Assessment of leucoreduction of sickle cell trait blood: quality of the filtered product

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Background. With the implementation of universal leucoreduction of blood components in several industrialised countries, the problems associated with leucocyte filtration of sickle cell trait blood have been reconsidered. In this study, we assessed the use of high performance filters for leucoreduction of packed red blood cells donated from subjects with sickle cell trait and evaluated the incidence and recurrence of altered red blood cell filterability.

Materials and methods. Twenty-one volunteer donors with HbAS were compared to 21 donors with HbAA selected at random. The main parameters analysed were residual white blood cell count and post-filtration haemolysis. Filtration times, flow, volume and haemoglobin loss of the packed red blood cells were also determined.

Results. In all, 33% of HbAS red blood cell units with slow flow and prolonged filtration time had high residual white blood cell counts. In 7.7% of cases, despite flow through the filter, the units were not leucoreduced properly. Haemoglobin and volume loss were significantly greater in the slow filtration group. Significant post-filtration haemolysis was present in half of the units with high residual white blood cell counts.

Discussion. Despite the development of new technology for filtration, the problem of filterability of blood from donors with sickle cell trait is not yet resolved. Altered filterability of blood from sickle cell trait donors cannot be predicted from the donors' characteristics and recurrence of the problem is not observed between donations. Screening blood donors for sickle cell trait to ensure the safety and quality of blood products for transfusion does, therefore, remain a relevant issue.

Keywords: sickle cell trait, blood donors, leucoreduction, altered filterability.

Introduction

Several reports suggest that red blood cells (RBC) collected from blood donors with sickle cell trait (SCT) and stored under conventional conditions can be difficult to filter and may not be leucoreduced satisfactorily[1-3]. Not all blood donated by subjects with SCT is filtered poorly, but when abnormal filtration does occur, the donated blood cannot be used for transfusion. It has been demonstrated that the filterability of blood units from donors with SCT can be improved by enhanced oxygenation, suggesting that some degree of red cell sickling could be responsible for the poor filterability indices associated with SCT[4,5].

The Martinique Blood Centre of the French Blood Establishment, located in the Caribbean area, collects approximately 12,000 units of RBC each year. In conformity with French transfusion safety regulations, since 1998 we have performed 100% leucoreduction of red cell units. In order for a RBC unit to be called leucoreduced in France, its residual white blood cell (WBC) count must be less than $1x10^6$ leucocytes.

SCT affects approximately 8% of the population in Martinique. We estimate that 32,000 people in Martinique could have SCT and each year about 700-900 are potential blood donors, representing 8-10% of our blood donor community. Neonatal testing to detect sickle cell disease has been performed since 1978; however, many individuals do not know their sickle trait status. From 1985 to June 2010, the policy in our transfusion centre was systematic screening of all new blood donors for pathological haemoglobin, the destruction of all blood packs containing HbAS and exclusion of SCT-positive carriers from blood donation[6]. However, to comply with national regulations, in July 2010, this systematic screening of haemoglobin was stopped and no more first-time donors have since been excluded from donating blood[7]. Indeed, there are no specific recommendations in France about the screening or use of blood from SCT donors. The phenotype of haemoglobin in donated units of blood is unknown because screening donors for HbS is not standard French practice. We estimate that approximately 1% of

our donations could now come from SCT-positive new donors and could cause filter failure with destruction of the donated blood product.

With the implementation of universal leucoreduction in several countries, the problems associated with leucocyte filtration of SCT blood have been reconsidered. Currently, there is no unified standard practice for SCT donors. Some blood centres screen all ethnic African donors for HbS, others do not. There are differences in opinion about whether SCT red cells should be considered as equivalent to non-SCT red cells. Some blood centres do not give red cells from a SCT donor to neonates or patients undergoing general anaesthesia as a safety transfusion measure[8]. Nevertheless, in order to avoid transfusing RBC from SCT donors to patients with sickle cell anaemia (whose blood oxygenation might be compromised) or neonates, we must know the HbAS status of our RBC units. Furthermore, besides filtration failure, in the SCT red cell units that are successfully filtered the number of residual WBC is usually abnormal. Thus, there is a real risk of producing RBC units with a high WBC count and delivering non-compliant ones.

In order to study the leucoreduction of packed RBC from donors with SCT, in particular the residual white cell counts and haemolysis rate in relation to the time of filtration, and to assess the quality of the filtered product, we compared two groups of blood donors: one group of 21 donors with HbAA and another group of 21 donors with HbAS. The main goal of this study was to estimate whether people with HbAS, or some of them, should remain eligible for whole blood donation and provide arguments for the national authorities on the need to resume systematic screening of blood donors for HbS, especially in the Caribbean area.

Materials and methods

Donor selection

Forty-two donors, selected at random from normal donors (with HbAA) and SCT donors (with HbAS), were divided into two groups, each of 21 donors. The SCT donors were identified from a database of donors who had been tested using an isolectrofocusing technique. All donors recruited were eligible to donate blood according to current French guidelines. All gave informed consent to participation in this study.

Blood collection and pre-storage

Whole blood (8 mL/kg) was collected into CPD (66.5 mL) using quintuple bags (Fenwall Inc., Lake Zurich, USA) with an integral red cell filter (Sepacell, Asahi pure RC, Tokyo, Japan). Before separation of the blood components, the units of blood were pre-stored for 2.5 hours at 22±2 °C, according to usual practice in our centre, to allow the WBC to exert their bactericidal activity.

Processing

The whole blood was centrifuged for 20 min (22±2 °C) at 3,998 *g* and then separated using Compomat G4 technology (Fresenius NPBI Transfusion Technology, Emmer-Compascuum, The Netherlands) according to the manufacturer's recommendations. The red cells were transferred into bags containing SAG-mannitol preservative solution (105 mL) and filtered at room temperature (20-25 °C) through an in-line leucoreduction filter (Sepacell, Asahi Pure RC, Tokyo, Japan) within 6-8 hours after collection.

Filtration performance and laboratory analysis

The filtration time (in minutes) was recorded for each unit. The units that were not filtered completely or demonstrated prolonged filtration (≥2 hours) were transferred overnight to an environment at 4 °C in order to see whether the process was or was not completed eventually. The pre-filtration and post-filtration volumes and haemoglobin levels of the packed red cells were determined by, respectively, weighing and measurement on a XE 2100D Sysmex automated device in order to determine haemoglobin and volume loss. Post-filtration haemolysis was measured using a HemoCue Plasma/Low Hb System (Meaux, France), according to the manufacturer's recommendations. Residual WBC counts were measured by flow cytometry (LeucoCount reagent, FACScalibur flow cytometer, Becton Dickinson Biosciences, Le Pont-de-Claix, France).

Statistical analysis

Mean values ± standard deviation, as well as minimum and maximum values are given. For multiple comparisons (HbAA group *versus* HbAS subgroups), statistical analysis was undertaken using computer software (IBM SPSS statistics for Windows, Version 19.0, Chicago, IL, USA). To test for group differences for each of the measures, a series of one-way analyses of variance (ANOVA) with Bonferroni's adjustment were conducted. Student's *t*-test was used to compare the two groups: HbAA *vs* HbAS. All P values less than 0.05 were considered statistically significant.

Results

The analysis of the main outcome parameters of the study, residual WBC count and haemolysis, showed that there was a significant difference ($P < 0.01$) in residual WBC count between the groups of units of HbAA and HbAS blood, but no difference in haemolysis (Table I). Based on the time of filtration and the residual WBC counts of the packed red cell units, three sub-groups were distinguished within the HbAS group: group 1: subnormal filtration (mean of 1 h) with normal leucocyte reduction (13/21); group 2:

slow filtration (>2 h) with normal leucocyte reduction (2/21) and group 3: slow filtration (>2 h) with high residual WBC count (6/21). The results are reported in Table II.

Overall, the HbAS subgroups exhibited a significant difference (P <0.001) in terms of time and flow of filtration. Furthermore, when we compared subgroup 3 units (HbAS with high WBC counts and slow filtration) *vs* the HbAA group of units, we found statistically significant differences (P <0.01) for both residual WBC counts and levels of post-filtration haemolysis (Tables III and IV). No statistically significant difference was observed in terms of volume loss between the groups. There was, however, a statistically significant difference (P =0.01) in haemoglobin loss between HbAA units and subgroup 3 HbAS units. No difference was observed between groups in terms of donors' haematological parameters, in particular for the WBC counts. In addition there was no relationship between donor characteristics and filterability (Table V).

Discussion

Our results are comparable to those previously published[3], despite technological developments and the use of new generation, high performance filters. However, we noted that there is a gradient in the alteration of the filterability of SCT blood. Indeed, 33% of units of HbAS packed red blood cells could not be filtered and had high residual WBC counts and significant post-filtration haemolysis. Nevertheless, we found higher residual WBC counts in normally filtered HbAS units than in the HbAA units, so there is a risk of transfusing poorly leucoreduced RBC. It is important to appreciate that there is interindividual variability in the occurrence of abnormal filtration. Packed red cells from

Table I - HbAA group *vs* HbAS group: WBC counts and haemolysis.

	HbAA (n=21)	HbAS (n=21)	P value
WBC counts >1x10^{6a}	0	7 (33.3%)	<0.01
Haemolysis (≥0.5% at D+1)^{b}	3 (14.3%)	4 (19%)	NS^{c}

[a]Regulatory standard in France: residual leucocytes <1x10^{6}/RBC unit; [b] day after donation; acceptable limit (according to the French Blood Establishment) <0.5%; [c] not statistically significant.

Table II - HbAA group *vs* HbAS subgroups: WBC counts and haemolysis.

	HbAA (n=21)	HbAS group 1 (n=13)	HbAS group 2 (n=2)	HbAS group 3 (n=6)
WBC counts >1x10^{6a}	0	1 (7.7%)	0	6 (100%)
Haemolysis (≥0.5% at D+1)^{b}	3 (14.3%)	1 (7.7%)	0	3 (50%)

[a]Regulatory standard in France: residual leucocytes <1x10^{6}/RBC unit; [b] day after donation; acceptable limit (according to the French Blood Establishment) <0.5%.

Table III - HbAA group *vs* HbAS subgroups: comparison of the different parameters (mean±SD).

	HbAA	HbAS group 1	HbAS group 2	HbAS group 3
N (%)	21	13/21 (62%)	2/21(9.5%)	6/21 (28.6%)
Sex ratio	0.6	1.1	1	1
History of failure in filtration (same donor) (%)	-	5/13 (38.5%)	1/2 (50%)	2/6 (33%)
PRC post-filtration volume (mL)	254±16.4	259±25	250±34	231±36.8
PRC post-filtration Hb content (g)	47±5.6	51±8	46±11	42±8.4
Volume loss (%)	11±0.01	11±0.01	11±0.02	16±0.1
Hb loss (%)	24±0.02	24±0.02	35±0.1	34.5±0.2
Time of filtration at 22 °C (minutes)	42±10	55± 22	240	221±35.6
Flow (mL/min)	7±1	6±2	1.1±0.2	1.3±0.3
Residual WBC counts (min-max)	0.001±0.012 (0.001-0.005)	0.08±0.28 (0.01-1.01)	0.34±0.3 (0.12-0.55)	7.21±8.1 (1.96-23.2)
Haemolysis (%) (min-max)	0.21±0.29 (0.06-0.55)	0.15±0.10 (0.1-0.47)	0.3±0.08 (0.36-0.24)	0.63±0.5 (0.12-1.51)

PRC: packed red cells; Hb: haemoglobin.

Table IV - P value of the comparison between the HbAA group and HbAS subgroups for the main parameters (ANOVA one-way comparison).

	Group 1 *vs* AA	Group 2 *vs* AA	Group 3 *vs* AA
Volume loss	NS	NS	NS
Haemoglobin loss	NS	NS	0.01
Time of filtration	0.02	<0.001	<0.001
Flow of filtration	0.03	<0.001	<0.001
Residual WBC count	NS	NS	<0.01
Haemolysis	NS	NS	0.03

NS: not significant.

Table V - Comparison of haematological parameters between the normal (HbAA) and SCT (HbAS) donors.

	HbAA	HbAS Group 1	HbAS group 2	HbAS group 3	P value[a]
Haemoglobin (g/dL) Mean±SD (min-max)	13±0.88 (11.5-14.4)	13.7±1.4 (11.7-15.7)	12±2.5 (10.3-13.8)	12.9±1.1 (11.8-14.7)	NS
Haematocrit (%) Mean±SD (min-max)	40.9±2.18 (37-43.6)	41.8±3.9 (36-47)	36.1±7.2 (31-41.2)	39.9±2.4 (37-43.4)	NS
MCV (fL) Mean±SD (min-max)	86±6.24 (71.9-96)	85.4±5.4 (73-91)	79.5 ± 7.8 (74-85.1)	84.7±5.6 (75-90)	NS
MCHC (g/dL) Mean±SD (min-max)	31.9±1.2 (29.4-33.8)	32.7±0.6 (31.8-33.8)	33.2±0.4 (32.9-33.5)	32.5±0.9 (31.5-33.9)	NS
MCH (pg) Mean±SD (min-max)	27.4± 2.5 (23.1-31.1)	27.9 ± 1.8 (23.7-30.7)	26.3±3 (24.2-28.5)	27.6±2.2 (24-30.2)	NS
Platelet count (x10^9/L) Mean±SD (min-max)	270±49 (175-367)	268±55.2 (188-407)	204±78.5 (149-260)	239±56.4 (164-303)	NS
WBC count (x10^9/L) Mean±SD (min-max)	6.1±1.8 (2.8-9.9)	5.7±1.3 (3.3- 9.2)	4.5±1.8 (3.2-5.8)	6.8±2.2 (4.6-9.1)	NS

[a] ANOVA one-way comparison.
MCV: mean corpuscular volume; MCHC: mean cell haemoglobin content; MCH: mean corpuscular haemoglobin; NS: not significant.

a SCT donor may be blocked at one donation and not at the next donation. We did not find any predictive factors or correlation between altered filterability and donors' characteristics, such as haematological parameters, or a personal or family history in the questionnaire or during the medical interview.

The variables affecting filter blockage are unclear. In order to identify critical variables, some authors have investigated the relationship between filter blockage and donors' characteristics, processing conditions, platelet activation and microvesicle formation in donations from subjects with or without SCT. It was concluded that filter blockage in SCT donors cannot be predicted by the donors' characteristics or filter type and is not related to platelets or coagulation activation, but can be reduced by storing units at 4 °C before filtration[9]. Factors and their combinations which have been suggested to be likely to contribute to the filtration failure of sickle trait products are: temperature, pH, osmolarity, type of anticoagulant, time of storage, and oxygen saturation of the blood unit[10].

In a comparison of blood from normal and SCT subjects stored at 4 °C, over a 21-day period after blood filtration, sickling was seen in all HbAS samples. HbAS blood filtered significantly slower than HbAA blood at 4, 25, and 37 °C and filterability became progressively prolonged as temperature rose. In addition, HbAS samples with high percentages of HbS filtered significantly more slowly than did those with a smaller proportion of HbS[1]. It is known that polymerisation of HbS is exacerbated by acidic and hyperosmotic citrate anticoagulant solutions and often results in occlusion of leucoreduction filters by RBC from SCT donors. Using a metered anticoagulation instrument, Bryant *et al.* demonstrated a potential for successful leucoreduction of HbAS units[11]. Stroncek *et al.* showed that storage of HbAS whole blood in large-capacity oxygen-permeable bags increases haemoglobin oxygen saturation allowing successful filtration of blood from SCT donors and more effective leucoreduction[12]. A major cause of filter failure of RBC components from donors with SCT is the polymerisation of haemoglobin. The oxygen saturation

(sO_2) of blood stored in various plastics and different volumes of air was assessed. Blood from ten HbAS donors was collected and divided into two bags, one with air added, one without. Bags with added air had increased sO_2 levels. Filtration was successful for nine of ten components with added air, but for only one of the ten without added air. Successful filtration of RBC components occurs when the sO_2 is increased[5]. *In vitro*, RBC from subjects with SCT have the potential to sickle, with HbS polymerisation, at low oxygen saturations and high haemoglobin concentrations. To determine whether the low pH and high osmolarity of the CP2D used in the collections contributes to filter failure, the filterability of RBC collected from SCT donors into CP2D was compared with that of RBC from the same donors collected into heparin. Filtration of five of the six SCT components collected into CP2D remained incomplete, whereas all six RBC components collected into heparin filtered completely. RBC components collected into CP2D from four other SCT donors were divided in two parts and one part was then treated with carbon monoxide to convert HbS to its liganded form to prevent HbS polymerisation. All four carbon monoxide-treated components filtered within 9 minutes, but only one untreated component filtered completely. RBC components collected by apheresis contain less CP2D and in a study examining this aspect, five of seven SCT apheresis components filtered completely: four of the five filtered rapidly (<15 minutes) and one filtered in 100 minutes. Haemoglobin oxygen saturation was greater in the four RBC apheresis components that filtered rapidly (68±9%) than in the three that filtered slowly or incompletely. HbS polymerisation appears to be responsible for the failure of filters to reduce the WBC content of RBC units.

Citrate anticoagulant and low oxygen saturation are responsible in part for HbS polymerisation in this setting[13]. However, in an *in vitro* comparison of quality of RBC collected by multicomponent apheresis *versus* manually collected RBC, Picker *et al.* found that initial pH value was slightly higher in manually collected RBC than in RBC obtained by apheresis, although the difference was not statistically significant. The mean citrate content (mL/mL RBC mass) was 5.6 in apheresis RBC *versus* 1.88 in manually collected RBC[14].

Although there might be disadvantages of transfusing SCT blood, there is no means other than biological screening to determine which of the 12,000 units collected each year in Martinique are positive for SCT. Screening blood donors by questionnaire would not be conclusive although screening all blood donations for HbS would not help to identify the subgroup at risk of filtration failure. This policy should be considered to prevent the use of RBC units with a high WBC count. This matter is of importance because of the high incidence of SCT in our donor population and for particular recipients, especially patients with sickle cell anaemia.

Indeed, we will soon conduct a study in order to verify whether there is an association between abnormal filtration, impaired haemorheology and increased concentration of microparticles in donors with SCT compared to in control subjects. This could provide support for the hypothesis that SCT carriers who exhibit this type of abnormality express an inflammatory profile amplified by cellular activation and altered red blood cell rheology. We expect that SCT donors whose blood does not exhibit abnormal leucoreduction filtration have a normal concentration of microparticles and a normal haemorheological profile. These subjects could be eligible for blood donation. Finally, the results obtained should provide a better understanding of pathophysiological mechanisms reported in a subpopulation of subjects of SCT carriers, as well as in patients with sickle cell anaemia.

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References

1) Hipp MJ, Scott RB. Altered filterability of CPD-stored sickle trait donor blood. Transfusion 1974; **14**: 447-52.
2) Bodensteiner D. White cell reduction in blood from donors with sickle cell trait. Transfusion 1994; **34**: 84.
3) Ould Amar K, Césaire R, Maier H, et al. Altered filterability of fresh sickle cell trait donor blood. Vox Sang 1997; **73**: 55-6.
4) Hillyer KL, Rapaport H, Roback JD, Hillyer CD. Pre-filtration oxygenation abrogates the leukoreduction filtration lesion in haemoglobin AS whole blood units. Blood 2002*;* **100** (suppl. 11s): 287a.
5) Byrne KM, Leitman SF, Schechter AN, Stroncek DF. Increasing oxygen tension improves filtration of sickle trait donor blood. Br J Haematol 2003; **122**: 678-81.
6) Ould Amar K, Kérob B, Leconte C, et al. Assessment of qualitative functional parameters of red blood cells from donors with sickle cell trait (AS) and with heterozygote (AC) status. Transfus Clin Biol 1996*;* **4**: 225-33.
7) Ould Amar K. Red blood cells from donors with sickle cell trait: a safety issue for transfusion? Transfus Med 2006*;* **16**: 248-53.
8) Krailadsiri P, Gilcher R, Seghatchian J. Leukoreduction of sickle cell trait blood: an unresolved issue. Transfus Apher Sci 2001; **24**: 223-5.
9) Beard MJ, Cardigan R, Seghatchian J, et al. Variables determining blockage of WBC-depleting filters by Hb sickle cell trait donations. Transfusion 2004; **44**: 422-30.
10) Schuetz AN, Hillyer KL, Roback JD, et al. Leukoreduction filtration of blood with sickle cell trait. Transfus Med Rev 2004; **18**: 168-76.

11) Bryant B, Bianchi M, Wesley R, et al. Leukoreduction filtration of whole-blood units from sickle trait donors: effects of a metered citrate anticoagulant system. Transfusion 2007; **47**: 2233-41.
12) Stroncek DF, Byrne KM, Noguchi CT, et al. Increasing hemoglobin oxygen saturation levels in sickle trait donor whole blood prevents hemoglobin S polymerization and allows effective white blood cell reduction by filtration. Transfusion 2004; **44**: 1293-9.
13) 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.
14) Picker SM, Radojska SM, Gathof BS. In vitro quality of red blood cells (RBCs) collected by multicomponent apheresis compared to manually collected RBCs during 49 days of storage. Transfusion 2007; **47**: 687-96.

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