

Targeting Perioperative Hemoglobin in Major Abdominal Surgery

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Abstract

Background: Perioperative transfusion optimization may result in blood saving and minimization of complications associated with blood transfusions. The study aimed to compare units transfused, cytokines and patient outcome in a restrictive versus a liberal transfusion strategy.

Materials and methods: We conducted a randomized-controlled study, in a single center, from December 2004 to May 2007. Of the 75 patients scheduled for major abdominal surgery and assessed for eligibility, 58 were randomized and 52 completed the study. Preoperatively, patients were randomly assigned to the Hb 7.7 g dL⁻¹ (restrictive) or to the Hb 9.9 g dL⁻¹ (liberal) group to receive blood transfusion intraoperatively and postoperatively if hemoglobin was below 7.7 g dL⁻¹ or Hb 9.9 g dL⁻¹ respectively. The follow-up for hemoglobin and intervention lasted five days, for cytokine measurements three days and for complications till discharge from the hospital. Units of red blood cells (RBC) per patient and the incidence of transfused patients in each group were the main outcome measure.

Results: Median RBC transfused (units/patient) was 0 [interquartile range 0,2] in the restrictive versus 1 [0,3] in the liberal group ($p=0.013$), and the percentage of patients transfused 36% versus 70% respectively ($p=0.027$). Postoperative IL-10 levels were higher in the liberal transfusion group 24 h postoperatively ($p<0.05$). Pooled peak postoperative IL-10 levels correlated with the overall number of units of blood transfused ($r^2 = 0.38$, $p = 0.032$) as well as with the overall mean duration of storage of blood transfused (in days) ($r^2 = 0.52$, $p = 0.007$). Complications or time to discharge from hospital did not differ between the groups.

Conclusion: In major abdominal surgery, restrictive transfusion decreases RBC requirements and IL-10 levels. The association between IL-10 and transfusion variables indicates that IL-10 may play a role in transfusion-associated immunomodulation.

Keywords: Blood transfusion; Abdominal surgery; Cytokines; Complications

Introduction

Major Perioperative blood loss results in impaired oxygen transport and requires red blood cell (RBC) transfusion. Blood transfusion, though in some occasions lifesaving, may cause a number of adverse effects, like transmission of viral infections, decreased capacity of the transfused red cells to deliver oxygen to the tissues [1-3], immune suppression resulting in an increased risk for recurrence of malignancies and/or nosocomial infections [4-6], tumour necrosis factor α (TNF α) and interleukin changes in the stored blood but also in the recipient's blood [7-9]. Red blood cells stored for longer periods and immunoparalysis involving factor IL10 may be implicated in transfusion-induced immunosuppression and jeopardize the clinical outcome [10-13]. The amount of transfused blood may also be associated with a risk for adverse postoperative outcomes [14]. The high cost of one blood unit should also be considered along with the patient's net benefit effect and the problems of blood shortage.

Transfusion strategies have been reconsidered and studies have come to conclusions depending upon the patients' condition and disease, like elderly, critically ill, surgical patients, unstable angina, or acute myocardial infarction [15-17]. With the exception of ischemic heart disease, a restrictive strategy of transfusing RBC when hemoglobin concentration falls below 7 g/dL was found at least as safe and possibly superior to a more liberal transfusion policy targeting to hemoglobin concentrations of 10 g/dL, since it demonstrated that in-hospital mortality was higher in the liberal transfusion group [18]. However, most of the studies have been performed in critically ill patients, and

literature data regarding the target of hemoglobin levels postoperatively are very limited.

A blood-sparing transfusion approach is of special interest in the general surgery population. The net savings of a reduction in RBC usage, when extrapolated to the large number of surgical procedures performed annually, can be substantial, given limited supply and the escalating cost of blood transfusion. To address this issue, we designed this prospective randomized open-labeled study aiming to compare two transfusion strategies, restrictive versus liberal, in patients undergoing major abdominal surgery. Main outcome measures were the number of blood units transfused and the incidence of transfusions in each group. Secondary endpoints were clinical outcome measures, as expressed by time to patient mobilization, time of first liquid and solid food intake and duration of hospital stay. We also sought to determine whether there are any differences in postoperative immunologic response between the two transfusion strategies, as expressed by changes in inflammatory mediators IL-6, IL-10 and TNF α .

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Received November 28, 2011; Accepted February 11, 2012; Published February 15, 2012

Citation: Markatou M, Theodoraki K, Demetrios Rizos, Fassoulaki A (2012) Targeting Perioperative Hemoglobin in Major Abdominal Surgery. J Anesth Clin Res 3:190. doi:10.4172/2155-6148.1000190

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Methods

Patient population and randomization

The study protocol was approved by the Institutional Review Board of the Aretaieion University Hospital. After evaluation of eligible patients during the preoperative visit and after obtaining written informed consent from each participant, 58 adult patients, American Society of Anesthesiologists (ASA) distribution I-III, scheduled for elective upper major abdominal surgery were enrolled in the study.

Exclusion criteria were a history of bleeding diathesis associated with thrombocytopenia, hereditary hemostatic defects such as hemophilias or chronic anticoagulant administration, refusal of transfusions for religious reasons, ischemic heart disease (unstable angina or myocardial infarction within the last six months), and preexisting infectious or autoimmune diseases as well use of corticosteroids or immunosuppressive drugs within the last six months.

Preoperatively patients were randomly assigned to the Hb 7.7 g dL⁻¹ or to the Hb 9.9 g dL⁻¹ group to receive blood transfusion intra- and postoperatively if hemoglobin was below 7.7 g dL⁻¹ or Hb 9.9 g dL⁻¹ respectively. The postoperative target hemoglobin values were between 7.7 g dL⁻¹ and 9.9 g dL⁻¹ for the Hb 7.7 g dL⁻¹ group and 10 g dL⁻¹ or above for the Hb 9.9 g dL⁻¹ group. Randomization was done by means of sealed opaque envelopes containing odd and even numbers for the Hb 7.7 g dL⁻¹ and the Hb 9.9 g dL⁻¹ group respectively.

Transfusion management and postoperative follow-up

All patients were operated using standardized anesthesia, antibiotic prophylaxis and postoperative analgesia. Both the surgical team and the anesthesiologist responsible for the patient were aware of the study protocol and group assignment. Intra- and postoperative transfusions were prescribed by an anesthesiologist participating in the study and as indicated by the group assignment. Transfusions were administered one unit at a time and the length of storage of each unit transfused was recorded. Hemoglobin concentration was measured daily in all patients with the HemoCue 201 DM device (HemoCue, Inc, Cypress, CA 90630, USA)

The number of units transfused during the first five postoperative days was recorded and the mean length of storage of all units transfused during this period was calculated for each patient. Enrolled patients were followed-up on a daily basis during their hospital stay for the incidence of postoperative infectious complications including: 1) pulmonary complications diagnosed by clinical examination and chest radiograph, corresponding to pleural effusion, atelectasis or pneumonia; 2) presence of septic fluid accumulation in the peritoneal space with the initial suspicion raised by ultrasonography and definitive confirmation by needle aspiration or surgical drainage and bacteriologic culture; 3) urinary infection diagnosed by leukocyturia in sediment with clinical signs and symptoms of infection or isolation of a pathogenic organism from urine culture; 4) wound infection manifested by erythema and purulent exudate or pus from the wound incision. Finally, the number of units transfused and the mean length of storage of all blood units transfused during the first five postoperative days were correlated to peak postoperative cytokine levels.

Prior to the study, sample size estimation indicated that approximately 29 patients should be included in each transfusion strategy group in order to detect a clinically relevant reduction of 40% in the number of units transfused by introducing a more restrictive transfusion policy with a power of 0.80 and an alpha error of 0.05. With a

transfusion threshold of 9.9 g dL⁻¹, mean RBC units transfused perioperatively in patients subjected to major abdominal surgery in our institution had been determined to be approximately 2.25 ± 1.20 from hospital records.

Primary outcome measure was the number of units transfused per patient as well as the incidence of blood transfusions in each group. Secondary end-points were time of mobilization, time of first liquid and solid food intake, the incidence of postoperative infectious complications as defined before, length of postoperative hospital stay and changes in the inflammatory mediators in each group.

Cytokine determinations

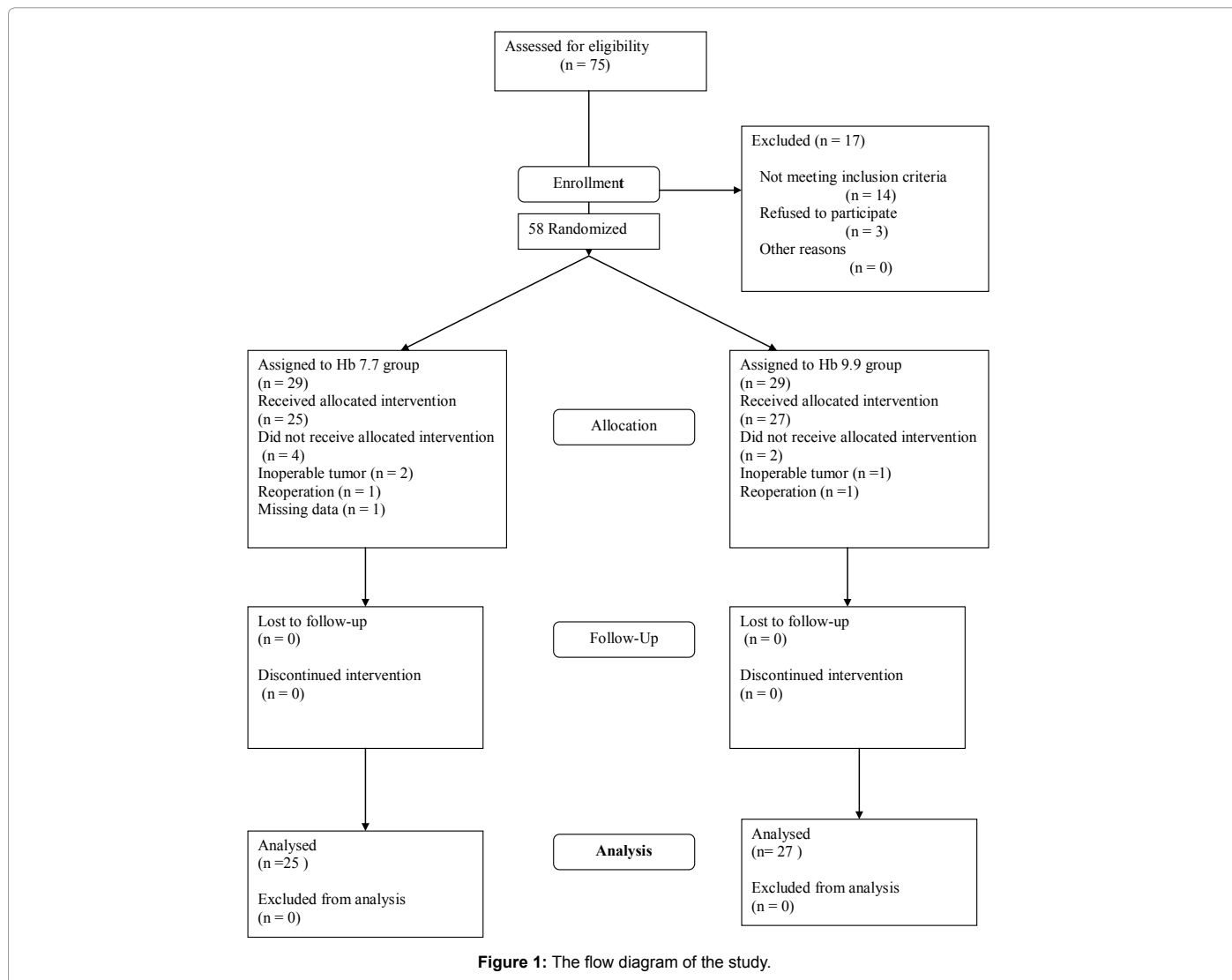
In ten patients randomly selected from each transfusion policy allocation group IL-10, IL-6 and TNF α were measured. Peripheral venous blood was drawn preoperatively, six, 24 and 72 hours postoperatively. Samples were collected in sterile tubes (Vacutainer, Becton-Dickinson, Heidelberg, Germany), were immediately centrifuged and the supernatant was stored at -60^o C until assay. Quantitative determination of cytokine levels was performed using commercially available sensitive immunoassay kits (Quantikine[®] HS human IL-10, Quantikine[®] HS IL-6 and Quantikine[®] HS human TNF α for IL-10, IL-6 and TNF α respectively) (R&D Systems Inc. 614 Mc Kinley Place NE, MN 55413, USA), according to the recommendations of the manufacturer. Detection sensitivity was 3.9 pg mL⁻¹ for IL-10, 0.039 pg mL⁻¹ for IL-6, and 0.106 pg mL⁻¹ for TNF α . The coefficient of variability of the method was 4.3-7.5% for IL-10, 6.5-9.6% for IL-6 and 5.3-16.7% for TNF α . All assays were performed in duplicate and averaged data were used in the subsequent analysis.

Statistical analysis

Variables were tested for normality of distributions with the Kolmogorov-Smirnov test. Comparisons of numeric data between the two groups were performed with the unpaired t-test or the Wilcoxon rank sum test for independent samples, depending on whether the variables followed a normal or non-normal distribution. The chi-square test or Fisher's exact test, as appropriate was used for comparisons of categorical data. Correlation between data was tested by using the Pearson product moment correlation coefficient test. The postoperative Hb levels as well as serial changes in IL-10 IL-6, and TNF α levels were analysed with two-factor mixed design analysis of variance with repeated measures for one factor (time). The two factors were the subject group and time and the Student-Newman-Keuls method was used *post hoc* for pairwise multiple comparisons. Results are expressed as mean \pm SD or as median [25th-75th percentiles] depending on normality of distributions. A value of $p < 0.05$ was considered as statistically significant. Statistical analysis was performed by the use of SPSS for Windows v.16.0 statistical software (SPSS Inc., Chicago IL, USA).

Results

From December 2004 until May 2007, 75 patients were assessed for eligibility of which 58 fulfilled criteria for enrolment and were included in the study protocol, with 29 of them allocated in the Hb 7.7 group and 29 in the Hb 9.9 group. Patient dropouts before and after randomization are shown in the flow diagram of the study (Figure 1). Of the 29 patients assigned to the Hb 7.7 group, two patients with inoperable tumor and one patient reoperated due to massive bleeding did not receive intervention. Additionally, one patient's charts were missing. In the 9.9 Hb group, one patient with inoperable tumor and one reoperated due to massive postoperative bleeding also did not receive intervention. The



excluded patients were treated regarding RBC transfusion according to their individual needs. Figure 1 shows the study design and dropouts.

Patient characteristics, type and duration of surgery and preoperative hemoglobin levels did not differ between the two groups (Table 1).

In comparison to the Hb 9.9 group, the Hb 7.7 group was associated with less units of blood transfused per patient ($p = 0.013$), a lower proportion of patients transfused (36% versus 70%, $p = 0.027$), lower average postoperative hemoglobin values ($p < 0.001$) and a shorter duration of storage of the blood transfused ($p = 0.002$) (Table 2). Daily hemoglobin concentrations in each group over the first five postoperative days are shown in Figure 2.

The two groups of patients did not differ regarding the parameters evaluating the outcome. Though the incidence for pulmonary complications was more than threefold in the Hb 9.9 group (37% versus 12%), the difference was not statistically significant (Table 2). Two patients in the Hb 9.9 group subjected to major hepatectomy died after 15 and 17 days in hospital respectively, due to ensuing severe hepatic failure (Table 2).

IL-10 levels were significantly higher in the Hb 9.9 group compared

to the Hb 7.7 group 24 hours postoperative and TNF α levels were lower 72 hours postoperatively ($p < 0.05$ and $p < 0.05$ respectively) (Table 3). Pooled peak postoperative IL-10 levels correlated with the overall number of units of blood transfused ($r^2 = 0.38$, $p = 0.032$) as well as with the overall mean duration of storage of blood transfused (in days) ($r^2 = 0.52$, $p = 0.007$). We found no such correlations for the other two mediators.

Discussion

The main finding of our study is that targeting lower hemoglobin values postoperatively is associated with a reduction in transfusion requirements and consequently in the actual number of blood units transfused.

In the Transfusion Requirements in Critical Care (TRICC) study, it was shown that a lower transfusion threshold did not affect the outcome of critically ill patients when compared to a more liberal transfusion strategy [18]. Literature of relating lower hemoglobin concentrations to postoperative complications is very limited, if any. In the limited number of patients we studied, patients randomized to the Hb

Parameter	Hb 7.7 group group (n=25)	Hb 9.9 group group (n=27)	P value
Age (years)	58.2 ± 11.7	63.4 ± 11.3	0.103
Sex (male/female)	13/12	16/11	0.805
Weight (kg)	77.7 ± 10.3	76.4 ± 12.1	0.677
Height (cm)	168.3 ± 9.9	167.4 ± 8.9	0.749
ASA score (I/II/III)	3/15/7	5/17/5	0.643
Operative procedure			0.574
Hepatectomy for benign disease	3	2	
Hepatectomy for malignant disease	15	18	
Whipple's	1	3	
Esophagectomy	2	0	
Gastrectomy	4	4	
Duration of surgery (min)	193.8 ± 61.0	216.2 ± 59.8	0.186
Preoperative Hb level (g dL-1)	13.1 ± 1.97	13.1 ± 1.40	0.887

Values are mean ± SD or number; ASA, American Society of Anesthesiologists; Hb, hemoglobin

Table 1: Patient characteristics, surgical procedure, duration of surgery and preoperative hemoglobin levels in the Hb 7.7 and Hb 9.9 group.

Parameter	Hb 7.7 group (n = 25)	Hb 9.9 group (n = 27)	P value
RBC usage (units/patient)	0 [0,2]	1 [0,3]	0.013
Proportion transfused [n (%)]	9 (36)	19 (70)	0.027
Average postoperative Hb (g dL-1)	9.6 ± 1.0	10.8 ± 1.1	<0.001
Duration of blood storage (days)	21.9 ± 8.5	28.4 ± 7.3	0.002
Time of mobilization (days)	1 [1,2]	1 [1,2]	0.786
Time of first liquid intake (days)	2 [2,3]	2 [1,2]	0.554
Time of first solid intake (days)	3 [2,3]	2.5 [2-5]	0.958
Length of hospital stay (days)	7 [7,9]	8 [7,12]	0.501
Mortality [n (%)]	0 (0)	2 (7.4)	0.493
Pulmonary complications	3	10	0.0708
Intrabdominal collection	2	2	1.000
Urinary infection	1	0	0.481
Wound infection	0	2	0.491

Values are mean ±SD for parametric numeric data, median [25th-75th percentiles] for non-parametric numeric data and number (percentage) for categorical data; RBC, red blood cells; Hb, hemoglobin

Table 2: Primary and secondary outcome data in patients randomly assigned to restrictive and liberal transfusion thresholds.

7.7 group were not exposed to increased risks at least during the early postoperative period that the follow-up lasted.

Considering the postoperative cardiovascular risks, which are not negligible in the major abdominal surgery population, we adopted relatively higher hemoglobin levels to transfuse our patients than those adopted in the TRICC study. Myocardial ischemia and infarct as well as delayed wound healing are potential postoperative complications when factors affecting oxygen transport become inadequate to meet oxygen demand requirements, especially in vulnerable populations [19,20]. In fact, in a subgroup analysis of the TRICC trial in patients thought to be at increased risk for complications associated with anemia because of coronary artery disease, the authors showed a non-significant trend towards increased mortality in the restrictive strategy group [21].

In our study, patients transfused with targeting hemoglobin levels 9.9 g dL-1 received more units of RBC and of older age compared to the age of units transfused to patients with targeting hemoglobin levels Hb 7.7 g dL-1. Large transfusion requirements increase the possibility of transfusion of units with long storage time. Additionally, as at most hospital transfusion services, it is our blood bank policy to release the oldest RBC units first. Thus, patients requiring a higher number

of erythrocytes have a higher probability of receiving transfusion with older units. According to universal blood bank policies, the maximum duration of storage of erythrocyte units is 42 days [22]. In a recent observational study, 3130 patients undergoing cardiac surgery and transfused with RBC stored for longer than 14 days exhibited a higher risk of a combination of severe postoperative complications and decreased short- and long-term survival than those (2872 patients) transfused with blood with a shorter duration of storage [23]. Both groups of patients in this study received similar number of RBC units, so the difference in outcome can not be correlated to the number but to the age of RBC units transfused. In the limited number of patients that we examined, we found no difference regarding postoperative complications between the liberal and restrictive transfusion strategy for mean duration of blood storage 28.4 and 21.9 days, respectively. Perhaps an association between duration of storage of transfused blood and potential complications would be more solidly detectable by trials randomizing patients to different lengths of storage of transfused units but such randomization might not be ethically acceptable and conclusions can mostly be drawn from observational studies.

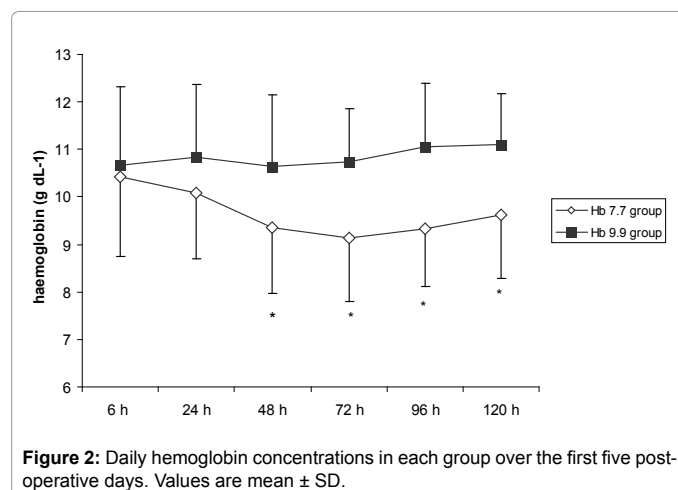


Figure 2: Daily hemoglobin concentrations in each group over the first five postoperative days. Values are mean ± SD.

	Preoperatively	6 h postoperatively	24 h postoperatively	72 h postoperatively
IL-10				
Hb 7.7 group	1.24 ± 2.77	54.36 ± 17.21	15.83 ± 13.22	6.33 ± 11.59
Hb 9.9 group	3.86 ± 8.48	46.83 ± 22.94	49.82 ± 29.07 *	11.39 ± 21.70
95% CI	-8.54 to 3.30	-11.52 to 26.58	-55.21 to -12.77	-21.41 to 11.29
IL-6				
Hb 7.7 group	4.34 ± 2.87	11.26 ± 0.76	11.56 ± 0.42	11.83 ± 0.44
Hb 9.9 group	5.50 ± 3.74	11.40 ± 0.58	11.27 ± 0.55	11.37 ± 1.06
95% CI	-4.30 to 1.97	-0.77 to 0.50	-0.16 to 0.75	-0.30 to 1.22
TNFα				
Hb 7.7 group	2.12 ± 0.76	2.07 ± 0.88	2.36 ± 1.07	2.84 ± 0.96
Hb 9.9 group	1.64 ± 0.53	1.50 ± 0.63	2.08 ± 0.47	1.65 ± 0.51 *
95% CI	-0.14 to 1.09	-0.14 to 1.29	-0.49 to 1.06	0.45 to 1.90

Values are mean ± SD and 95% CI of the difference in means. For the IL-10 measurements: p < 0.001 for the effect of time and p < 0.001 for group by time interaction; *p < 0.05 for intergroup comparison. For the IL-6 measurements P < 0.001 for the effect of time; p = 0.462 for group by time interaction and p=0.746 for intergroup comparison. For the TNFα measurements P = 0.842 for the effect of time; p = 0.029 for group by time interaction and *p < 0.05 for intergroup comparison.

Table 3: Interleukin-10 (IL-10) (pg mL⁻¹), Interleukin-6 (IL-6) (pg mL⁻¹), and tumor necrosis factor α (TNFα) (pg mL⁻¹) preoperatively, as well as 6, 24 and 72 hours after surgery.

Blood transfusion is associated with immunomodulation and the impact on the outcome is highly debatable [24]. The Hb 9.9 group of our patients exhibited a threefold higher incidence in pulmonary complications than the Hb 7.7 group but this difference was not statistically significant. Of course, the small number of patients limits the potential of the study to show differences in the secondary outcome measures. The mechanisms involved in the immunomodulatory effect of allogeneic blood transfusion have not been elucidated yet but it has been suggested that these adverse effects may be mediated by white blood cells present in transfused cellular blood components and/or the generation of inflammatory mediators [9].

Inflammatory cytokines are increased after major surgery [25,26]. Blood transfusion might serve as a second inflammatory insult, which amplifies the initial inflammatory response [8,9]. In the present study, a two-fold increase in IL-6 levels was observed in both targeting lower and higher hemoglobin concentration groups and this increase was sustained even 72 hours postoperatively. The absence of differentiation between the two groups shows that the surgical impact itself is predominantly responsible for IL-6 changes and that the role of blood transfusion may be less significant. In contrast, IL-10 remained increased and significantly higher only in the Hb 9.9 group 24 hours postoperatively, so this difference might be attributable to the different transfusion approach. This fact is also supported by the correlation we found between peak postoperative IL-10 levels with the overall number of units of RBCs transfused as well as with the overall mean age (in days) of the transfused blood.

IL-10 is mainly considered anti-inflammatory and the predominance of antiinflammation may lead to immunosuppression ("immunoparalysis"). It acts as a down-regulator of a number of proinflammatory actions by preventing migration of polymorphonuclear leukocytes and eosinophils to sites of inflammation [12,13]. IL-10 has also been shown to inhibit the release of interleukins IL-1, IL-6 and IL-8 from human peripheral blood monocytes and macrophage cells [27]. It also down-regulates TNF α production and TNF α gene expression in both peripheral blood monocytes and alveolar macrophages [28,29]. This is consistent with our findings, where TNF α levels in the Hb 9.9 group were significantly lower than in the Hb 7.7 group on the third postoperative day, following the increase of IL-10 in the same group. This shows that the time course and variation of TNF α may be regulated by the presence of anti-inflammatory IL-10.

The immunosuppression clinically manifested by blood transfusions seems to be mediated at least partly via the release of IL-10. The significantly higher levels of IL-10 and the trend for a higher incidence of pulmonary complications in the Hb 9.9 group may be related to more and/or older blood units transfused, though the number of patients overall assigned and the number of patients in whom we measured cytokines are small. Nevertheless, to our knowledge a correlation between the IL-10 and the age of transfused blood has not been demonstrated before.

Our study was underpowered as to the detection of postoperative complications or to a difference in other clinical outcome measures, which opens the possibility of a type- β error and may be considered as a limitation. Firmer conclusions can of course be reached with larger randomized studies sufficiently powered to detect differences in outcome.

In conclusion, under the present experimental design and in light of the limitations of the present study, we accomplished a reduction in blood transfusion requirements by targeting lower hemoglobin values

postoperatively. In this day and age, cost-effectiveness and optimization of resource utilization are considered fundamental requirements of hospital practice. Therefore, strategies such as a restrictive approach to blood administration, which lead to a more rationalized use of an already limited resource, are of great importance. An additional finding of our study is that increases in IL-10 seem to be associated with the volume of transfused blood and the duration of blood storage. Considering the IL-10 immunosuppressive and anti-inflammatory effects, this mediator may be an important factor contributing to transfusion related immunosuppression.

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