J Anesth (2009) 23:61–66
DOI 10.1007/s00540-008-0706-3

Journal of
Anesthesia

Difference in autologous blood transfusion-induced inflammatory responses between acute normovolemic hemodilution and preoperative donation

Yoshifumi Kotake[1], Michiko Yamamoto[1], Midori Matsumoto[2], Takashige Yamada[1], Hiromasa Nagata[1], Hiroshi Morisaki[1], and Junzo Takeda[1]

[1] Department of Anesthesiology, Keio University, Tokyo, Japan
[2] Department of Anesthesiology, Tachikawa Kyosai Hospital, Tokyo, Japan

Abstract
Purpose. The inflammatory response triggered by transfusion is implicated in the pathophysiology of transfusion-related immunomodulation. The authors hypothesized that two distinctive autotransfusion methods, acute normovolemic hemodilution (ANH) and preoperative donation (PD), have different influences on both inflammatory mediator generation during storage and the inflammatory response after a transfusion. The purpose of this study was to compare the plasma concentrations of neutrophil elastase (NE), interleukin (IL)-6, IL-8, and IL-10 in patients who underwent either of these two autologous transfusion methods.
Methods. With institutional review board approval, the plasma concentrations of the above inflammatory mediators were determined in 23 patients with ANH and 8 patients with PD at the following time points: after anesthetic induction, at the end of the operation, and the morning of postoperative day 1. The concentrations of these inflammatory mediators were also measured in the donated blood obtained by either ANH or PD before retransfusion.
Results. The mean storage durations were 3.7 h and 6.1 days for ANH and PD, respectively. Higher concentrations of NE and IL-10 were detected in the PD blood than in the ANH blood. Long duration of storage and/or low temperature may have been responsible for the increased NE and IL-10 concentrations in the PD blood. However, the difference between the two groups in the extent of increased plasma concentrations of these inflammatory mediators was not statistically significant.
Conclusion. Inflammatory mediators were significantly increased in PD blood during storage compared to the blood obtained by ANH. However, their effects on the inflammatory response elicited in the recipients were not significantly different.

Address correspondence to: Y. Kotake, Department of Anesthesiology, Toho University, 6-11-1 Ohmori-Nishi, Ohta-ku, Tokyo 143-8540, Japan
Received: April 28, 2008 / Accepted: October 10, 2008

Key words Preoperative donation · Acute normovolemic hemodilution · Neutrophil elastase · Interleukin · HES 70/0.5

Introduction

The complications of allogeneic transfusions, such as transfusion-related immunological modulation and the transmission of infectious agents, are widely acknowledged [1–3]. Biological substances released from blood components during storage play important roles in these side effects [4–6]. For example, the cause of the febrile nonhemolytic transfusion reaction was previously attributed to the immune system's reaction against donor leukocytes. However, several studies have implicated inflammatory cytokines generated during storage for this syndrome [7,8]. Furthermore, proteins and lipids released during storage prime neutrophils and may result in transfusion-related acute lung injury [9,10]. In this respect, several studies have compared the generation of inflammatory mediators during storage and the reactions of the recipients of autologous and allogeneic blood transfusions [11–13]. These studies mainly focused on the difference between preoperative donation (PD) and allogeneic blood transfusions and concluded that autologous blood elicited less of an inflammatory response. However, recent reports have demonstrated that even PD blood triggers an inflammatory response in the recipient under certain conditions [14,15]. To clarify the possible effects of autologous transfusion on the inflammatory response, we compared the inflammatory response of PD and acute normovolemic hemodilution (ANH), another method for autologous transfusion.

The purpose of this prospective, observational study was to investigate the production of inflammatory mediators in ANH and PD during storage and the subsequent inflammatory responses in the transfused hosts.

Subjects and methods

The institutional review board of Tachikawa Kyosai Hospital approved the study protocol, and informed consent from each patient was obtained before the study started. Consecutive patients undergoing major abdominal, urological, and orthopedic surgery with 800 ml of ANH or PD at Tachikawa Kyosai Hospital during a 30-month period were prospectively examined. Patients who were transfused with less than 800 ml of autologous blood and those who received concomitant allogeneic blood products were excluded from the analysis. The general anesthesia applied to all the patients was sevoflurane and nitrous oxide.

Autologous transfusion

The inclusion criteria of ANH were as follows: estimated intraoperative blood loss more than 1000 ml and preoperative hemoglobin (Hb) more than 11 $g \cdot dl^{-1}$. After the induction of general anesthesia, 800 ml of autologous blood was drawn into a blood bag containing citrate-phosphate-dextrose (CPD; Terumo, Tokyo, Japan) via an intravenous catheter placed in the right internal jugular vein. After blood collection, 1000 ml of 6% hydroxyethyl starch solution (Saline-Hes HES 70/0.5; Kyorin Pharmaceuticals, Tokyo, Japan) was administered to maintain normovolemia. The autologous blood, stored at room temperature, was retransfused to maintain Hb at more than 8 $g \cdot dl^{-1}$ during surgery. Other anesthetic management, including fluid administration, was at the discretion of the attending anesthesiologist.

In the PD group, orthopedic patients whose estimated intraoperative blood loss exceeded 1000 ml were included. Autologous blood (800 ml) was donated before surgery and was stored in a CPD-containing bag at 4°C. Patients received supplemental $FeSO_4$ and recombinant human erythropoietin (rhEPO; Daiichi Pharmaceuticals, Tokyo, Japan). The same anesthetic management as that in the ANH group was applied, including the trigger for transfusion of autologous blood.

Measurements of neutrophil elastase (NE) and interleukin (IL)-6, IL-8, and IL-10

The sample of donated blood was obtained at the time of retransfusion. Arterial blood was obtained at the following time points: after anesthetic induction, at the end of the operation, and the morning of postoperative day 1 (POD 1). All samples were immediately centrifuged at 4°C and the plasma was stored at –80°C until assay. Plasma concentrations of the following inflammatory mediators were assayed with commercially available enzyme immunoassay systems: NE, with PMN Elastase (Merck, Darmstadt, Germany); and IL-6, IL-8, and IL-10, with Biotrak cytokine human EIA systems (Amersham, Buckinghamshire, UK). All the assays were duplicated and averaged data were used in the subsequent analysis. The clinical presentations of any types of transfusion reactions were recorded.

Statistical methods

The values for demographic and surgical data were expressed as means ± SD. The values for the concentrations of the inflammatory mediators were expressed as medians and 25th–75th percentiles. Friedman's test assessed changes within the groups. If the P value was less than 0.05, post-hoc comparisons were performed for the change from the preoperative value by a two-tailed Wilcoxon test for pair-wise comparisons. Because multiple comparisons were required to evaluate statistically significant change within a group, $P < 0.01$ was used. Comparisons between the ANH and PD groups, were performed with the Mann-Whitney test and differences were considered significant if the P value was less than 0.05.

Results

The patient demographics are summarized in Table 1. There were no significant differences in any parameters between the two groups. The ANH group ($n = 23$) consisted of patients who underwent major gastrointestinal ($n = 10$) and urological ($n = 11$) surgery for malignancy, as well as patients who underwent gynecological and spinal surgery ($n = 1$ each). The PD group ($n = 8$) consisted of patients who underwent orthopedic surgery (spinal or hip replacement surgery).

The mean storage duration of ANH blood and PD blood was 3.7 h and 6.1 days, respectively. All the donated blood was retransfused and there were no clinically relevant transfusion reactions after retransfusion in either of the groups.

The concentrations of the inflammatory mediators in the donated blood at the time of retransfusion are sum-

Table 1. Patient characteristics

	ANH ($n = 23$)	PD ($n = 8$)
Age (years)	52 ± 12	50 ± 13
Sex (M/F)	18/5	5/3
Duration of surgery (min)	327 ± 156	227 ± 88
Blood loss (g)	833 ± 397	780 ± 570
Hb on POD 1 ($g \cdot dl^{-1}$)	11.6 ± 1.5	11.1 ± 1.0
WBC on POD 1 (mm^{-3})	12 160 ± 2770	11 540 ± 1610

Data values are expressed as means ± SD. No significant differences were found in any of the parameters

Table 2. Concentrations of inflammatory mediators in the autologous blood

	ANH (n = 23)	PD (n = 8)
Neutrophil elastase (ng·ml^{-1})	251 (151–280)	506 (479–633)*
IL-6 (pg·ml^{-1})	4.9 (2.3–4.7)	5.3 (2.0–5.5)
IL-8 (pg·ml^{-1})	10.0 (2.8–11.7)	18.1 (8.2–22.0)
IL-10 (pg·ml^{-1})	11.9 (5.9–18.2)	28.0 (11.4–42.6)*

* $P < 0.05$ vs ANH group
Concentration was determined at the time of retransfusion. Data values are expressed as means (25th–75th percentiles)

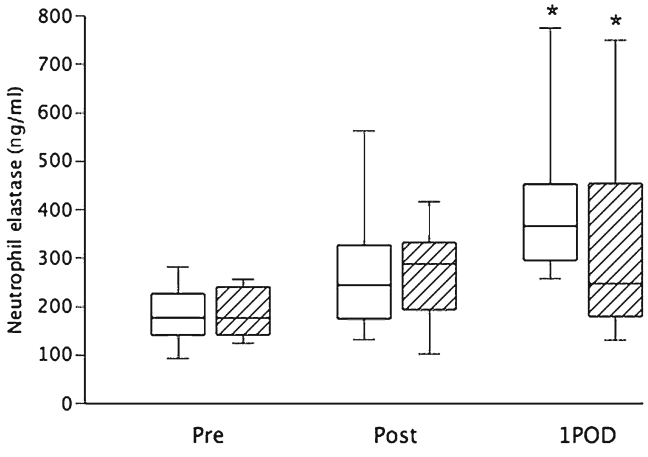

Fig. 1. The plasma concentrations of neutrophil elastase in the acute normovolemic hemodilution (ANH) group (n = 23; *open boxes*) and PD group (n = 8; *shaded boxes*) are summarized in this box plot. The median values, 25th–75th percentiles, and 10th–90th percentiles are given. *Pre*, after anesthetic induction; *Post*, at the end of operation; *1POD*, the morning after the operation. *$P < 0.01$ vs preoperative value with Wilcoxon rank sum test. No significant differences were noted between the ANH group and the PD group

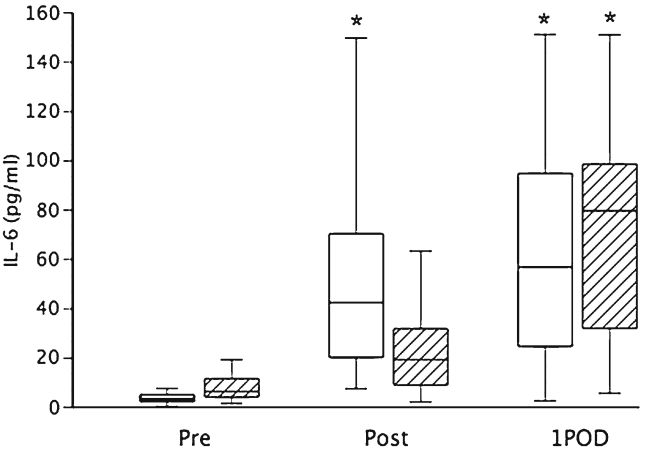

Fig. 2. The plasma concentrations of interleukin-6 (*IL-6*) in the ANH group (n = 23; *open boxes*) and PD group (n = 8; *shaded boxes*) are summarized in this box plot. The median values, 25th–75th percentiles, and 10th–90th percentiles are given. *Pre*, after anesthetic induction; *Post*, at the end of operation; *1POD*, the morning after the operation. *$P < 0.01$ vs preoperative value with Wilcoxon rank sum test. No significant differences were noted between the ANH group and the PD group

marized in Table 2. The concentrations of NE and IL-10 in the donated blood were significantly higher in the PD group than in the ANH group. However, neither IL-6 nor IL-8 levels in the donated blood differed between the two groups.

Changes in the plasma concentrations of the studied inflammatory mediators during the perioperative period are summarized in Figs. 1 to 4. There was a significant increase in the plasma NE concentration on POD 1 compared to the preoperative value in both groups, but there was no significant difference between the groups (Fig. 1). Plasma IL-6 also steadily increased immediately postoperatively and on POD 1 in the ANH group (Fig. 2). In the PD group, it remained unchanged immediately postoperatively, but was significantly increased on POD 1. Plasma IL-8 was significantly increased immediately postoperatively in the ANH group (Fig. 3) and then decreased significantly on POD 1 and returned to the preoperative level. In the PD group, no significant change in plasma IL-8 was noted during the study period. The plasma IL-10 level did not change during the study period in either of the groups (Fig. 4). Although the changing profiles of these inflammatory mediators in the two groups were somewhat different over time, the plasma concentrations of NE, IL-6, IL-8, and IL-10 at equivalent time points were not significantly different between the two groups.

Discussion

This study demonstrated that NE and IL-10 were significantly higher in PD blood than in ANH blood (Table 2). This finding indicates that the generation of inflammatory mediators is, to some extent, affected by storage conditions. The risks associated with allogeneic blood transfusions have been well recognized, and autologous

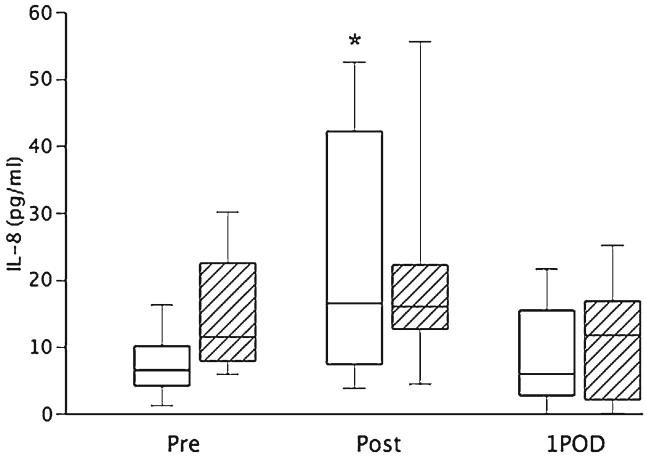

Fig. 3. The plasma concentrations of IL-8 in the ANH group ($n = 23$; *open boxes*) and the PD group ($n = 8$; *shaded boxes*) are summarized in this box plot. The median values, 25th–75th percentiles, and 10th–90th percentiles are given. *Pre*, after anesthetic induction; *Post*, at the end of operation; *1POD*, the morning after the operation. $*P < 0.01$ vs preoperative value with Wilcoxon rank sum test. No significant differences were noted between the ANH group and the PD group

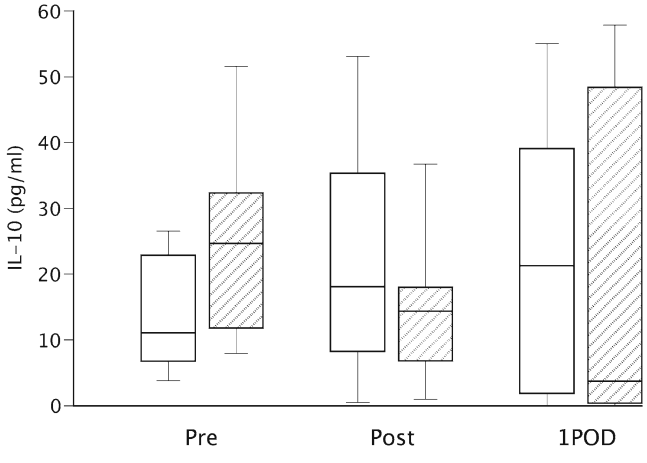

Fig. 4. The plasma concentrations of IL-10 in the ANH group ($n = 23$; *open boxes*) and the PD group ($n = 8$; *shaded boxes*) are summarized in this box plot. The median values, 25th–75th percentiles, and 10th–90th percentiles are given. *Pre*, after anesthetic induction; *Post*, at the end of operation; *1POD*, the morning after the operation. No significant differences were noted between any time points and the preoperative value, nor were any significant differences noted between the ANH group and the PD group

blood transfusion is now widely preferred [16]. One of the major advantages of autologous transfusion is that it causes less of a proinflammatory response when compared to allogeneic blood transfusions.

Recently, much attention has focused on the accumulation of inflammatory mediators during the retrieval and storage of donated blood. Possible relationships between the accumulation of inflammatory mediators and the side effects of blood transfusions, such as nonhemolytic febrile reaction, transfusion-related lung injury, and multiple organ failure after a massive transfusion have been investigated. For example, several studies have reported increased concentrations of NE, IL-1β, IL-6, and IL-8 during storage [17–20]. Additionally, Biedler et al. [13] reported that banked whole blood had an immunosuppressive effect that was largely attributable to storage-dependent factors. Jensen et al. [4] reported an increased IL-6 concentration 3 days after colorectal surgery in patients who underwent allogeneic transfusion, and they noted that the increase was attenuated by leukocyte depletion before storage.

Because these findings suggest that the presence of leukocytes during storage significantly augments the inflammatory response, leukocyte depletion in autologous transfusion may have the potential to attenuate the inflammatory response. Additionally, the storage duration is obviously longer in PD blood than in ANH blood. This storage period may have a significant impact on the transfusion-related inflammatory response. Recent investigations, which have reported that the prolonged storage of allogeneic blood might increase morbidity, may support this possibility [21–23]. The temperature during storage may also affect the inflammatory response. ANH blood is typically stored at room temperature in order to preserve platelet function [24,25]. Based on these possibilities, we hypothesized that the methods of autologous transfusion may affect the perioperative inflammatory response, because different storage durations and conditions may cause a distinct pattern of inflammatory mediator generation.

We found no differences in the IL-6 and IL-8 concentrations in the stored blood between our two groups. These cytokines are, presumably, released from the neutrophils and monocytes contained in the stored blood. Kristiansson et al. [18] reported increased concentrations of these cytokines in red blood cell concentrates during storage. Interestingly, they reported that the increase of IL-6 was independent of the length of storage, but the increase of IL-8 was dependent on the length of storage. In contrast, we found that the IL-10 concentration in the PD blood increased significantly during storage (Table 2). Hodge et al. [26] reported that IL-10 production during storage was decreased at room temperature and increased at 4°C. Our data correspond with their conclusion that temperature plays an important role in IL-10 production during storage.

The plasma concentrations of the investigated cytokines increased at some points of measurement in each study group, as shown in Figs. 1 through 4. However, there were no significant differences in the plasma concentrations of IL-6, IL-8, and IL-10 between the two

groups. Additionally, there was no apparent relationship between the concentration in the stored blood and the plasma sampled from the patients. Avall et al. [12] found that patients who were transfused with PD blood demonstrated higher IL-6 and IL-8 concentrations in plasma than patients who received allogeneic blood transfusion. They concluded that an attenuated cytokine response to allogeneic transfusion was a sign of immunosuppression. Heiss et al. [11] reported a significant increase in plasma IL-10 after an allogeneic transfusion but not after an autologous transfusion in patients undergoing colorectal cancer surgery. Tylman et al. [27] reported that reinfusion of salvaged blood resulted in an increased plasma IL-10 concentration. These results suggest that the difference between PD and ANH had less of an impact on the transfusion-triggered inflammatory response compared to allogeneic transfusion or the reinfusion of salvaged blood.

There are some limitations in the present study. First, the present study population varied in terms of background and surgical procedure. These differences were mainly caused by the fact that the decision to apply autologous transfusion was at the discretion of the surgeons and was based on their clinical preferences. These differences make the interpretation of the data somewhat difficult. The different baseline characteristics of the subjects, especially the presence of malignancy, may have affected the results. Previous investigations have demonstrated that preoperative values of the inflammatory cytokines that we investigated were similar in patients undergoing either surgery for cancer removal or orthopedic reconstructive surgery [4,11,12,27]. However, it is still possible that the transfusion-related inflammatory response was actually less in our ANH group, but the difference may have been undetected due to the influence of a more stressful surgical procedure in the ANH group. Second, the numbers of participants in the two groups were small and unevenly distributed. Because the cytokine concentrations were not normally distributed, formal power analysis was not feasible for our study. However, we do not think that increasing the number of participants would drastically change the results. Third, other medications may also affect the inflammatory response. For example, erythropoietin [28] and $FeSO_4$ [29] were administered to the PD group, while HES 70/0.5 was infused to maintain normovolemia in the ANH group. Thus, the anti-inflammatory properties of the HES solutions may have contributed to the results [30]. However, the preparations used in the present study have characteristics different from those of commonly used HES preparations, such as HES 130/0.4 or 200/0.5, so this possibility remains to be clarified [31]. Despite these limitations, the present study provided previously unknown information about the differences in inflammatory mediator generation during storage and the inflammatory response elicited in the recipients of PD blood and ANH.

In conclusion, this study demonstrated higher NE and IL-10 concentration over time in predonated autologous blood than in instantaneous autologous blood obtained by normovolemic hemodilution. This difference may be related to the differences in storage conditions between the two methods. However, the plasma concentrations of these inflammatory mediators were not different between the study groups after retransfusion, indicating that the inflammatory response was not affected by the method of autologous transfusion.

References

1. Landers DF, Hill GE, Wong KC, Fox IJ. Blood transfusion-induced immunomodulation. Anesth Analg. 1996;82:187–204.
2. Blajchman MA. Allogeneic blood transfusions, immunomodulation, and postoperative bacterial infection: do we have the answers yet? Transfusion. 1997;37:121–5.
3. Heiss MM. Risk of allogeneic transfusions. Br J Anaesth. 1998; 81(Suppl 1):16–9.
4. Jensen LS, Hokland M, Nielsen HJ. A randomized controlled study of the effect of bedside leucocyte depletion on the immunosuppressive effect of whole blood transfusion in patients undergoing elective colorectal surgery. Br J Surg. 1996;83:973–7.
5. Innerhofer P, Luz G, Spotl L, Hobisch-Hagen P, Schobersberger W, Fischer M, Nussbaumer W, Lochs A, Irschick E. Immunologic changes after transfusion of autologous or allogeneic buffy coat-poor versus white cell-reduced blood to patients undergoing arthroplasty. I. Proliferative T-cell responses and the balance of helper and suppressor T cells. Transfusion. 1999;39:1089–96.
6. Weisbach V, Wanke C, Zingsem J, Zimmermann R, Eckstein R. Cytokine generation in whole blood, leukocyte-depleted and temporarily warmed red blood cell concentrates. Vox Sang. 1999; 76:100–6.
7. Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME. Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. Transfusion. 1993;33: 195–9.
8. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG. The role of the plasma from platelet concentrates in transfusion reactions. N Engl J Med. 1994;331:625–8.
9. Silliman CC, Voelkel NF, Allard JD, Elzi DJ, Tuder RM, Johnson JL, Ambruso DR. Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. J Clin Invest. 1998;101:1458–67.
10. Looney MR, Gropper MA, Matthay MA. Transfusion-related acute lung injury: a review. Chest. 2004;126:249–58.
11. Heiss MM, Fraunberger P, Delanoff C, Stets R, Allgayer H, Strohlein MA, Tarabichi A, Faist E, Jauch KW, Schildberg FW. Modulation of immune response by blood transfusion: evidence for a differential effect of allogeneic and autologous blood in colorectal cancer surgery. Shock. 1997;8:402–8.
12. Avall A, Hyllner M, Bengtson JP, Carlsson L, Bengtsson A. Postoperative inflammatory response after autologous and allogeneic blood transfusion. Anesthesiology. 1997;87:511–6.
13. Biedler AE, Schneider SO, Seyfert U, Rensing H, Grenner S, Girndt M, Bauer I, Bauer M. Impact of alloantigens and storage-associated factors on stimulated cytokine response in an in vitro model of blood transfusion. Anesthesiology. 2002;97: 1102–9.

14. Domen RE. Adverse reactions associated with autologous blood transfusion: evaluation and incidence at a large academic hospital. Transfusion. 1998;38:296–300.
15. Covin RB, Ambruso DR, England KM, Kelher MR, Mehdizadehkashi Z, Boshkov LK, Masuno T, Moore EE, Kim FJ, Silliman CC. Hypotension and acute pulmonary insufficiency following transfusion of autologous red blood cells during surgery: a case report and review of the literature. Transfus Med. 2004; 14:375–83.
16. Lisander B, Ivarsson I, Jacobsson SA. Intraoperative autotransfusion is associated with modest reduction of allogeneic transfusion in prosthetic hip surgery. Acta Anaesthesiol Scand. 1998;42: 707–12.
17. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, Denny C, Silliman CC. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. Am J Surg. 1999;178:570–2.
18. Kristiansson M, Soop M, Saraste L, Sundqvist KG. Cytokines in stored red blood cell concentrates: promoters of systemic inflammation and simulators of acute transfusion reactions? Acta Anaesthesiol Scand. 1996;40:496–501.
19. Shanwell A, Kristiansson M, Remberger M, Ringden O. Generation of cytokines in red cell concentrates during storage is prevented by prestorage white cell reduction. Transfusion. 1997; 37:678–84.
20. Tolksdorf B, Frietsch T, Quintel M, Kirschfink M, Becker P, Lorentz A. Humoral immune response to autologous blood transfusion in hip surgery: whole blood versus packed red cells and plasma. Vox Sang. 2001;81:180–6.
21. Biffl WL, Moore EE, Offner PJ, Ciesla DJ, Gonzalez RJ, Silliman CC. Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: abrogation by poststorage washing but not prestorage leukoreduction. J Trauma. 2001;50: 426–31.
22. Leal-Noval SR, Jara-Lopez I, Garcia-Garmendia JL, Marin-Niebla A, Herruzo-Aviles A, Camacho-Larana P, Loscertales J. Influence of erythrocyte concentrate storage time on postsurgical morbidity in cardiac surgery patients. Anesthesiology. 2003; 98:815–22.
23. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med. 2008;358:1229–39.
24. Goodnough LT. Acute normovolemic hemodilution. Vox Sang. 2002;83 (Suppl 1): 211–5.
25. Jamnicki M, Kocian R, van der Linden P, Zaugg M, Spahn DR. Acute normovolemic hemodilution: physiology, limitations, and clinical use. J Cardiothorac Vasc Anesth. 2003;17:747–54.
26. Hodge G, Markus C, Nairn J, Hodge S. Effect of blood storage conditions on leucocyte intracellular cytokine production. Cytokine. 2005;32:7–11.
27. Tylman M, Bengtson JP, Avall A, Hyllner M, Bengtsson A. Release of interleukin-10 by reinfusion of salvaged blood after knee arthroplasty. Intensive Care Med. 2001;27:1379–84.
28. Aguilera A, Selgas R. Effect of recombinant human erythropoietin on inflammatory status in dialysis patients. Nephrol Dial Transplant. 2004;19 (Suppl 5): V46–53.
29. Oldenburg B, van Berge Henegouwen GP, Rennick D, Van Asbeck BS, Koningsberger JC. Iron supplementation affects the production of pro-inflammatory cytokines in IL-10 deficient mice. Eur J Clin Invest. 2000;30:505–10.
30. Lang K, Suttner S, Boldt J, Kumle B, Nagel D. Volume replacement with HES 130/0.4 may reduce the inflammatory response in patients undergoing major abdominal surgery. Can J Anaesth. 2003;50:1009–16.
31. Ickx BE, Bepperling F, Melot C, Schulman C, Van der Linden PJ. Plasma substitution effects of a new hydroxyethyl starch HES 130/0.4 compared with HES 200/0.5 during and after extended acute normovolaemic haemodilution. Br J Anaesth. 2003;91: 196–202.