Release of cytokines, polymorphonuclear elastase and terminal C5b-9 complement complex by infusion of wound drainage blood

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25 patients undergoing total hip replacement surgery were studied in an investigation of release of cytokines (interleukin-1ß, IL-1ß; interleukin-6, IL-6; interleukin-8, IL-8; and tumor necrosis factor-α, TNF-α), PMN elastase and terminal C5b-9 complement complexes (TCC) at the time of collection and transfusion of autologous blood. 15 patients received wound blood that was washed and centrifuged before being transfused as an erythrocyte suspension. In this blood there were no elevations in the concentrations of cytokines, TNF-α, PMN elastase or TCC, and there was no increase in these variables in plasma after transfusion of wound blood. 10 patients received postoperatively-collected drainage blood. There were high amounts of cytokines, PMN elastase and TCC in this blood, and filtration of the collected drainage blood did not reduce the concentrations of these factors, except those of TCC. When the collected drainage blood was infused, elevated plasma concentrations of IL-6, IL-8 and PMN elastase were observed 1 and 60 minutes after completing the transfusion. No differences regarding blood pressure, oxygen saturation (SpO_2), and hemoglobin concentration between the groups were recorded.

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The available techniques for collection and transfusion of wound blood lead to changes in the blood shed (Silva et al. 1984, McShane et al. 1987). Activation of the coagulation, fibrinolytic and complement systems have often been reported with transfusions of autologous blood (Griffith et al. 1989, Bengtson et al. 1990) and other investigators have described such complications as hemolysis, air embolization and the formation of microaggregates at the time of such procedures (Bretton et al. 1985). Sieunarine et al. (1990) showed that leukocytes are activated when systems for transfusion of washed erythrocytes are used, and that the washing procedure removes the formed polymorphonuclear (PMN) elastase. Activation of complement with extensive formation of the anaphylatoxins C3a and C5a was also found during the collection of drainage blood (Bengtson et al. 1990).

We studied two techniques for collection and transfusion of autologous blood, regarding the release of cytokines, PMN elastase and complement split products, and whether the washing procedure diminishes the infusion of inflammation-mediating substances.

Patients and methods

25 patients (20 women and 5 men) undergoing elective hip replacement were investigated. Spinal anesthesia with bupivacaine 15–20 mg and 0.2 mg morphine was used. All of the patients studied required blood transfusions and received autologous blood intraoperatively (wound blood group) or postoperatively (drainage blood group). The indications for infusion of collected blood were the same as those for transfusion of bank blood. The infusion was started when the patient had a hemoglobin value below 100 g/L or showed signs of hypovolemia. Blood pressure, heart rate, pulse oximetry saturation (SpO_2) and hemoglobin levels were recorded before and after transfusion of collected blood. Febrile reactions after transfusion were also recorded.

Wound blood group

Patients requiring blood transfusions intraoperatively (15) received blood via a Haemonetics Cell Saver® device. The collected wound blood was washed and centrifuged before transfusion and mixed with sodi-

um citrate in the suction equipment. The erythrocytes were concentrated by centrifugation and then washed with isotonic saline. Centrifuge bowels of 225 mL and a wash volume of 1500 mL were used. The supernatant was discarded and the washed red cells were relayed to the infusion bag. The supernatant was collected in the waste bag. Transfusion of washed erythrocytes was performed in two portions and blood samples from the patients were drawn preoperatively, 1 min before and 1 min after each transfusion.

5 mL blood was taken from the wound before starting the transfusion for determinations of IL-1ß, IL-6, IL-8, TNF-α, PMN elastase and C5b-9 complement complex (TCC) concentrations in the wound blood.

Washed erythrocytes were drawn from the infusion bag 1 min before the transfusion. Samples were also drawn from the waste bag containing the supernatant and saline.

Drainage blood group

Patients not requiring blood transfusion until the postoperative period (10) received an autologous drainage blood transfusion. The patients had not received homologous blood transfusions or autologous erythrocyte transfusions before the infusion of wound drainage blood. Shed drainage blood was mixed with 40 mL anti-coagulating citrate-dextrose solution (ACD) in the suction equipment (40 mL ACD solution contains 0.32 g citric acid, 0.88 g sodium citrate and 0.98 g glucose). A drainage suction system allowing infusion of aspirated wound drainage blood was employed (Solcotrans®, Solco Basle Ltd., UK) (Bengtson et al. 1990). A maximum negative pressure of 100 mm Hg was used.

IL-1ß, IL-6, IL-8, TNF-α, PMN-elastase and TCC determinations

The samples were taken 1 min before starting the infusion. All blood samples were drawn into tubes containing 0.054 mL of 0.34 M EDTA. The tubes were immediately centrifuged to remove the cells. Samples were frozen in separate tubes for each determination and the tubes were frozen within 30 min and stored at –80 °C. All determinations were performed in duplicate.

IL-1ß and IL-8 were determined with commercially available ELISA systems (Biotrak cytokines human ELISA systems, Amersham). The plasma concentration of IL-6 was measured with an immunoassay on microplates. A mouse monoclonal anti-human IL-6 antibody (5E1) for coating, a rabbit polyclonal anti-human IL-6 as the second antibody (the antibodies were a gift from W. Buurman, Maastricht, Netherlands) and recombinant human IL-6 (a gift from Dr. P Mayer, Sandoz Research Institute, Vienna, Austria) as the standard were used. The plasma concentrations of TNF-α were calculated according to the immunoassay described by Engelberts and coworkers (1991).

PMN elastase was determined with by the PMN elastase IMAC immunoassay (E. Merck, Darmstadt, Germany) (Fink et al. 1984, Dreher et al. 1989). For evaluation of complement activation, TCC was measured. The complex was determined with a double-antibody enzyme-linked immunosorbent assay (Mollnes et al. 1985). All the results are given as means of duplicate determinations.

Statistics

The results are given as medians and 25%–75% range of values. ANOVA-repeated measurements and Wilcoxon's test for unpaired comparisons were used for statistical evaluations. Differences were considered significant for $p < 0.05$.

Results

Age, peroperative bleeding, operating time and volume of returned autologous blood in the patients receiving drainage blood or washed erythrocyte transfusion, prosthesis type, fixation technique, indication for operation, blood pressure, heart rate, SpO_2 and hemoglobin levels before and after transfusions are given in Tables 1 and 2. One febrile reaction > 39 °C was recorded in the drainage blood group. There were no postoperative infections and there were no clinical or radiographic signs of implant loosening during the one-year follow-up in either of the two groups.

Wound blood group

In the wound blood there were no increased concentrations of IL-1ß, IL-6, TNF-α and PMN elastase compared to the concentrations 1 min before the start of the transfusion. The concentrations found in the suspension of washed erythrocytes were not increased, compared to the plasma concentrations. The IL-8 and TCC concentrations were increased in the samples drawn from the wound, compared to the concentrations found in the plasma (Table 3).

There were no differences in the plasma concentrations of cytokines, PMN elastase and TCC before and after the completed transfusions. The concentrations were not increased, compared to the concentrations found preoperatively or 1 min before the start of the transfusion (Table 3).

Table 1. Clinical data. Median (25%–75% range)

	Wound blood group	Drainage blood group
No. of patients	15	10
Age	69 (56–72)	62 (48–71)
Diagnosis: Arthrosis	12	10
Rheumatoid arthritis	3	–
Peroperative bleeding (mL)	930 (370–1560)	800 (500–1350)
Volume returned (mL)	490 (220–800)	400 (300–530)
Prosthesis type and fixation technique [a]	8 Harris-Galante II/Spectron (UC/C) 1 Harris-Galante II/PCA long (UC/C) 1 Harris-Galante II/Anatomica (UC/C) 1 Harris-Galante II (UC) 4 Spectron (C)	1 Harris-Galante II/Spectron (UC/C) 1 Harris-GalanteII/Ti-fit (UC/C) 3 PCA-E (UC) 5 Spectron (C)
Operation time (min)	165 (140–202)	135 (120–210)

[a] C cemented, UC Uncemented

Table 2. Physiological data related to blood transfusion

	Transfusion wound blood group		Transfusion drainage blood group	
	Before	After	Before	After
MAP (mmHg)	77 (69–86)	92 (83–93)	78 (70–83)	86 (77–88)
HR (beats/min)	80 (79–86)	75 (60–91)	88 (70–110)	80 (65–105)
SpO_2 (%)	99 (98–100)	99 (97–100)	97 (96–98)	98 (97–99)
Hb (g/L)	91 (80–117)	99 (97–120)	96 (90–111)	109 (107–113)

Table 3. Concentrations of cytokines, TNF-α, PMN elastase and TCC in the collected blood and in the plasma of the patients receiving wound blood. Median (25%–75% range)

	IL-1ß (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (pg/mL)	PMN elastase (pg/mL)	TCC (AU/mL)
Blood from the wound	<1.8 (<1.8)	<50 (<50–66)	88 (85–110)*	<5.0 (<5.0)	52 (<40–90)	3.4 (2.0–6.1)*
Reservoir blood	<1.8 (<1.8)	<50 (<50–428)	<47 (<47)	<5.0 (<5.0)	44 (40–120)	1.8 (1.2–2.8)
Waste bag fluid	<1.8 (<1.8)	<50 (<50–129)	<47 (<47–123)	<5.0 (<5.0)	77 (53–105)	<1.0 (<1.0–1.5)
Transfusion blood from the reinfusion bag	<1.8 (<1.8)	<50 (<50–53)	85 (<47–101)	<5.0 (<5.0)	50 (40–100)	<1.0 (<1.0)
Preoperatively	<1.8 (<1.8)	<50 (<50–53)	<47 (<47)	<5.0 (<5.0)	<40 (40–49)	<1.0 (<1.0)
1 min before transfusion 1	<1.8 (<1.8)	<50 (<50–56)	<47 (<47)	<5.0 (<5.0)	41 (<40–72)	<1.0 (<1.0)
1 min after transfusion 1	<1.8 (<1.8)	<50 (<50–65)	<47 (<47)	<5.0 (<5.0)	51 (<40–90)	<1.0 (<1.0)
1 min before transfusion 2	<1.8 (<1.8)	<50 (<50–66)	<47 (<47)	<5.0 (<5.0)	<40 (<40–73)	<1.0 (<1.0)
1 min after transfusion 2	<1.8 (<1.8)	56 (<50–72)	<47 (<47)	<5.0 (<5.0)	<40 (40–132)	<1.0 (<1.0)

Significance compared to the preoperative value; *$p < 0.05$

Drainage blood group

In the aspirated wound drainage blood, the concentrations of IL-1ß, IL-6, IL-8, PMN elastase and TCC were increased compared to the concentrations found in systemic blood. No elevated concentrations of TNF-α were found. There were differences regarding IL-1ß, IL-6, IL-8 , TNF-α and PMN elastase concentrations before and after the microporous filter (Table 4). The TCC concentrations were lower after filtration compared to those before the filter. However, the concentrations after the filter were higher than those in the systemic blood.

There were increased concentrations of IL-6 and IL-8 both 1 and 60 min after the transfusion when compared with the concentrations found preoperatively and 1 min before the start of the transfusion. The plasma concentrations of PMN elastase were also increased both 1 and 60 min after the transfusion compared to those found preoperatively and 1 min before the start of the transfusion. No alterations in plasma concentrations of IL-1ß, TNF-α and TCC

Table 4. Concentrations of cytokines, TNF-α, PMN elastase and TCC in the collected blood and in the plasma of the patients receiving drainage blood. Median (25%–75% range)

	IL-1ß (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (pg/mL)	PMN elastase (pg/mL)	TCC (AU/mL)
Transfusion blood						
before the filter	5.2 (<1.8–10)*	8900 (4000–11400)**	960 (280–4200)*	<5.0 (<5.0)	525 (318–640)**	18 (16–32)**
after the filter	3.9 (2.2–8.7)*	8800 (3100–10200)**	1490(380–5450)*	<5.0 (<5.0)	522 (229–660)**	9.7 (5.9–21)**
Preoperatively	<1.8 (<1.8)	<50 (<50)	<47 (<47–49)	<5.0 (<5.0)	<40 (<40–47)	1.6 (1.3–1.8)
1 min before tf [a]	<1.8 (<1.8)	93 (<50–132)	<47 (<47–66)	<5.0 (<5.0)	68 (<40–92)	1.1 (1.0–1.7)
1 min after tf [a]	<1.8 (<1.8)	244 (119–458)**	160 (<47–320)*	<5.0 (<5.0)	168 (114–276)*	1.7 (1.1–2.9)
60 min after tf [a]	<1.8 (<1.8)	125 (88–202)**	133 (115–210)*	<5.0 (<5.0)	116 (61–229)*	1.3 (<1.0–1.5)

[a] tf transfusion.
Significance compared to the preoperative value; *$p < 0.05$, **$p < 0.001$.

were found in association with transfusion of collected wound drainage blood (Table 4).

Discussion

Two essentially different types of equipment for transfusion of shed blood are available: the centrifuge-based cell salvage system and the canister collection system. In the centrifuge-based method the blood is washed and centrifuged before transfusion as an erythrocyte concentrate. Blood collected in a canister can be concentrated and washed with standard blood-bank washing instruments, or it can be transfused after filtration only.

The safety of the blood collecting systems has been discussed. Especially the transfusion of unwashed, filtered, shed blood may be hazardous as the coagulation and fibrinolytic systems are already activated in the wound blood (Griffith et al. 1989). Complement activation in collected drainage blood has also been pointed out (Bengtson et al. 1990). Split products of the complement cascade (C3a and C5a) are potent activators of leukocytes (Webster et al. 1980). Such an activation may lead to the release of lysosomal enzymes and cytokines (Okusawa et al. 1988, Scholz et al. 1990). The cytokines IL-1, IL-6, IL-8 and TNF-α are known to be released in septic situations and in association with trauma and hemorrhage (Waage et al. 1989, Redl et al. 1991). Thus, there is a risk of the occurrence of microaggregate formation (Yawn 1989), hemolysis and renal dysfunction (Emergency Care Research Institute 1990). The hemolysis may be caused by mechanical damage to erythrocytes during processing of the blood by negative pressure during suction, foaming, and contact with air and foreign material. Mediators are activated when blood comes in contact with foreign materials and activation of complement, secretion of PMN elastase and release of cytokines have been demonstrated in various types of extracorporeal circulation procedures (Haeffner-Cavaillon et al. 1989a, b, Arnestad et al. 1992, Butler et al. 1993). Complement activation and the formation of TCC may also lead to hemolysis (Bengston et al. 1990). After transfusion of unwashed, filtered, shed blood, hypotension, hypothermia, and febrile reactions have been reported (Faris et al. 1991, Clements et al. 1992). It has also been observed that stored platelet concentrates contain increased levels of cytokines (Stack and Snyder 1994). Febrile non-hemolytic reactions in patients receiving platelet transfusion have been demonstrated (Muylle et al. 1993). In our study one of the patients transfused with drainage blood had a febrile reaction. In a recent case report of upper airway edema after whole blood transfusion, the possible role of complement activation was discussed (Woda and Tetzlaff 1992). Complement-induced granulocyte aggregation has been suggested as an etiologic factor in non-cardiogenic pulmonary edema. There is a risk of prolonged anemia when unwashed, filtered, shed blood is used, as inflammatory cytokines play a role in the regulation of erythropoietin production. IL-1ß as well as TNF-α are capable of suppressing hypoxic induction of erythropoietin, whereas IL-6 seems to have a stimulating effect (Faquin et al. 1992).

When a small volume of blood is infused, there are few side-effects. With large volumes, there is substantial evidence that unwashed, filtered, shed blood is dangerous due to activation of the coagulation and fibrinolytic system, activation of complement, activation of leukocytes, release of cytokines and a large amount of free hemoglobin.

In our study, intraoperative infusion of centrifuged and washed autologous erythrocytes did not alter the plasma concentrations of IL-1ß, IL-6, IL-8, TNF-α, PMN elastase or TCC in the suspension of erythrocytes and in the patient. This was not the case when

drainage blood was infused postoperatively. However, when large volumes are needed, we suggest the use of a centrifuge-based cell salvage device instead of a canister collection system.

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