Intensive Care Med (1995) 21:989–995

ORIGINAL

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Cytokine and coagulation characteristics of retrieved blood after arthroplasty

Received: 18 February 1994
Accepted: 14 January 1995

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Abstract *Objectives:* To investigate cytokine and coagulation/fibrinolysis characteristics in blood retrieved from wounds using an autotransfusion system, and to compare the cytokine pattern in the retrieved blood with those in the systemic circulation and in the initial portion of drainage blood from the wound.
Design: Prospective controlled clinical study.
Setting: The postoperative ward of a University hospital.
Patients and participants: Blood retrieval was performed over a period of 4–6 h on patients who had just undergone arthroplasty (nine hips, one knee). In five other cases involving hip arthroplasties, the initial portion of drainage blood was studied.
Measurements and results: Coagulation/fibrinolysis parameters were analyzed in blood retrieved using the Stryker Consta Vac system. Concentrations of tumor necrosis factor α (TNF), interleukin-1 β (IL-1) and interleukin-6 (IL-6) were analyzed in the retrieved blood, in the systemic circulation of the patients at the beginning and at the end of blood retrieval and in the initial portion of drainage blood from the surgical area. In the retrieved blood, the activities of thrombin, kallikrein and plasmin were increased, antithrombin and free protein S were decreased, and in all samples IL-6 was >1000 pg/ml. Postoperative plasma concentrations of IL-6 rose from a median value of 0 to 116 pg/ml ($p<0.01$). Four patients had circulating TNF concentrations (range: $<15-50$ pg/ml). Plasma IL-1 was not detected. TNF and IL-1 were detected in all samples of initial blood from the surgical area and IL-6 in one sample.
Conclusion: Hypercoagulability and high concentrations of IL-6 were present in the retrieved blood. The cytokine pattern in the initial portion of blood from the surgical area differed from those in the retrieved blood and in the systemic circulation.

Key words Interleukin-1 β · Interleukin-6 · Tumor necrosis factor α · Autotransfusion · Fibrinolysis · Coagulation

Introduction

The transfusion of allogeneic blood and of blood products involves the risk of classical transfusion reactions. The risk of transferring infectious agents is also a consideration [1]. Furthermore, immunologic dysfunction, reflected by such indicators as decreased natural killer-cell activity, has been associated with allogeneic transfusions [2, 3]. Although the clinical significance of these findings

is uncertain, increased subsequent risk of infection and of tumor relapse has been proposed [4, 5]. Accordingly, for reasons of both economy and safety, increasing interest has developed in systems for the salvage and subsequent retransfusion of autologous blood.

Shed blood is processed to differing degrees in the various blood retrieval systems available. In advanced systems, such as the Cell Saver system (Haemonetics, Boston, Mass., USA), the blood is anticoagulated, washed and filtrated [6]. In closed-loop systems, the processing of the shed blood is less sophisticated and is performed both with and without anticoagulants [7]. In one study, patients who had undergone hip arthroplasty received intraoperatively shed blood that had been anticoagulated with citrate using a cell-saver autotransfusion system, Electromedic Autotrans AT-100 (Englewood, Colo., Mass. USA). The coagulation factors and inhibitors did not differ postoperatively from those of a group that had received bank blood [8]. However, variables demonstrating whether thrombin had been present were not investigated.

Stryker Consta Vac (Stryker, Kalamazoo, Mich., USA) is a closed-loop retransfusion system in which no anticoagulant is used. In one study using a similar system, Solcotrans (Solco Basle, Hingham, USA), an increase in the concentration of fibrin cleavage products and a decrease in the concentration of antithrombin were observed in salvaged blood [9]. A slight decrease in antithrombin was found in patients 24 h after infusion of two units of salvaged blood. Fibrin cleavage products increased 1 h after transfusion but returned to normal values after 24 h.

Tissue trauma involves a local response: the acute inflammatory reaction. Cytokines such as tumor necrosis factor α (TNF), interleukin-1 β (IL-1) and interleukin-6 (IL-6) – important mediators in trauma [10, 11] and in infection [12, 13] – are secreted locally [14]. The extent of the systemic response is due to various factors, e.g. the magnitude of the surgical procedure [11] and of hemorrhage [15, 16]. Some of the cytokines (e.g. TNF and IL-1) have been reported to activate the coagulation system [17, 18].

The objectives of this study were firstly to characterize blood retrieved using the Stryker Consta Vac system with respect to coagulation and fibrinolysis and, secondly, to compare the concentrations of TNF, IL-1 and IL-6 in retrieved blood with those in the systemic circulation during the period of blood salvage and with those in the initial portion of drainage blood from a surgical wound.

Materials and methods

In an inital study (study A), ten patients, aged 58–86 years (median: 73) and undergoing arthroplasty due to arthrosis, were investigated. In this study, the coagulation and cytokine characteristics of retrieved blood maintained in the Stryker Consta Vac® system were analyzed. The type of arthroplasty and anesthesia, the duration of surgery and the number of units of red cells transfused are given in Table 1. Epidural anesthesia was performed with etidocain combined with adrenaline and spinal anesthesia, with hyperbaric bupivacaine. General anesthesia was performed with thiopentone, midazolam and fentanyl, supplemented with isoflurane in nitrous oxide and oxygen. A lumbar approach was used for regional blocks and the level of anesthesia varied between T-7 and T 10. Muscle relaxation during general anesthesia was achieved by suxamethonium for intubation and by pancuronium for peroperative maintenance. Three patients (3, 4 and 9, Table 1) had been treated preoperatively with nonsteroid antiinflammatory drugs (NSAID) and two patients (5 and 7, Table 1), with beta-adrenoceptor-blocking drugs. There were no cases of renal or hepatic failure. All patients preoperatively received 500 ml of dextran 70 (Macrodex, Kabi Pharmacia, Uppsala, Sweden), as thromboprophylaxis. Local anesthetics (bupivacaine) were administered postoperatively via the epidural catheter for pain relief. In the other patients, postoperative analgesia was accomplished with meperidine. The Stryker Consta Vac drainage

Table 1 Individual data (*IL* interleukin, *TNF* tumor necrosis factor)

Patient no.	Arthroplasty	Anesthesia	Age (years)	Sex (M/F)	Height/weight (cm/kg)	Duration of surgery (h)	Erythrocytes (number of units[a] transfused)	TNF[b] (pg/ml)	IL-6[b] (pg/ml)
1	Hip arthroplasty	Epidural	86	F	172/64	1.3	5	<15/<15	48/227
2	Hip arthroplasty	Epidural	74	F	159/60	2.3	0	<15/<15	0/47
3	Hip arthroplasty	Spinal	73	F	155/64	2.3	0	<15/15	3/22
4	Hip arthroplasty	Epidural	58	F	155/60	1.3	1	<15/<15	0/79
5	Hip arthroplasty	Spinal	72	M	183/105	1.25	0	50/<15	0/161
6	Hip arthroplasty	General	73	F	159/50	1.6	3	20/<15	0/271
7	Hip arthroplasty	Epidural	84	M	178/70	2.1	0	<15/<15	0/58
8	Hip arthroplasty	Epidural	71	M	175/77	1.25	0	15/<15	0/178
9	Knee arthroplasty	General	68	F	159/77	2.0	0	<15/<15	0/54
10	Hip arthroplasty	Epidural	72	F	172/88	2.3	0	<15/<15	0/154
Mean ± SEM			73 ± 2		167 ± 3/72 ± 5	1.9 ± 0.2			5 ± 4.8/ 125 ± 27

[a] One unit ≈ 300 ml of packed red cells in SAGM solution
[b] Circulating plasma concentrations at the beginning/end of the period of blood retrieval

was adapted at the end of the operation using a suction level of 60 mmHg. The blood was collected via the polyvinylchloride tubing system, which drains through a 240-μm prefilter into an 800-ml container. The retrieval of blood ceased after 6 h or when the capacity of the container (800 ml) was reached. After salvage, the blood was transferred from the container into a transfusion bag and immediately passed via a 40-μm screen filter before sampling. No blood was retransfused to the patients.

In a second study (study B) involving five patients undergoing hip arthroplasty due to arthrosis, the initial portion of blood drained from the wound was analyzed for cytokine content. All patients in the second study received epidural anesthesia performed as described above. Two patients (12 and 15, Table 3) had been treated preoperatively with NSAID.

Cytokine concentrations in healthy volunteers, six males and six females, aged 18–56 years (median: 36), were used for comparison. The coagulation/fibrinolysis and plasma (P) hemoglobin values in the retrieved blood were compared with normal reference values. Informed consent was obtained. The protocol was approved by the local Ethics Committee.

Experimental protocol and sampling

In study A, samples were obtained from the transfusion bags in the Stryker Consta Vac system by allowing 4.5 ml of blood to drip into 5-ml open vacutainer test tubes containing one part of 0.5 ml 0.129 M trisodiumcitrate, pH 7.4, and nine parts of blood. Tubes without additive were used for serum. The tubes were turned several times and, after 10 min of centrifugation at 700 G, the plasma was aliquoted into plastic tubes and frozen at −70 °C until analysis. A 10-ml blood sample was collected from the transfusion bags for determination of TNF, IL-1 and IL-6 and was handled in the same way, except that heparinized tubes were used. Blood samples for analysis of P-hemoglobin in retrieved blood were collected from the transfusion bags in citrated tubes. The blood was allowed to drip into the tubes. The tubes were gently mixed and centrifuged at 800 G for 10 min. The supernatant plasma was removed and recentrifuged for 5 min to assure that all erythrocytes were removed. The plasma samples were then stored at −70 °C until analysis.

In study A, venous blood samples for cytokine analysis were obtained, one at the time of the application of the drainage system (approximately 2 h after skin incision) and one at the end of the period of blood retrieval (6–8 h after skin incision) for determination of circulating TNF, IL-1 and IL-6 concentrations. These blood samples were obtained in heparinized tubes and handled as described above.

In a separate experiment, a 5-ml sample of blood from one transfusion bag of the Stryker Consta Vac was taken in a 10-ml tube without anticoagulant and immediately centrifuged. The supernatant was then added to a coagulation system, using fibrinogen as a substrate. This experiment was designed to test the thrombin activity.

In study B, designed to study the cytokine content in the initial portion of drainage blood, a 10-ml blood sample was obtained immediately after the drainage was adapted for determination of plasma concentrations of TNF, IL-1 and IL-6. The samples were obtained by allowing blood to drip via the drainage tubing into a Vacutainer tube. These samples were handled as outlined above.

In the control group, venous blood samples for plasma cytokine analyses were obtained between 7:00 and 7:30 a.m., and were handled in the same way. All tubes were from Becton Dickinson, Plymouth, England.

Analytical procedures

P-protein C [19], P-antithrombin [20] and soluble P-fibrin (earlier named fibrin monomers) [21] were analyzed with amidolytic methods, using chromogenic peptide substrates with kits from Chromogenix (Mölndal, Sweden). P-thrombin-antithrombin complexes [22] and P-prothrombin fragment 1+2 [23] were analyzed by enzyme-linked immunosorbent assays (ELISA) using kits from Behringwerke (Marburg, Germany). Free P-protein S [24] and P-D-dimer [25] were analyzed by ELISA with kits from Stago (Asnières, France) and Mabco (Brisbane, Australia), respectively. Spontaneous proteolytic activity was measured in principle according to Blombäck et al. [26] using chromogenic substrates S-2238, S-2302 and S-2403 from Chromogenix (Mölndal, Sweden). As the substrates are sensitive to several enzymes, inhibitors (soybean trypsin inhibitor, Sigma St. Louis, Mo., USA and hirudin from Pentapharm, Basle, Switzerland) were added to make the system more enzyme specific. Direct thrombin activity was measured using 0.4% human fibrinogen (Imco, Stockholm, Sweden) in TRIS buffer as substrate. A mixture consisting of 0.2 ml fibrinogen and 0.2 ml supernatant of the retrieved blood or 0.15 M NaCl as control was incubated at 37 °C for 24 h and clot formation was studied at regular intervals.

Cytokine analyses (TNF, IL-1, IL-6) were performed using commercial enzyme immunoassays (EASIA, Medgenix Diagnostics, Fleurus, Belgium). These tests are based on an oligoclonal capture antigen system in which several monoclonal antibodies directed against distinct epitopes of the cytokines are used, allowing for high sensitivity and a short incubation time. The assays were performed according to the manufacturer's instructions directly on thawed samples without any treatment or extraction. The detection limits for TNF and IL-1 is 15 pg/ml. The coefficient of variation is $< 10\%$ between assays.

The spectrophotometric method described by Standefer and Vanderjagt [27] with the modifications of Geissler and Stith [28] was used for the P-hemoglobin assay.

Statistical analyses

Values are presented as the means ± SEM. Medians are given within parentheses. Comparisons of the plasma cytokine concentrations in retrieved blood with the circulating concentrations in patients and in controls were made using the Wilcoxon two-sample test (Mann-Whitney). This test was also used for comparison of patient plasma cytokine concentrations with that of controls. The increase in circulating plasma IL-6 concentrations during the study was analyzed using the Wilcoxon matched-paired rank test. A P-value of < 0.05 was considered significant. The concentrations of the coagulation and fibrinolysis parameters in retrieved blood were evaluated against the normal reference plasma values of our laboratory.

Results

The amount of retrieved blood (study A) ranged from 350 ml to 800 ml (median: 525 ml). The duration of blood salvage was 6 h in all patients except 4 and 5 (Table 1). In these patients, the 800-ml container was filled to capacity in 4 h 40 min and 4 h, respectively.

Elevated concentrations of P-hemoglobin were found (Table 2). Spontaneous proteolytic activity measured using substrate S-2238, which is mainly sensitive to throm-

Table 2 Plasma concentrations of hemoglobin, coagulation and fibrinolysis parameters in retrieved blood (study A) from ten patients

Variable	Retrieved blood (range)	Normal (range)
Hemoglobin (mg/l)	49 – 356	< 5
Thrombin-antithrombin complexes (μg/l)	> 200	< 4.1
Prothrombin fragment 1 + 2 (nmol/l)	> 15	< 1.11
Fibrin, soluble (nmol/l)	> 200	< 25
Antithrombin (units/ml)	0.16 – 0.51	0.80 – 1.20
D-Dimer (μg/l)	1530 – 3612	< 80
Protein C (units/ml)	0.59 – 1.24	0.68 – 1.20
Protein S, free (units/ml)	0.15 – 0.27	0.20 – 0.50
S-2238[a] (optical density/2 h)	2.15 – 3.09	0.012 – 0.074

[a] S-2238 is a substrate that measures the spontaneous proteolytic activity

bin and activated protein C, was elevated (Table 2). Half of the spontaneous proteolytic activity was quenched by hirudin, which is a specific thrombin inhibitor. High levels of thrombin-antithrombin complexes, soluble fibrin, prothrombin fragment 1 + 2 and D-dimer, markers of thrombin activity, were found (Table 2). Finally, thrombin activity was tested directly on one sample fom one transfusion bag in the separate experiment. A clot was formed after 21 min, indicating that thrombin was present. The clot dissolved after 24 h. High levels of kallikrein activity were detected by the more specific substrate S-2302 and the presence of plasmin, by substrate S-2403. In these assays, soybean trypsin inhibitor (SBTI) was used to quench any free activity by kallikrein or plasmin.

The concentrations of the coagulation inhibitors antithrombin and free protein S were considerably decreased (Table 2). Protein C values were slightly decreased (Table 2). Venous plasma IL-6 concentrations in the control group were 16 ± 13. TNF and IL-1 were not detected in controls.

The circulating plasma IL-6 concentrations in the patients (study A) increased from (0) to 125 ± 27 (116) pg/ml ($p < 0.01$) during the postoperative 4 to 6-h period of blood retrieval, at the end of which they were increased in comparison with control values ($p < 0.001$). No patient had a measurable circulating IL-1 concentration. Circulating TNF concentrations (range: is < 15 – 50 pg/ml) were detected in four patients (3, 5, 6 and 8, Table 1).

The IL-6 concentration in retrieved blood (study A) obtained from the Stryker Consta Vac transfusion bags were > 1000 pg/ml in all samples, thus elevated in comparison with systemic plasma IL-6 concentrations in controls ($p < 0.0001$) and patients ($p < 0.001$). TNF and IL-1 were not detected.

In the initial portion of drainage blood from the wound (study B), TNF was observed in three samples and IL-1 in four samples, while IL-6 was detected in one sample (Table 3).

Table 3 Cytokine concentrations (pg/ml) in the initial portion of drainage blood from the surgical area (study B)

Pat. no.	TNF	IL-1	IL-6
11	< 15	18	0
12	88	< 15	0
13	< 15	26	0
14	277	125	23
15	100	49	0

Discussion

The present findings of elevated concentrations of thrombin-antithrombin complexes, prothrombin fragment 1 + 2, soluble fibrin and D-dimer, all indicating increased thrombin activity, suggest that the retrieved blood (study A) was characterized by marked hypercoagulation. Half of the activity on the peptide substrate was quenched by the thrombin inhibitor hirudin. The rest of the activity could thus be due to either free thrombin activity or thrombin activity bound to α-2-macroglobulin. The increased D-dimer levels also showed that fibrinolysis had taken place. The results may reflect coagulation/fibrinolysis occurring in the transfusion bag but also the impact of hip replacement surgery on these parameters [29].

Spontaneous proteolytic activity measured with low-molecular-weight synthetic peptides sensitive to thrombin, kallikrein, etc. was very high in the retrieved blood (study A). However, it is known that thrombin, kallikrein and plasmin are bound not only to their specific inhibitors, but also to α-2-macroglobulin [30]. The complexes formed have little or no activity on the natural substrates, such as fibrinogen, the natural substrate for thrombin [31]. Free thrombin activity was also found when one sample of retrieved blood in the separate experiment was added to fibrinogen solution. It must be remembered that in principle, the retrieved blood is serum, as no anticoagulant is given. It has been demonstrated in animal experiments that under conditions of stasis in the animal, infusion of serum will give rise to a thrombus [32]. This is a very well-known experimental model for inducing thrombosis.

As the thrombin activity on fibrinogen was not very strong, it is hoped that thrombin produces no harmful effects in vivo. If the patient is in an unbalanced hemostatic state, however, retransfusion of retrieved blood might be harmful. Furthermore, the blood should probably not be retransfused immediately after collection from the wound, as the thrombin may require some time to be neutralized by the antithrombin. On the other hand, more kallikrein activity may develop during storage. The

increased levels of D-dimer suggest that even if fibrin had been formed, it had been degraded by the fibrinolytic system (plasmin). Plasmin was found in six samples of retrieved blood (study A). Fibrinolytic activity, i.e. dissolution of the clot, also occurred in the separate experiment. All these findings indicate the need for further clinical studies on the effect of retransfused blood on hemostasis in vivo. With regard to the increased thrombin activity in the retrieved blood, the use of low-molecular-weight heparin as thromboprophylaxis [33, 34, 35] may be more efficient than dextran 70 or standard heparin in preventing postoperative thrombosis.

In a study using the Solcotrans blood retrieval system (Solco Basle, Hingham, Mass., USA) after arthroplasty, decreased plasma levels of fibrinogen, coagulation factors V and VII, antithrombin and plasminogen were found in the salvaged blood. The level of protein C was normal but the concentrations of fibrin degradation products were increased regardless of whether an anticoagulant was used [13]. Surprisingly, the levels of coagulation factor VIII and protein C were significantly lower in anticoagulated than in nonanticoagulated retrieved blood. Results from that study suggest a less pronounced derangement of coagulation and fibrinolysis than is observed in the present one. The soluble fibrin, thrombin-antithrombin complexes and the prothrombin fragment 1+2 analyzed in the present study are new methods that are more sensitive than previous ones in detecting thrombin formation. Soluble fibrin represents an intermediate state in the development of cross-linked polymerized fibrin. Thrombin-antithrombin complexes appear when formed thrombin is deactivated by firm binding to antithrombin. Prothrombin fragment 1+2 is produced when prothrombin is activated to thrombin, and the amount formed reflects the absolute amount of thrombin generated. D-Dimer is a specific degradation product of cross-linked fibrin. Thus, the presence of this product shows that fibrin has been formed and degraded by fibrinolytic activity. The low concentrations of the coagulation inhibitors indicate consumption. The increased levels of D-dimer suggest that even if fibrin has been formed, it has been degraded by the fibrinolytic system (plasmin). In the Stryker Consta Vas system, anticoagulants were not used, which may have resulted in hypercoagulability.

TNF and IL-1 concentrations were found in the first portion of blood from the wound (study B), which probably reflected local activation of cells. In the wound, TNF and IL-1 are initial circulating mediators in plasma in septic shock syndrome [10, 13] but have to our knowledge not previously been demonstrated in drainage blood from a surgical area in patients. Interestingly, no TNF or IL-1 concentrations were detected in the retrieved blood that had residued in the transfusion bag (study A). In contrast, very high concentrations of IL-6 were consistently found in the transfusion bag. This discrepancy between the cytokine pattern of the initial portion of blood from the wound and that of salvaged blood from the transfusion bag may be attributed to the fact that the two studies were conducted on different sets of patients. On the other hand, both studies involved the same types of patients and surgery. It is therefore likely that the first portion of blood entering the container contained TNF and IL-1 and small amounts of IL-6. The absence of TNF and IL-1 in the transfusion bag after 4–6 h may be explained otherwise. Assuming that TNF and IL-1 are produced only transiently in the wound, their concentrations in the initial portion of blood would be diluted to undetectable levels.

Alternatively, is is possible that TNF and IL-1 were produced continuously in the wound but rapidly degraded or bound. Similarly, the high levels of IL-6 in the transfusion bag may have several explanations. Firstly, cells in the wound may have synthesized and released IL-6 into drainage blood during the main part of the period of blood savage. Secondly, activated cells from the wound may have been transported into the system for blood retrieval and have continued to produce IL-6 there as a consequence of activation in the wound. Thirdly, the material in the container and tubings may have stimulated IL-6 production/release [36]. Finally, it cannot be excluded that tissue debris and/or bacterial contamination may have stimulated cytokine production/release from cells within the container.

The cytokine pattern in the initial portion of drainage blood from the wound (study B) – considerable TNF and IL-1 concentrations and low IL-6 concentrations – was almost inverse to that observed in the systemic circulation 4–6 h postoperatively (study A), where IL-6 was elevated, TNF was detected in 40% of patients and no IL-1 was detected.

No causal relationship between the different cytokine patterns in blood from the wound (study B), in the transfusion bag (study A) and in the postoperative circulation (study A) is evident. One interpretation is that surgical trauma triggered the synthesis/release of the initial mediators TNF and IL-1 within the wound. According to this hypothesis, these mediators induced synthesis/release of IL-6 [37] in the wound, which was reflected by the high concentrations of IL-6 in retrieved blood 4–6 h postoperatively and the consistent occurrence of IL-6 in the systemic circulation at this time. As IL-1 was no found in the peripheral blood and TNF was detected only sporadically, it can be hypothesized that the occurrence of these cytokines in the wound was more transient. Interestingly, in two patients (5 and 6, Table 1) systemic TNF concentrations decreased during the postoperative period, perhaps reflecting an initial spillover of TNF activity. Thus interpreted, the present data, to some extent, lend support to the notion that the cytokine characteristics of the wound are propagated into the salvaged blood as well as into the systemic circulation.

It is conceivable that coagulation as well as the cytokine findings in the present study were to some extent specific to traumatized bone tissue and to the exposition of bone marrow into the wound. The incidence of coagulation disorders and susceptibility to disseminated intravascular coagulation (DIC) in connection with accidental or surgical trauma skeletal structures containing bone marrow is in line with this assumption. Interestingly, the abnormalities of coagulation and fibrinolysis in retrieved blood (study A), including increased concentrations of thrombin-antithrombin complexes, prothrombin fragment 1+2 and soluble fibrin, in combination with decreased concentrations of coagulation inhibitors (antithrombin and protein C), were similar to those that appear in connection with DIC [38].

High plasma cytokine concentrations have been reported in connection with septicemia [12, 13], a condition frequently associated with coagulation abnormalities and DIC. Preliminary data from our laboratory indicate elevated circulating cytokine concentrations in response to major accidental orthopedic trauma. Recently, a causal relationship between cytokines and activation of the coagulation system has been demonstrated. Accordingly, TNF infusion to volunteers resulted in activation of the coagulation system [18]. We have not been able to find data in the literature regarding the influence of IL-6 on coagulation and fibrinolysis. The present data suggest an association between these variables. Whether a causal relationship prevails is uncertain, as both elevated IL-6 concentrations and coagulation abnormalities in the salvaged blood may have been induced by initial but transient TNF activity. Cytokine-induced coagulation abnormalities may represent the initial steps of the normal healing process when restricted to the local traumatized area, but it can be anticipated that they result in DIC when propagated into the systemic circulation. The cytokine content of blood intended for transfusion may be of concern for reasons other than its possible coagulation effects. IL-6 is pyrogenic [10], and high concentration of it in transfused blood products might account for subsequent febrile reactions [7].

In summary, retrieved blood from the wound (study A) was characterized by hypercoagulability and a high concentration of IL-6 when collected using a nonanticoagulated retrieval system. The initial portion of blood from the wound (study B) had high concentrations of TNF and IL-1. We believe the elevated cytokine concentrations may have induced hypercoagulability, the normal hemostatic mechanism in the wound.

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