.75) n = 14[c]	1.56 (0.69 to 2.75) n = 13[b]	1.47 (1.19 to 2.08) n = 3	1.52 (0.51 to 2.72) n = 8[b]
Difference between medians	−0.35 (−0.51 to −0.22)	−0.27 (−0.50 to −0.13)	−0.42 (−0.74 to −0.09)		−0.44 (−0.95 to −0.18)
Controls					
Before filtration	1.27 (0.44 to 2.42) n = 38				
After filtration	1.48 (0.36 to 2.82) n = 38[a]				
Difference between medians	−0.30 (−0.41 to −0.19)				
F1 + 2 (nmol/L)					
Sickle trait					
Before filtration	0.61 (0.23 to 1.04) n = 38	0.66 (0.23 to 1.04) n = 14	0.60 (0.46 to 1.01) n = 13	0.70 (0.50 to 0.80) n = 3	0.54 (0.42 to 0.85) n = 8
After filtration	0.72 (0.36 to 1.58) n = 38[c]	0.66 (0.38 to 0.99) n = 14	0.71 (0.51 to 1.58) n = 13[b]	0.83 (0.73 to 1.40) n = 3	0.73 (0.36 to 1.54) n = 8
Difference between medians	−0.10 (−0.22 to −0.04)	−0.02 (−0.09 to 0.06)	−0.12 (−0.46 to −0.03)		−0.26 (−0.63 to 0.03)
Controls					
Before filtration	0.65 (0.33 to 1.04) n = 38				
After filtration	0.65 (0.35 to 1.15) n = 38				
Difference between medians	0 (−0.3 to 0.3)				

* Results represented by median (range) and difference between medians (95% CI). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.005$, and [d]$p < 0.001$ before versus after filtration. There was no significant difference between sickle trait and control units before filtration for all measures (difference between medians): soluble CD62P −4.8 (−11.4 to 1.3); FXIIa, −0.08 (−0.30 to 0.15); and F1 + 2, −0.02 (−0.09 to 0.06). The change induced by filtration was significantly different between control and sickle units for F1 + 2 ($p < 0.005$) but not for FXIIa or sCD62P. Statistical analysis of data on less than 6 units was not performed.

did not appear to improve the LD success rate. Our findings suggest that the minimum core temperature that packs of RBCs reach at any time before filtration may be an important factor in determining blockage rate of sickle cell trait units. The solubility of deoxygenated HbS at pH 7.2 decreases as the temperature increases between 10 and 25°C and then remains constant up to 40°C.[9] Increased HbS gelation at higher temperatures is therefore not unexpected.

Although the number of units investigated with each filter type is small, we also observed that the filter type has some influence on blockage rate. None of the eight whole-blood filters investigated, used on Day 1 following a 4°C overnight hold, had a 100 percent LD success rate. Two filters produced 100 percent blockage, but of more concern, one filter type never blocked but resulted in 50 percent of final components failing to meet UK specification for residual WBC counts. In addition, several other filter types occasionally produced units with a high residual WBC content. In units that blocked it is difficult to say whether the WBC content of the partially filtered unit would be representative of the WBC content had the entire

unit filtered. Nevertheless, with one type of RBC filter, it has been demonstrated that WBC removal is not consistent during filtration, with a gradual decrease in removal efficiency throughout the filtration period.[22] Whether the same relationship holds with whole-blood filters used in this study or with sickle trait units is not known, but the previous findings suggest that a high WBC concentration in a partially filtered unit is likely only to worsen if the unit had filtered completely. These data indicate the importance of testing 100 percent of sickle trait donations for residual WBCs.

The mechanism by which large numbers of WBCs pass through the LD filter membranes in apparently nonblocked sickle cell trait units is not understood. It has been suggested that partial filter blockage causes blood channeling, reducing contact with filter membranes and reducing the LD efficiency,[4] and it may be that minor undetectable blockage of filter channels occurs more frequently in HbAS donations. Consistent with this is the tendency toward increased levels of F1 + 2 in units that complete filtration but that contain greater than 5×10^6 WBCs. During this study two manufacturers updated their filter design by adding more filter medium to improve LD performance; this appeared to increase the percentage of sickle trait donations that blocked the filter.

It is difficult to conclude from our study whether filter blockage is donor-related, because only a relatively small number of donors gave repeat donations and eight filter types were used, resulting in many possible combinations. Our results show that the same donor can have different outcomes with different filters, but the study design did not allow any conclusions to be drawn regarding whether a donor is likely to have a predictable outcome from repeated filtrations with the same filter type. Nevertheless, we found no donor-related characteristics that adequately predicted filtration outcome across filters. There was no significant difference in HbS levels or most other RBC parameters between donations that blocked and those that completed filtration successfully, except for the mean RBC volume, which tended to be higher in units that blocked. Although increased HbF reduces RBC sickling in vivo, and therapies that increase HbF levels are used to reduce symptoms of sickle cell disease,[23] we found no relationship between donor HbF level and filter blockage rate. Despite previous evidence that RBC microvesicles are related to irreversible sickling in sickle disease patients,[15] RBC microvesicle levels did not differ before filtration among controls, sickle trait units that blocked LD filters, and those that filtered successfully. Nevertheless, the LD process did remove a significant number of microvesicles.

Of measures that could differ because of collection variables, pO_2 level before filtration appeared to be a partial predictor of LD filter blockage. In all sickle trait units that completed filtration, the pO_2 level was at least 5.0 kPa, whereas in 9 of 17 units that blocked LD filters, the pO_2 was below this level, with 4 of 17 below 4.0 kPa. This is consistent with a previous study in which Hb oxygen saturation was greater in HbAS components that filtered more quickly.[14] In addition, washed HbAS RBCs filter more slowly and contain more polymerized HbS as Hb oxygen saturation decreases.[11] The pO_2 level at which RBC sickling occurs depends on the percentage of HbS present. In blood from sickle cell disease patients, sickling has been demonstrated to commence at a pO_2 of 8.0 kPa (60 mmHg) with the majority of cells sickling at 5.3 kPa (40 mmHg).[10] For blood containing 40 percent HbS, sickling commences at 5.3 kPa and for 25 percent HbS at 3.3 kPa.[10] Sickle cell trait donors in our study had HbS levels of between 29 and 40 percent, suggesting that a pO_2 below 5.0 kPa would cause HbS gelation, with a few of the RBCs starting to sickle in the whole blood before filtration.

The factors determining the pO_2 level in a blood pack are unclear, although others have shown that this may be related to the influence of the anticoagulant on pH and osmolarity.[14] Nevertheless, the differences in pO_2 levels between donors before filtration suggests that other factors that are related to the donor or collection procedure may be important but are not well understood. One possible means of improving the pO_2 levels before filtration is by the use of gas-permeable collection packs, as suggested by others.[14] This would be particularly useful if the external pO_2 could also be increased before LD. The addition of air to HbAS units, in combination with the use of gas-permeable collection bags, enables complete filtration of units.[24] Nevertheless, deliberate addition of air to blood packs is generally discouraged, because of the possibility of bacterial contamination from the environment. The use of alternative anticoagulants, such as heparin, have also been shown to improve LD performance in HbAS units,[14] but heparin is not optimal for the storage of blood components for transfusion.

Patients with sickle cell disease are known to have increased levels of PLT[16,19] and coagulation activation markers,[16-18] and a previous study showed increased coagulation activation in individuals with sickle trait.[20] In our study, there were no differences in either cell surface or soluble CD62P levels among sickle cell trait units that blocked filters, those that filtered completely, and control units. The contact system of coagulation is activated when plasma FXII is autoactivated to FXIIa[25] by binding to a negatively charged surface, such as certain LD filters.[26] Our results show that some activation of the contact factor pathway occurred in all donations after filtration, but there was no difference between sickle trait donations that blocked or filtered completely. We did not observe a significant difference in prefiltration levels of prothrombin F1 + 2 between sickle trait and control donations, but lev-

els increased after filtration in sickle cell trait units that blocked or failed to LD. This was shown in the HbAA donations to be reproduced by a slow flow rate, so that although coagulation activation is unlikely to be the primary cause of filter blockage, secondary activation resulting from slow flow once the blockage commences may worsen the situation.

In the National Blood Service, England & North Wales, we are currently working toward national policies relating to LD of HbAS donors. Currently, any donations, whether HbAS or not, are discarded if they fail to filter within 3 hours. Selected donors will be screened for sickle trait; donations from positive donors will not be filtered on Day 0 and will all be tested for residual WBC content. Furthermore, donors who repeatedly block LD filters will be tested for HbAS if they have previously not been tested.

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REFERENCES

1. Williamson LM. Leucocyte depletion of the blood supply—how will patients benefit? (review). Br J Haematol 2000; 110:256-72.
2. Hipp MJ & Scott RB. Altered filterability of CPD-stored sickle trait donor blood. Transfusion 1974;14:447-52.
3. Mijovic V & Kruse A. Filtration of blood from donors with HbAS: an unexpected problem. In: Brozovic B, editor. The role of leucocyte depletion in blood transfusion practice: proceedings of the international workshop. Oxford: Blackwell; 1988:48-50.
4. Bodensteiner D. White cell reduction in blood from donors with sickle cell trait. Transfusion 1994;34:224.
5. Ould Amar AK, Cesaire R, Kerob-Bauchet B, Robert P, Maier H, Bucher B. Altered filterability of fresh sickle cell trait donor blood. Vox Sang 1997;73:55-6.
6. Williamson LM, Beard M, Seghatchian MJ, et al. Leukocyte depletion of whole blood and red cells from donors with hemoglobin sickle trait. Transfusion 1999;39(Suppl): 108S.
7. Beard MJ, Seghatchian J, Cardigan R, Bennett J, Smith KM, Williamson L. Leucofiltration of sickle cell trait blood "the blocker": NBS London & S.E. experience. Transfus Sci 2000;22:71-3.
8. Guidelines for the blood transfusion services in the United Kingdom. 5th ed. London: The Stationery Office; 2001.
9. Sickle cell disease—molecular and cellular pathogenesis. In: Bunn HF, Forget BD, editors. Haemoglobin: molecular, genetic and clinical aspects. Philadelphia: Saunders; 1986: 453-501.
10. Sickle-cell haemoglobin (HbS). In: Lehmann H, Huntsman RG, editors. Man's haemoglobins. Oxford: Holland-North; 1974:141-76.
11. Hiruma H, Noguchi CT, Uyesaka N, Schechter AN, Rogers GP. Contributions of sickle hemoglobin polymer and sickle cell membranes to impaired filterability. Am J Physiol 1995;268:H2003-8.
12. Sears DA. Sickle cell trait. In: Embury SH, Hebbel RP, Mohandas N, Steinberg MH, editors. Sickle cell disease: basic principles and clinical practice. New York: Raven Press; 1994:381-94.
13. Gorlin JB & Gudino MD. A sticky situation: WBC reduction of RBCs from donors with sickle cell trait. Transfusion 2001;41:1192.
14. Stroncek DF, Rainer T, Sharon V, et al. Sickle Hb polymerization in RBC components from donors with sickle cell trait prevents effective WBC reduction by filtration. Transfusion 2002;42:1466-72.
15. Allan D, Limbrick AR, Thomas P, Westerman MP. Micro-vesicles from sickle erythrocytes and their relation to irreversible sickling. Br J Haematol 1981;47: 383-90.
16. Francis RB Jr. Platelets, coagulation, and fibrinolysis in sickle cell disease: their possible role in vascular occlusion. Blood Coagul Fibrinolysis 1991;2:341-53.
17. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared with healthy Black controls. Blood Coagul Fibrinolysis 1995;6:93-9.
18. Liesner R, Mackie I, Cookson J, et al. Prothrombotic changes in children with sickle cell disease: relationships to cerebrovascular disease and transfusion. Br J Haematol 1998;103:1037-44.
19. Wun T, Paglieroni T, Rangaswami A, et al. Platelet activation in patients with sickle cell disease. Br J Haematol 1998;100: 741-9.
20. Westerman MP, Green D, Gilman-Sachs A, et al. Coagulation changes in individuals with sickle cell trait. Am J Haematol 2002;69:89-94.
21. Krailadsiri P, Seghatchian J, Williamson LM. Platelet storage lesion of WBC-reduced, pooled, buffy coat-derived platelet concentrates prepared in three in-process filter/storage bag combinations. Transfusion 2001;41:243-50.
22. Williamson LM, Wimperis JZ, Williamson P, et al. Bedside filtration of blood products in the prevention of HLA alloimmunization—a prospective randomized study. Blood 1994;83:3028-35.
23. Serjeant GR. The emerging understanding of sickle cell disease. Br J Haematol 2001;112:3-18.
24. Byrne KM, Stroncek DF, Leitman S. Increasing oxygen tension prevents the occlusion of leukocyte reduction filters

by sickle cell trait donor RBCs. Transfusion 2002;42(Suppl): 15S.

25. Salzman EW, Merril EW, Kent KC. Interaction of blood with artificial surfaces. In: Coleman RW, Hirsh J, Marder VJ, Salzman EW, editors. Haemostasis and thrombosis: basic principles and clinical practice. Philadelphia: Lippincott; 1994:1469-85.
26. Cardigan R, Sutherland J, Garwood M, et al. The effect of leucocyte depletion on the quality of fresh frozen plasma. Br J Haematol 2001;114:233-40.