The role of cell savers and filters in cardiac surgery

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Chapter 5

Clinical efficacy and biocompatibility of three different leukocyte and fat removal filters during cardiac surgery

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Abstract

Background: Activated leukocytes and fat particles are associated with organ injury after a cardiac surgery. Filters are currently used to remove either leukocytes or fat particles. A novel approach with a filter that combines leukocyte and fat removal might be clinically useful. As it is not known which type of filter has a good and safe performance in both leukocyte and fat removal, we measured in this study the leukocyte and fat removal properties and the biocompatibility of three different filters.

Methods: We used six Pall RS1 (Pall, Portsmouth, England) leukocyte removal filters, six Pall LipiGuard fat removal filters, and six Fresenius Biofil 02 (Fresenius, Emmer-Compascuum, The Netherlands) leukocyte removal filters and measured the passage times of 500 and 1000 mL of residual heart–lung machine blood. We determined the circulating leukocyte and platelet counts, and total haemoglobin, triglyceride, and free fatty acid concentration after the filters. In addition, we measured free haemoglobin, plasma elastase (Merck, Darmstadt, Germany), and complement C5–9 (Quidel, San Diego, CA, U.S.A.) to assess the biocompatibility of the filters. The circulating fat particles were calculated with an automated haematology analyzer.

Results: The passage time for the blood was shortest for the Biofil filter ($P = 0.02$, analysis of variance). The total leukocyte counts ($P = 0.04$) and fat particles ($P = 0.02$) were higher after the LipiGuard filter. This filter also had a higher increase in free haemoglobin concentration ($P = 0.03$).

Conclusions: We conclude that the leukocyte removal filters were superior to the fat removal filter both in leukocyte and fat removal.

Introduction

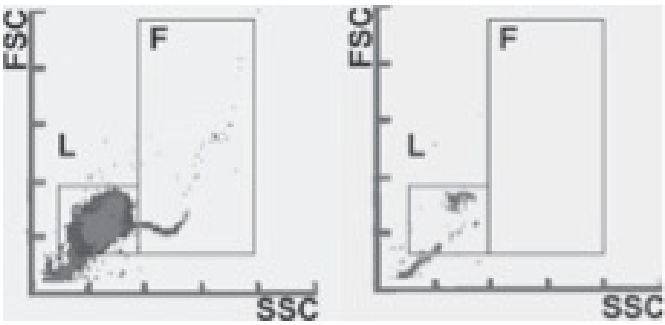
Activated leukocytes and fat particles are associated with organ injury in patients after a cardiac surgery. The effects on the lungs, brain, and kidneys have been documented [1-3]. Leukocyte depletion by means of filtration has been proposed as a method to reduce this organ injury [4,5]. Recently, it was suggested to remove, in addition, fat particles from the circulation by filtration [6]. Filters may therefore be used to remove either leukocytes or fat particles during a cardiac surgery. However, by its structure and nature a leukocyte removal filter also removes fat particles [7], and a fat removal filter also removes leukocytes, be it not at the same efficiency [8]. A novel approach with a filter that combines both properties might be clinically useful. However, we do not know which type of filter has a good performance in a clinical setting during a cardiac surgery in both leukocyte and fat removal, while at the same time the blood damage and blood activation are minimal. Most clinical filtration procedures are currently performed during a cardiopulmonary bypass (CPB) with its concomitant haemodilution. Haemodilution may increase the volume capacity of the filters, but to what extent is unknown. A large filter capacity would improve the clinical acceptability as it minimizes filter changes.

Therefore, we measured in this study the leukocyte and fat removal properties and the biocompatibility of three different filters using residual heart–lung machine blood.

Methods

After a local ethics committee approval and patient consent, we collected in this prospective randomized study the residual blood that was left in the heart–lung machine after a CPB of 18 consecutive cardiac surgical patients. The patients underwent either a coronary artery bypass grafting and/or a valve replacement. This blood was filtered before re-transfusion in a patient with a leukocyte or a fat removal filter under gravity from a height of 140 cm. This height equals a pressure of 100 mm Hg. We used one filter for each patient. As filters, we used six Pall RS1 (Pall, Portsmouth, England) leukocyte removal filters, six Pall LipiGuard fat removal filters, and six Fresenius Biofil 02 leukocyte removal filters (Fresenius, Emmer-Compascuum, The Netherlands). We

measured the time of the passage of 500 and 1000 mL of the blood. We also took three blood samples: one sample from the transfusion bag with the residual blood, one sample after the filter at 500 mL, and one sample after the filter at 1000 mL. From these blood samples, we determined the circulating leukocyte and platelet counts, and the total haemoglobin, triglyceride, and free fatty acid concentration. In addition, two blood samples were collected in ethylenediaminetetraacetic acid medium: one sample from the residual blood bag and one sample after the filter at 1000 mL, or less when the filter was blocked. These samples were immediately centrifuged at 1000 ×g for 10 min. The plasma was collected and stored at -80°C until further analysis. From these samples, we measured free haemoglobin as a measure of erythrocyte lysis, the elastase (Merck, Darmstadt, Germany) concentration as a measure of leukocyte activation, and the complement C5–9 (Quidel, San Diego, CA, U.S.A.) complex concentration as a measure for complement system activation using an enzyme immunoassay. These three measurements served to estimate the biocompatibility of the filters.



A B

Figure. 1. Two plots from an automated haematology analyzer taken from the residual heart–lung machine blood (A) and from blood that has passed the filter (B). The plots are constructed using front-scattered (FSC) and side-scattered (SSC) laser light. Leukocytes are in the rectangular L; fat particles form a sigmoid- shaped curve in the rectangular F. In panel B, both leukocytes (L) and fat particles (F) are reduced.

The circulating fat particles were calculated with an automated haematology analyzer based on fluorescence flow cytometry (Sysmex XE-2100; Sysmex, Kobe, Japan) as previously described [9]. Briefly, cells are counted in the first channel of the analyzer using front- and side-scattered (SSC) laser light. Fat particles also diffract the laser light resulting in a sigmoid-shaped curve in this channel (Fig. 1A).

The fat particles are thus also counted. The cells are differentiated and again counted in the second channel using fluorescence and SSC laser light. The cell counts from the second channel are subtracted from the total counts from the first channel. The difference reflects the circulating fat content. Statistical analysis was performed using Student's t-test for paired values and analysis of variance (ANOVA) with Bonferroni post hoc analysis as appropriate. Two-way ANOVA for repeated measurements was used to determine the effects of quantity, group, and interaction over the three measurement points. $P < 0.05$ was considered significant. Values are given as mean ± standard deviation. For the fat and particle counts, values are given as a percentage of the individual patient's starting value.

Result

The patient demographics are shown in Table 1, and indicate that the groups were not different. The volume of the residual blood was 1197 ±323 mL and was not statistically different between the groups.

Tabel 1: Demographics

	RS 1 (n=6)	LipiGuard (n=6)	Biofil 02 (n=6)	
Age (year)	65 ± 9	61 ± 11	70 ± 7	NS
Height (cm)	176 ± 5	174 ± 15	175 ± 6	NS
Weight (kg)	79 ± 7	85 ± 7	87 ± 17	NS
Male	6	5	6	NS
CABG	3	4	3	NS
Valve	2	2	1	NS
CABG + valve	1	0	2	NS
CPB time (min)	133 ± 73	121 ± 38	102 ± 26	NS

CABG, coronary artery bypass graft; CPB, cardiopulmonary bypass; NS, not significant.

In three patients, the total amount of residual heart–lung machine blood was <1000 mL (550 and 950 mL RS 1 group, and 850 mL LipiGuard group). In these patients, the third (1000 mL) sample was collected at these blood quantities. Three of the LipiGuard filters became blocked before 1000 mL had passed (at 500, 600, and 650 mL, respectively). In these patients, the third (1000 mL) sample was collected at these blood quantities and the passage time was set at 600 s. The total passage time for the blood was shortest for the Biofil filter ($P = 0.02$, Fig. 2).

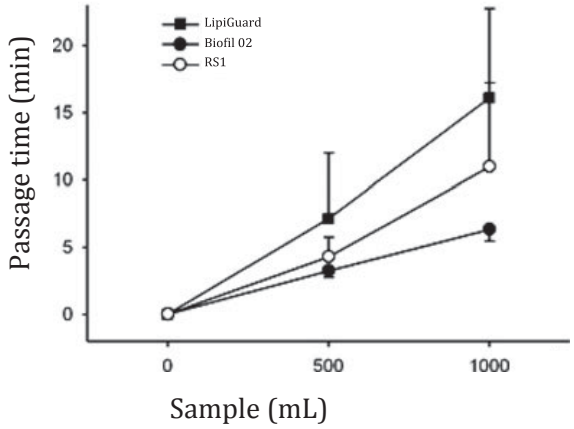


Figure. 2. Passage time for residual heart–lung machine blood through three types of leukocyte and fat removal filters. Error bars indicate standard deviation. Repeated measurement analysis indicates a difference (P = 0.03) between the filter types.

This was not only true for the first 500 mL of the blood, but especially for the second 500 mL. All filters removed leukocytes, but the total leukocyte counts were significantly higher after the LipiGuard filter ($P = 0.04$) at 500 and 1000 mL (Table 2). The leukocyte counts after the second 500 mL were slightly higher in all filters than after the first 500 mL (Table 2). The circulating platelet counts were lowest after the RS1 filter ($P = 0.05$, Table 2). The concentration of free fatty acids in the blood after passage through the three filter types was almost similar. However, fat particles were less reduced in the LipiGuard filter at 500 mL ($P = 0.02$). A typical example of these fat particles in the blood before filtration and after 500 mL of the blood had passed through the filter is shown in Fig. 1.

Table 2: Composition of the residual heart–lung machine blood before and after filtration with three different filters

Filter	RS 1			LipiGuard			Biofill		
Sample point (mL)	0	500	1000	0	500	1000	0	500	1000
n	6	6	5	6	6	4	6	6	6
Haemoglobin (mmol/L)	4.6±0.6	-	-	4.4±1.1	-		4.2±1	-	-
Leukocytes (x10^9/L)	8.3±2.6	0.13±0.05‡	0.26±0.18‡	7.3±3.2	3.0±2.3‡‡	4.1±2.5‡‡	7.8±4.2	0.13±0.05‡	0.22±0.12‡
Platelets (x10^9/L)	129±32	4.8±2.9*‡	5.6±1.8*‡	151±40	64±38‡	87±118‡	154±66	56±72‡	83±17
Triglycerides (mmol/L)	0.41±0.07	0.37±0.06	0.37±0.06	0.54±0.23	0.53±0.23	0.48±0.13	0.53±0.17	0.51±0.15	0.51±0.16
Free fatty acids (µmol/L)	1011±445	852±391	797±410‡	943±260	881±266	815±194	961±222	865±171‡	868±174
Elastase (µg/L)	822±179	-	1167±102‡	929±411	-	1357±338‡	820±282	-	1044±264‡
C5-9 (ηg/L)	1454±756	-	1877±771‡	1874±516	-	1994±509	1568±968	-	1854±889
Plasma hemoglobin (mg/L)	356±175	-	388±156	305±54	-	1247±973‡‡	185±123	-	207±143
Fat particle decrease (%)	-	86±8	86±8	-	50±22‡	38±17‡	-	83±9	92±8

*P ≤ 0.05 for RS 1 versus LipiGuard and Biofil; ‡P ≤ 0.05 for LipiGuard versus RS 1 and Biofil; ‡P≤ 0.05 to residual blood.

The increase in the elastase concentrations was slightly lower after the Biofil filter ($P = 0.26$, Table 2). The increase in the complement C5–9 concentration was slightly, but not significantly, lower in the LipiGuard filter ($P = 0.21$, Table 2). This filter also showed a significant larger increase in plasma haemoglobin concentration than the other filters ($P = 0.03$, Table 2). There was no correlation between the elastase concentration or the complement C5–9 concentration in the residual blood and the duration of CPB or the quantity of residual blood. A small but significant negative correlation was observed between the quantity of the residual blood and the plasma haemoglobin concentration ($R = -0.51, P = 0$).

Discussion

This study shows a marked difference in passage time, in leukocyte and platelet removal, and in fat removal between the three filter types. Moreover, the biochemical measurements also demonstrate a difference between the three filter types in the increase in elastase, complement C5, and free haemoglobin after the passage of 1000 mL of residual heart–lung machine blood. The passage times for the residual heart–lung machine blood were different between the three groups. Both leukocyte depletion filters performed better than the fat removal filter. As these filters will be used clinically in the operating theater, a high flow rate and a large capacity are important. We used a constant pressure on the filter, and thus, the flow rate is reflected in the passage time. All filters showed an increase in the passage time for the second 500 mL of the blood. This suggests that the filter gradually becomes saturated with cells and particles. The capacity of the filters may therefore be estimated by the increase in the passage time for the second 500 mL and by the increase in the leukocyte counts. A small increase in passage time for the second 500 mL of the blood and in leukocyte counts suggests that 1000 mL of the residual heart–lung machine blood, with a low haemoglobin concentration, may be safely processed by the leukocyte filters. Unfortunately, our study cannot answer the question if, apart from debris and particles, the filters become blocked by the passage of the red blood cells or the leukocytes. The leukocyte load of the residual heart–lung machine blood was not particularly high compared to normal pre-operative patient values, but the leukocytes

have been activated by the CPB circuit. We have previously shown that activated leukocytes are preferentially trapped in the filter [10], and thus may lead to a rapid saturation of the filter. We did not differentiate the leukocyte counts into granulocyte and lymphocyte counts. Lymphocytes are mainly removed by trapping, whereas granulocytes also show adhesion [11]. An increase in circulating lymphocytes may therefore saturate the filter. In contrast, the composition of the storage solution for red blood cells influences filter efficacy [12] and suggests an interaction between the filter and the red blood cells. For the residual heart–lung machine blood in our clinical setting, no data are available. It has also been suggested that the efficacy of leukocyte depletion depends on the ratio of platelets to leukocytes [13]. Our platelet counts were relatively low, which suggests that not only the circulating leukocytes saturate the filter. The leukocyte counts showed a difference between the two leukocyte removal filters, on the one hand, and the fat removal filter, on the other hand, indicating that the leukocyte removal filters were superior in this respect. The filter efficiency of the Pall RS1 and the Fresenius Biofil 02 leukocyte removal filters in this setting was about 98%. This is lower than is expected from the data for blood bank use, but is in agreement with a previous study where for residual heart–lung machine blood, a removal rate of 95–97% was found [14]. The platelet counts in the 1000-mL samples were, in all filter types, higher than in the 500-mL samples. Platelets have a higher affinity for the filter material than leukocytes [15]. Thus, it is likely that during the first part of the filtration procedure, the fibers in the filters are coated by platelet deposition. This facilitates the adherence of the leukocytes on the fibers of the filter [16]. The overall reduction in platelet counts in the LipiGuard group and in the Biofil group is in agreement with our previous findings in residual heart–lung machine blood where we found a 50% reduction in platelet counts after the filter [14]. However, platelets were almost completely removed by the RS1 filter. This is a remarkable finding, because the filter material is polyester in all three filter types. Therefore, to explain this difference, an additional coating of the filter material must have been applied. The LipiGuard filter was specifically designed to remove fat particles. It may therefore not be surprising that this filter removed fewer leukocytes than the other two filters. However, with a 50% fat removal rate, fewer fat particles were also removed than what can be removed by the other two filters. This observed percentage of fat particle reduction is in

agreement with a previous study on cardiotomy suction blood in which the LipiGuard filter removed 46% of the free fatty acids and 30% of the triglycerides [8]. Also, Ramirez et al., using an automated fat particle analysis, demonstrated a moderate efficacy of this filter in orthopedic patients [9]. Booke et al., in contrast, demonstrated with 60% fat removal a higher efficacy of this filter in a laboratory study [7]. However, they used reconstituted blood with soy oil, in contrast to the more clinical approach that we chose, which may explain this discrepancy. Fat particles are largely composed of triglycerides, which are esters of fatty acids with glycerol [17]. When triglycerides are degraded, free fatty acids are formed. Therefore, we also measured the concentration of the triglycerides and free fatty acids before and after the filter. The concentration of the free fatty acids did not increase after filtration, nor was the concentration of triglycerides reduced. This suggests that the fat particles were trapped as a whole in the filter instead of being degraded by their passage through the filter. The fat removal did not decrease in the second 500 mL of the blood in the three filter types. This suggests that the fat removal capacity of the filters was not saturated after 1000 mL of the residual blood. The clinical measurement of fat particles is difficult for several reasons. First, it requires manual processing of the samples, which is expensive and may not be feasible when larger patient groups are involved. Second, the processed samples are assessed by phase contrast microscopy, which produces semiquantitative results. Recently, an automated method was proposed to measure the circulating fat content of the blood [9]. This method is based on the assessment of plots from automated haematology cell counters and would be suitable for larger patient groups. This method has been validated and applied to blood samples from orthopedic patients with good results [9]. Therefore, we also used this method in our study with cardiac surgical patients. The concentrations of elastase, complement, and free haemoglobin in the residual blood were highly variable between the patients. This might be caused by differences in CPB time, quantity of wound suction blood, and inflammatory reaction in the individual patient [18–20]. However, the surgical procedures were equally distributed over the three groups, and there was no correlation between the CPB time and the concentrations of elastase, complement, and free haemoglobin in the residual blood. We found only a small negative correlation between the quantity of residual blood and the plasma haemoglobin concentration. These findings suggest

that the differences in elastase and complement concentrations are caused by the individual patient's reaction, and thus, may be taken into account for the assessment of filter efficiency. The elastase and free haemoglobin concentrations increased more in the LipiGuard group than in the other two groups, indicating that under these specific clinical conditions, this filter damaged leukocytes and red blood cells by mechanical forces. In contrast, the low increase in complement C5–9 in the LipiGuard group suggests good blood compatibility. However, three LipiGuard filters became blocked before 1000 mL of the blood had passed. The trapped leukocytes also cannot explain the blocking of these filters as the leukocyte counts after the filter were higher than in the leukocyte removal filters. The activation of coagulation and platelets as well as cell debris caused by haemolysis might be an explanation for the blocking of these filters. Although this study is limited by the size of the three groups, we conclude that the two leukocyte removal filters were superior, both in leukocyte and fat removal, to the specific fat removal filter. Using a leukocyte removal filter, the capacity for residual heart–lung machine, with its concomitant low haemoglobin concentration, may safely be estimated to be 1000 mL. A short passage time and good biocompatibility may determine the choice for a leukocyte filter to remove leukocytes and fat particles from residual heart–lung machine blood.

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