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Complement split products and pro-inflammatory cytokines in salvaged blood after hip and knee arthroplasty

Purpose: To determine whether salvaged autologous blood collected postoperatively contains complement split products (SC5b-9), and pro-inflammatory cytokines (IL-6 and IL-8) and whether there are any differences between blood collected during hip or knee surgery.

Methods: Fifty-eight consecutive patients undergoing hip or knee replacement surgery were studied. Thirty-eight had postoperative bleeding large enough to require infusion of salvaged blood. The salvaged blood was filtered during collection through a 200 µm filter and before infusion a 40 µm filter was used. Samples for complement and cytokine determinations were drawn from the circulation and from the collected blood.

Results: High concentrations of SC5b-9, IL-6, and IL-8 were found in salvaged blood. The concentrations were higher than in the circulation ($P < 0.05$). The circulating concentrations of IL-6 and IL-8 were increased 60 min and 12-18 hr after transfusion. There were no differences regarding SC5b-9, IL-6, and IL-8 in the blood collected after hip or knee surgery.

Conclusion: Blood collected from a surgical wound contains large concentrations of inflammatory mediators. There were no differences between blood collected during hip or knee surgery.

Objectif : Déterminer si la récupération postopératoire de sang autologue contient des produits de dégradation du complément (SC5b-9) et des cytokines pro-inflammatoires (IL-6 et IL-8), et s'il y a des différences avec le sang prélevé pendant l'opération de la hanche ou du genou.

Méthode : L'étude a porté sur 58 patients successivement admis pour la mise en place d'une prothèse de hanche ou du genou. Chez 38 d'entre eux, les pertes sanguines postopératoires ont été assez importantes pour nécessiter la transfusion de sang récupéré. Ce sang a été filtré pendant le prélèvement avec un filtre de 200 µm et avant la transfusion avec un filtre de 40 µm. Des échantillons retenus de la circulation et du sang récupéré ont servi à déterminer la cytokine et le complément.

Résultats : De fortes concentrations de SC5b-9, IL-6 et IL-8 ont été trouvées dans le sang récupéré. Elles étaient plus élevées que celles de la circulation ($P < 0,05$). Les concentrations circulantes de IL-6 et de IL-8 ont augmenté 60 min et 12-18 h après la transfusion. Aucune différence n'a été enregistrée concernant les SC5b-9, IL-6 et IL-8 dans le sang prélevé après l'opération de hanche ou du genou.

Conclusion : Le sang prélevé au site d'incision chirurgicale contient de grandes concentrations de médiateurs de l'inflammation. Ce sang ne présente pas de différence avec celui qui est prélevé pendant une opération de hanche ou du genou.

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SEVERAL alternatives to allogeneic blood transfusion have been proposed.¹⁻³ Different techniques for autologous transfusions have been developed. Autotransfusion can be subdivided depending on the extent to which the scavenged blood is processed prior to infusion.⁴ In filter systems the salvaged blood is anti-coagulated and filtered, whereas in other autotransfusion systems the salvaged blood is washed and centrifuged prior to infusion.

Knee replacement surgery is normally performed in a blood-free field after the application of a tourniquet. The effect on complement activation and cytokine release by the blood-free field is not known. It has been demonstrated previously that collection and infusion of wound blood leads to activation of the coagulation, fibrinolytic and complement systems⁵⁻⁸ and TNF-, IL-6, IL-8 and PMN elastase are formed during salvage.⁸

The purpose of this study was to determine whether salvaged blood collected postoperatively contains complement split products (SC5b-9), and pro-inflammatory cytokines (IL-6 and IL-8) and whether there are any differences between blood collected during knee or hip surgery.

Methods

The Ethics Committee of Sahlgrenska University Hospital, Gothenburg, Sweden approved the study. Fifty-eight consecutive patients undergoing elective hip or knee replacement surgery were studied. Thirty patients underwent hip surgery and 28 knee surgery. Their ages ranged from 36-90 yr (median 72 yr). Spinal anesthesia with was used. Knee replacement surgery was performed in a blood-free field. The tourniquet was applied with a pressure of 100 mmHg above the patients systolic blood pressure. Postoperatively a drainage suction system allowing infusion of aspirated wound blood without using anticoagulation was used (Bellovac® A.B.T, AstraTech, Sweden).

Determinations of SC5b-9, IL-6, IL-8, PMN elastase, hemoglobin, free hemoglobin, hematocrit, leukocytes, platelets, sodium and potassium were made preoperatively, one minute before the start of infusion, and at 60 min and 12-18 hr after completed infusion. Samples were drawn from the infusion bag one minute before start of infusion and eight hours after start of collection of blood. SC5b-9 was determined with a double-antibody enzyme-linked immunosorbent assay. IL-6 and IL-8 were determined with an EIA test (R&D Systems, Abingdon, UK) and PMN elastase was determined by an ELISA method (DPC, Los Angeles, USA).

Bacterial cultures were performed from all the infusion bags.

Statistics

Median values and ranges are given. Comparisons were made by repeated ANOVA followed by Wilcoxon signed rank test.

Results

There were no differences between the volumes of collected blood obtained during hip and knee surgery. Sixteen of 28 knee surgery patients received a median of 504 mL salvaged blood and 22 of 30 hip surgery patients received a median of 363 mL salvaged blood.

Hemoglobin concentrations in patient blood were lower before the start of infusion, 60 min and 12-18 hr after infusion than the preoperative levels ($P < 0.05$). Higher concentrations of free hemoglobin were found in the infusion bags than in circulating

TABLE I Hemoglobin, free-hemoglobin, hematocrit, sodium, potassium, SC5b-9, IL-6, IL-8 and PMN elastase in shed blood just before reinfusion and eight hours after activation of drainage. Median and ranges are given.

	Before reinfusion (from bag)	8 hr after activation of drainage (from reinfusion bag)
<i>Hemoglobin (g·L⁻¹)</i>		
Hip surgery	81 (64-114)	74 (43-110)
Knee surgery	91 (60-120)	85 (49-117)
<i>Free hemoglobin (mg·L⁻¹)</i>		
Hip surgery	2350 (1390-5000)	2500 (1050-4800)
Knee surgery	932 (249-1750)	1170 (376-10800)
<i>Hematocrit (%)</i>		
Hip surgery	24 (19-35)	23 (13-34)
Knee surgery	28 (18-35)	26 (16-37)
<i>Leukocyte count (x10⁹·L⁻¹)</i>		
Hip surgery	3.0 (1.2-7.5)	3.2 (1.8-10.9)
Knee surgery	3.4 (0.5-6.5)	3.0 (0.4-9.8)
<i>Platelets (x10⁹·L⁻¹)</i>		
Hip surgery	30 (1-98)	52 (9-204)
Knee surgery	16 (1-56)	27 (1-201)
<i>Sodium (mmol·L⁻¹)</i>		
Hip surgery	140 (131-146)	139 (135-146)
Knee surgery	142 (139-146)	142 (135-147)
<i>Potassium (mmol·L⁻¹)</i>		
Hip surgery	4.9 (3.9-5.9)	5.0 (3.9-6.6)
Knee surgery	4.8 (4.0-6.0)	4.9 (3.8-7.9)
<i>SC5b-9 (ng·mE⁻¹)</i>		
Hip surgery	2471 (631-10000)	2370 (430-25415)
Knee surgery	829 (138-3400)	1220 (318-6480)
<i>IL-6 (pg·mL⁻¹)</i>		
Hip surgery	4780 (162-65700)	4532 (164-41400)
Knee surgery	4680 (252-56000)	5100 (218-36600)
<i>IL-8 (pg·mL⁻¹)</i>		
Hip surgery	1420 (55-10800)	1530 (82-6330)
Knee surgery	880 (90-10400)	1650 (44-8580)
<i>PMN-elastase (ng·mL⁻¹)</i>		
Hip surgery	1062 (480-2820)	1180 (68-3940)
Knee surgery	638 (127-2190)	925 (180-3007)

TABLE II Hemoglobin, free-hemoglobin, hematocrit, leukocyte count and platelet count, sodium, potassium, SC5b-9 and PMN elastase in the patient plasma in the patients receiving drainage blood. Median and ranges are given.

	<i>Preoperatively</i>	<i>1 min before reinfusion</i>	<i>60 min after reinfusion</i>	<i>12-18 hr after reinfusion</i>
<i>Hemoglobin (g·L⁻¹)</i>				
Hip surgery	133 (112-162)	108 (86-145)	105 (85-146)	102 (77-138)
Knee surgery	134 (98-162)	123 (94-137)	123 (89-135)	116 (82-128)
<i>Free-hemoglobin (mg·L⁻¹)</i>				
Hip surgery	83 (10-4920)	42 (10-1950)	60 (23-540)	44 (10-513)
Knee surgery	78 (11-2050)	51 (12-1210)	82 (21-1050)	41 (10-400)
<i>Hematocrit (%)</i>				
Hip surgery	41 (33-49)	34 (27-45)	33 (27-46)	31 (24-42)
Knee surgery	41 (30-49)	38 (27-41)	38 (29-41)	35 (24-39)
<i>Leukocyte count (x10⁹·L⁻¹)</i>				
Hip surgery	5.0 (3.4-11.0)	10.7 (6.3-14)	11.4 (6.6-18)	7.8 (5.4-13.8)
Knee surgery	5.0 (2.6-8.0)	7.3 (4.1-15.4)	9.5 (7.3-16.2)	8.5 (6.3-11.6)
<i>Platelets (x10⁹·L⁻¹)</i>				
Hip surgery	239 (168-459)	204 (137-313)	182 (116-300)	191 (113-305)
Knee surgery	225 (144-420)	198 (140-256)	195 (118-261)	185 (134-254)
<i>Sodium (mmol·L⁻¹)</i>				
Hip surgery	141 (137-147)	139 (137-145)	140 (136-145)	139 (134-144)
Knee surgery	142 (88-148)	141 (136-145)	141 (118-144)	139 (135-142)
<i>Potassium (mmol·L⁻¹)</i>				
Hip surgery	4.2 (3.3-5.5)	4.2 (3.5-5.1)	4.3 (3.5-5.0)	4.1 (3.4-4.6)
Knee surgery	4.1 (2.8-4.9)	4.2 (3.5-5.2)	4.1 (2.4-4.8)	4.0 (3.6-4.5)
<i>SC5b-9 (ng·mL⁻¹)</i>				
Hip surgery	119 (22-818)	107 (20-741)	121 (32-715)	121 (59-747)
Knee surgery	132 (29-892)	71 (0-742)	74 (39-790)	115 (51-696)
<i>PMN-elastase (ng·mL⁻¹)</i>				
Hip surgery	45 (0-201)	76 (32-133)	103 (0-290)	74 (19-158)
Knee surgery	40 (0-82)	62 (25-367)	78 (0-210)	76 (45-152)

blood ($P < 0.001$). There were no differences between hip and knee surgery regarding hemoglobin, hematocrit or free hemoglobin (Tables I, II). The leukocyte count was increased before the start of infusion, 60 min and 12-18 hr after infusion compared with preoperative levels ($P < 0.05$). The platelets count was decreased in the infusion bag compared with that in circulating blood ($P < 0.05$).

The concentrations of SC5b-9, IL-6, IL-8 and PMN elastase were increased in salvaged blood compared with systemic blood (Tables I, II). There were no differences in patients undergoing hip or knee replacement. There were increased concentrations of IL-6 and IL-8 both at 60 min ($P < 0.05$) and at 12-18 hr ($P < 0.05$) compared with concentrations found preoperatively (Figures 1, 2). The concentrations in plasma of SC5b-9 and PMN elastase remained unchanged.

One anaerobic culture was positive for Coagulase negative Staph Aureus bacteria. No cultures were positive from circulating blood.

Discussion

This study demonstrated that salvaged blood contains high concentrations of SC5b-9, IL-6, IL-8 and PMN

elastase. After infusion of filtered salvaged blood, hypotension, hyperthermia and febrile reactions have been reported.^{4,9,10} Febrile non-hemolytic reactions occur in patients receiving platelet transfusions.⁴ In the present study there were no signs of adverse reactions that could be explained by salvaged blood infusion. Salvaged blood also contains high concentrations of free hemoglobin. However, the concentrations of free hemoglobin in the circulation did not increase after infusion of salvaged blood.

The study demonstrated that complement is activated in salvaged blood. Increased plasma concentrations of complement split products, IL-6 and IL-8 may be due to either the infusion itself or the surgical trauma. However, it is not possible to draw any conclusions regarding the origin of the activation. The salvaged blood had been exposed to tissue factors at the operation site, to air, and to synthetic material of the collection equipment. These factors may contribute to the activation of the inflammatory systems.

Filter systems seem to be safe when a low volume of salvaged blood is returned. Filter systems are generally easier and less expensive to handle. In the present study no anticoagulation was used in the infusion

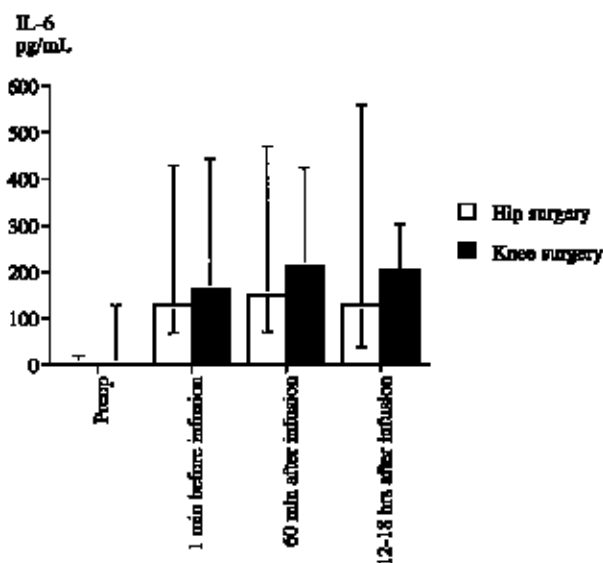


FIGURE 1 IL-6 preoperatively, one minute before start of infusion, 60 min and 12-18 hr after completed infusion in patients undergoing hip surgery (open bars) and patients undergoing knee surgery (filled bars). Median and ranges are given. In hip surgery the median IL-6 concentration in the infusion bag was one minute before start of infusion $4780 \text{ pg}\cdot\text{mL}^{-1}$ (range $162\text{-}65700 \text{ pg}\cdot\text{mL}^{-1}$) and eight hours after start of collection of salvaged blood the median concentration was $4532 \text{ pg}\cdot\text{mL}^{-1}$ (range $164\text{-}41400 \text{ pg}\cdot\text{mL}^{-1}$). In knee surgery the median IL-6 concentration in the infusion bag one minute before start of infusion was $4680 \text{ pg}\cdot\text{mL}^{-1}$ (range $252\text{-}56000 \text{ pg}\cdot\text{mL}^{-1}$) and eight hours after start of collection of salvaged blood the median concentration was $5100 \text{ pg}\cdot\text{mL}^{-1}$ (range $218\text{-}36600 \text{ pg}\cdot\text{mL}^{-1}$).

system. There were no reported problems with coagulation of the salvaged blood.

Depending on the surgical situation, the hemoglobin and hematocrit in unprocessed salvaged blood are low. If a small volume of blood is infused, there are few side effects. On the other hand, if a large volume of blood is needed, filtered salvaged blood may be dangerous due to activation of the complement cascade and the release of pro-inflammatory cytokines.

This study showed that blood collected from a surgical wound contains large concentrations of inflammatory mediators. There were no differences between blood collected during knee or knee surgery.

Acknowledgments

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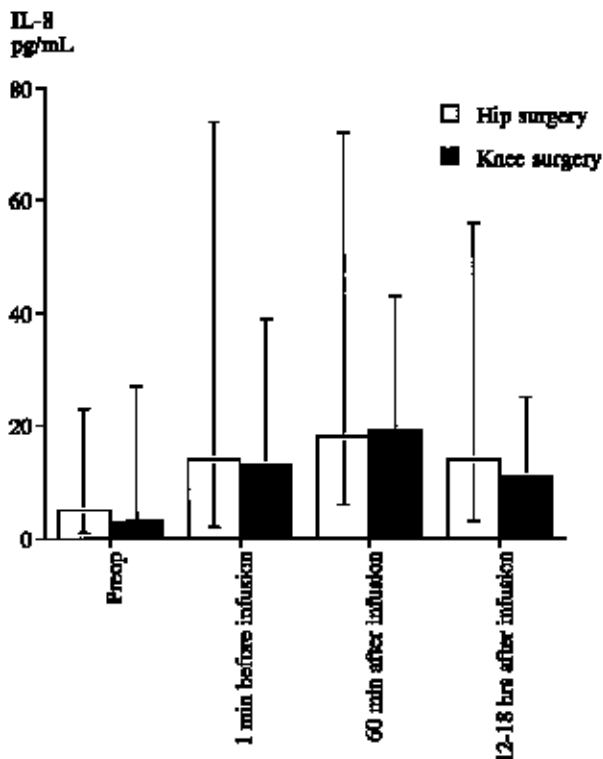


FIGURE 2 IL-8 preoperatively, one minute before start of infusion, 60 min and 12-18 hr after completed infusion in patients undergoing hip surgery (open bars) and patients undergoing knee surgery (filled bars). Median and ranges are given. In hip surgery the median IL-8 concentration in the infusion bag one minute before start of infusion was $1420 \text{ pg}\cdot\text{mL}^{-1}$ (range $55\text{-}10800 \text{ pg}\cdot\text{mL}^{-1}$) and eight hours after the start of collection of salvaged blood the median concentration was $1530 \text{ pg}\cdot\text{mL}^{-1}$ (range $82\text{-}6330 \text{ pg}\cdot\text{mL}^{-1}$). In knee surgery the median IL-8 concentration in the infusion bag one minute before start of infusion was $880 \text{ pg}\cdot\text{mL}^{-1}$ (range $90\text{-}10400 \text{ pg}\cdot\text{mL}^{-1}$) and eight hours after start of collection of salvaged blood the median concentration was $1650 \text{ pg}\cdot\text{mL}^{-1}$ (range $44\text{-}8580 \text{ pg}\cdot\text{mL}^{-1}$).

References

- 1 *Ereth MH, Oliver WC Jr, Santrach PJ.* Perioperative interventions to decrease transfusion of allogeneic blood products. *Mayo Clin Proc* 1994; 69: 575-86.
- 2 *Weiskopf RB.* More on the changing indications for transfusion of blood and blood components during anesthesia (Editorial). *Anesthesiology* 1996; 84: 498-501.
- 3 *Williamson KR, Taswell HF.* Intraoperative blood salvage: a review. *Transfusion* 1991; 31: 662-75.
- 4 *Bengtsson A, Bengtson JP.* Autologous blood transfusion: preoperative blood collection and blood salvage techniques. *Acta Anesthesiol Scand* 1996; 40: 1041-56.

- 5 Krohn CD, Reikerås O, Aasen AO. Inflammatory cytokines and their receptors in arterial and mixed venous blood before, during and after infusion of drained untreated blood. *Transfus Med* 1999; 9: 125–30.
- 6 Arnestad JP, Bengtsson A, Bengtson JP, Johansson S, Redl H, Schlag G. Release of cytokines, polymorphonuclear elastase and terminal C5b-9 complement complex by infusion of wound drainage blood. *Acta Orthop Scand* 1995; 66: 329–33.
- 7 Krohn CD, Reikerås O, Mollnes TE, Aasen AO. Complement activation and release of interleukin-6 and tumour necrosis factor- in drained and systemic blood after major orthopaedic surgery. *Eur J Surg* 1998; 164: 103–8.
- 8 Arnestad JP, Bengtsson A, Bengtson JP, Tylman M, Redl H, Schlag G. Formation of cytokines by retransfusion of shed whole blood. *Br J Anaesth* 1994; 72: 422–5.
- 9 Busund R, Balteskard L, Rønning G, Høgåsen K, Revhaug A. Fatal myocardial depression and circulatory collapse associated with complement activation induced by plasma infusion in severe porcine sepsis. *Acta Anesthesiol Scand* 1995; 39: 100–8.
- 10 Blevins F T, Shaw B, Valeri C R, Kasser J, Hall J. Reinfusion of shed blood after orthopaedic procedures in children and adolescents. *J Bone Joint Surg* 1993; 75-A: 363–71.