The effects of non-leukoreduced red blood cell transfusions on microcirculation in mixed surgical patients

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Abstract
Background: The impact of the storage process on oxygen-carrying properties of red blood cells and the efficacy of red blood cell (RBC) transfusions concerning tissue oxygenation remain an issue of debate in transfusion medicine. Storage time and leukocyte content probably interact since longer storage duration is thought to cause greater accumulation of leukocyte-derived cytokines and red blood cell injury.

Objectives: The aim of this study was to investigate the effects of storage and the efficacy of fresh (stored for less than 1 week) versus aged (stored for more than 3 weeks) non-leukoreduced RBC transfusions on sublingual microvascular density and flow in mixed surgical patients.

Methods: Eighteen surgical patients were included in this study. Patients were randomly assigned into two groups receiving fresh (Group A) and aged (Group B) RBC transfusions. Sublingual microcirculatory functional capillary density (FCD) and microvascular flow index (MFI) were assessed using orthogonal polarization spectral (OPS) imaging. Measurements and collection of blood samples were performed after induction of general anesthesia, before RBC transfusion and 30 min after the RBC transfusion ended.

Results: In both groups RBC transfusions caused an increase in hemoglobin concentration (p < 0.001). RBC transfusions increased FCD in Group A (p < 0.001), while FCD remained unaffected in Group B. Changes in MFI following RBC transfusion in both groups remained unaltered.

Conclusions: Fresh non-leukoreduced RBC transfusions but not RBCs stored for more than 3 weeks were effective in improving microcirculatory perfusion by elevating the number of perfused microvessels in mixed surgical patients.

1. Introduction
The objective of perioperative red blood cell transfusions (RBC) is to improve the oxygen-carrying capacity of blood by increasing the number of red blood cells (RBCs) and to deliver oxygen to tissues. The beneficial effects of RBC transfusion is being questioned as well. RBC transit time in the capillaries, local distribution of blood flow, capillary heterogeneity, and position of the oxygen dissociation curve, are all important for the transport of oxygen to the cell within the tissues [1,2]. In addition, the results of an increasing number of studies demonstrated that while RBC transfusions elevate the oxygen content of blood, transfusions of stored blood products did not improve impaired tissue oxygenation [3–7]. Nevertheless, despite the possibility of the use of different storage methods...
and liquids alternatives, storage-dependent alterations of RBCs cause a decrease on the oxygen-carrying properties of RBCs. During storage, RBCs undergo a number of changes that generally consist of two major alterations in biomechanical (cellular membrane, morphology and phospholipid content) and biochemical (2,3-diphosphoglycerate, 2,3-DPG) and ATP concentrations) [8–12]. Although some in vitro studies showed that these storage-dependent changes in RBCs occur between 7 and 21 days [13,14], other clinical studies did not find any relationship between storage time and patient outcome [15–17].

Several studies argue that storage time and/or contaminating intracellular materials from leukocytes in the packed RBC products may increase transfusion-related complications resulting in patient morbidity and mortality [18,19]. Storage time and white blood cell content can be detrimental to packed RBC products; lengthy storage times may cause accumulation of leukocyte-derived cytokines producing injury to packed RBCs. Anniss and Sparrow [20] observed that storage time and leukocyte burden of RBC increase the number and strength of adhesion of RBCs to vascular endothelium.

The microcirculatory network is an essential hemodynamic compartment and plays a crucial role in the interaction between blood and tissues. Thus, monitoring of the microcirculation might be of great importance during transfusion to determine the effects of RBC transfusions on the microcirculation. Orthogonal polarization spectral (OPS) imaging, a non-invasive imaging technique that is utilized to monitor tissue microcirculation, is used for the monitoring assessment and quantification of sublingual microvascular capillary density and blood flow at the bed-side [21]. There are only a few clinical studies, all in septic patients, that have investigated the effects of blood transfusions on the microcirculation [22,23].

Based on these observations we hypothesized that different storage times of non-leukoreduced RBC products may influence peripheral tissue perfusion dynamics and it can be detectable especially on the microcirculatory level. The aim of this study was to examine the efficacy of fresh (stored for less than 1 week) versus aged (stored for more than 3 weeks) non-leukoreduced RBC transfusions on sublingual microvascular density and flow intraoperatively.

2. Material and methods

2.1. Study design and patient selection

The institutional ethics committee approved the protocol and informed consent was from each patient. This study was design prospective, randomized and double-blind. Participants eligible for inclusion in this study were patients indicated to undergo major surgical procedures requiring intraoperative blood transfusion, the absence of iatrogenic bleeding, and transfusion of non-leukoreduced RBC suspensions from different storage time; less than 7 days and more than 21 days. Patients with hemodynamic instability prior to the operation, use of medications that cause metabolic and acid–base balance defects, steroids, antioxidant agents, drug allergies, and candidates with alcohol or -drug addictions were excluded. Preoperative laboratory findings of participants revealed no abnormal biochemical or hematological conditions and stable hemodynamic parameters. All measurements including systemic hemodynamic parameters, blood gas samples, and OPS imaging were performed at the same designated time points.

2.2. Randomization

Patients were randomly assigned to two groups; either receiving fresh blood stored for less than 7 days (Group A) or aged blood stored for more than 21 days (Group B). Randomization assignment of patients to Group A and Group B was prepared with a list of random numbers generated by computer software. The investigator who was analyzed OPS images and anesthesiologist who administered the blood transfusion to patients were blinded to the age of the blood being administered.

2.3. Preparation of packed red blood cells and transfusion practice

Packed red blood cell units were supplied from a regional blood center. Whole blood is collected into citrate–phosphate–dextrose anticoagulant and separated by centrifugation into red cell, platelet, and plasma components. Non-leukoreduced red cells are ultimately transferred into a pack containing an additive solution and stored at 4 ± 2°C. The currently used additive solution is a trehalose–adenine–glucose–mannitol (TAGM) solution. All donations were non-leukoreduced at the time of initial component preparation, plasma depleted, and suspended in TAGM (trehalose–adenine–glucose–mannitol). Both fresh blood and aged blood (maximum allowed storage time of 35 days) are used in daily practice in Hacettepe University, Medical Faculty Hospitals.

Observations were performed during standard transfusion practice. Measurements and collection of blood samples were performed after induction of general anaesthesia, before transfusion and 30 min after the blood transfusion ended. The administration of blood transfusion required approximately 60 min of infusion time. The red cell units were warmed to 37°C during infusion using a blood warmer. Blood samples were drawn at regular intervals from an arterial catheter. The frequency, volume, and age of the transfused blood was recorded for each patient. The stored blood products used in this study met both national and international criteria and guidelines for routine clinical use [24].

2.4. Anesthesia management

The day before surgery, patients were preanesthetically evaluated. All patients were premedicated with 5 mg diazepam orally, 1 h before the surgery. In the operating room; electrocardiogram (ECG), pulse oximetry and end-tidal CO2 and invasive arterial pressure (20-gauge cannula, right radial artery, internal volume 0.2 ml, BD floSwitch, Faraday Road, Swindon, UK) were monitored. Anesthesia was
intravenously induced with 1–2 mg kg\(^{-1}\) propofol (INN, marketed as Diprivan by AstraZeneca), 1 mcg kg\(^{-1}\) fentanyl citrate (Janssen-Cilag) and orotracheal intubation was performed after iv administration of vecuronium bromide (Organon, Turkey, marketed as Norcuron) 0.1 mg kg\(^{-1}\). Anesthesia was maintained with sevoflurane (Abbott Laboratories Limited, Berkshire, SL6 4XE, UK) 2%, \(\text{O}_2\), and \(\text{N}_2\text{O}\) at a total gas flow of 6 L min\(^{-1}\) using a semi-closed circle system with a soda lime canister. Vecuronium bromide was used for muscle relaxation during surgery. Ventilation was controlled to maintain carbon dioxide tension between 35 and 40 mmHg. Two peripheral venous (18 G and 16 G) and a bladder catheters were inserted. Percutaneous arterial blood oxygen saturation, end tidal CO\(_2\), and 16 G) and a bladder catheters were inserted. Per
tetration was set at 22 °C. End of surgery, residual neuromuscular blockade was antagonized using neostigmine methylsulfate (ADEKA, Samsun, Turkey) 1.5 mg and atropine sulfate (American Regent, Inc., Shirley, NY 11967) 0.5 mg and all patients (with the exception of cardiovascular surgery patients) were extubated and transferred to the post-anesthesia care unit. In cardiovascular surgery patients, anesthesia induction was performed with using 0.4 mg kg\(^{-1}\) etomidate (USAN, INN, BAN) (marketed as Amidate), 0.1 mg kg\(^{-1}\) vecuronium bromide, 1 mcg kg\(^{-1}\) fentanyl citrate. After induction, a 9.5 F three-lumen central venous catheter (Multicath, Vygon, Ecouen, France) was introduced via the right internal jugular vein, and a 20-gauge cannula (internal volume 0.2 ml, BD floSvitch, Faraday Road, Swindon, UK) via the right radial artery, and two peripheral venous lines (18 G and 16 G) were inserted. Anesthesia was maintained with sevoflurane 2% and \(\text{O}_2\). Remifentanil (GlaxoSmithKline and Abbott as Ultiva) infusion was administrated to all patients before and after CPB in a dose of 0.025–0.05 mg kg\(^{-1}\) min\(^{-1}\). During CPB, remifentanil was infused 0.025 mg kg\(^{-1}\) min\(^{-1}\) and sevoflurane was given with a vaporizer, inserted into the oxygenator’s gas supply line. Standart CPB procedures were performed [(Blood cardioplegia, mild hypothermia (28 °C), membrane oxygenator (COBE Cardiovascular, Inc., Colorado, USA) (Sarns 9000 Perfusion System, 3M Health Care Group, Michigan, USA), non-pulsatile flow 2.4 min\(^{-1}\) m\(^{-2}\), perfusion pressure 50–60 mmHg]). Anticoagulation was established with intravenous heparin (3 mg kg\(^{-1}\)) given 15 min before initiation of CPB. Target activated clotting time was 440 s. Hematocrit concentrations were maintained above 22%. At the end of the CPB, anticoagulation was antagonized with protamine sulfate to achieve a normal activated clotting time. At the end of the surgical procedure, CPB was weaned off slowly until adequate core body temperatures returned to normal. After decannulation, the chest was closed. Patients were transferred to the cardiovascular surgery intensive care unit and they were kept sedated with a continuous infusion of 0.3 mcg kg\(^{-1}\) min\(^{-1}\) remifentanil. At the achievement of hemodynamically stabilization, bleeding control and normothermia, the patients were weaned from the mechanical ventilation and extubated.

2.5. OPS imaging

Sublingual microcirculatory functional capillary density and perfusion were monitored using OPS (MicroVision Medical, Amsterdam, The Netherlands) imaging. OPS device is embodied in a hand-held instrument. OPS imaging illuminates tissues with polarized green light and measures the reflected light from the tissue surface after filtering out the polarized portion of the reflected light. Thus, surface reflections are filtered out and images can be observed of the microcirculation below the surface structures without transillumination. In OPS imagine method, the hemoglobin is used as a contrast agent, so as to red blood cells are imaged as dark moving globules against a white/grayish background. The vessel walls are not visualized directly, although pale contours can be identified depending on the presence of erythrocytes in the vascular lumen [21].

After the removal of saliva and other secretions using gauze, an OPS probe was covered by a sterile disposable cap, the OPS device was gently applied without any pressure on the sublingual mucosal at approximately 1.5–4 cm from the tip of the tongue. Five video image sequences of 20 s each from different adjacent areas were recorded using a computer (SONY Video Walkman GV-D1000E, Sony, Tokyo, Japan) and a video card (MicroVideo; Pinnacle Systems, Mountain Views, CA) and stored under a random number for offline analysis. Every effort was made to avoid movement and pressure artifacts to ensure accurate microcirculation image quality. Images stored on DVI tape were captured in 5–10 s video clips in DV-AV1 file format. OPS images were analyzed for functional capillary density which is defined as the length of red blood cell-perfused capillaries per observation area (FCD; mm capillary/mm\(^2\) tissue) and microvascular flow index (MFI; AU), providing an index for microcirculatory blood flow velocity (based on determining the predominant type of flow was scored as being (absent = 0, intermittent = 1, sluggish = 2, and continuous = 3) and these final score represents the averaged values of the four, was analyzed semiquantitatively in small- (diameter <25 mm) and medium-sized vessels (25 mm < diameter < 100 mm) as described previously [25,26]. OPS images were analyzed by using a computer software package (Automated Vascular Analysis Software (AVA) (Microvision Medical BV, Amsterdam, The Netherlands).

Baseline measurements were performed after induction of general anesthesia and repeated measurements were performed before and 30 min after the completion of RBC transfusion.

2.6. Statistical analysis

A study by Jhanji et al. [27] had shown that the decrease in perfused capillary density from a mean of 5.7 to a nadir of 3.8 mm\(^{-1}\) in patients (n = 25) after major abdominal surgery was associated with the development of postoperative complications. Hence, we calculated an anticipated
effects size (Cohen’s $d = 1.27$) from their results and the minimum sample size of 9 patients per group was required in our study to detect a minimum clinically significant difference of 2 units in perfused capillary density (FCD; $\text{mm capillary/mm}^2\text{ tissue}$) between the within group changes of the two groups with 80% power and 5% type I error level.

All data analysis was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software Inc., La Jolla, California, USA). Normal distribution of the data sets was confirmed using Kolmogorov–Smirnov/Shapiro–Wilk’s normality test. All data sets were presented as mean ± SD. Comparative analysis between FCD data sets was performed using a one-way analysis of variance (ANOVA) for repeated measurements with a Bonferroni post hoc test and MFI was evaluated using non-parametric statistic test. Differences between data sets with a $p$-value of <0.05 were considered statistically significant.

3. Results

3.1. Patients characteristics

Patient demographic characteristics are summarized in Table 1. Eighteen adult (7 male, 11 female), ASA physical status I–III patients scheduled for elective major surgery — ovarian cancer ($n$: 8), coronary artery bypass grafting (CABG) ($n$: 3), hip fracture ($n$: 2), osteosarcoma ($n$: 1), cervix cancer ($n$: 1), chondrosarcoma ($n$: 1), abdominal aneurysm ($n$: 1), and mitral valve replacement ($n$: 1) were admitted to this study.

No significant differences between Group A (<7 days) ($n$: 9) and Group B (>21 days) ($n$: 9) were identified with respect to the demographic characteristics and operative variables ($p > 0.05$). The volume of RBC transfusions in Group A was 752 ± 515 ml and storage on average was 3 ± 2 days. The volume of RBC transfusions in Group B was 712 ± 188 ml and storage on average was 24 ± 2 days. No patients received autologous and leukoreduced banked blood. No transfusion-related adverse reactions were observed during study.

3.2. Hemodynamic and microcirculatory parameters during surgery

In Group A, a significant decrease in systemic hemoglobin content at from $10.8 \pm 1$ to $8.1 \pm 1.1 \text{ g Dl}^{-1}$ ($p < 0.001$) and in Group B from $10.9 \pm 1.3$ to $8.7 \pm 1.8 \text{ g Dl}^{-1}$ ($p < 0.01$) was observed when compared with values of those after anesthesia induction and the values of those before transfusion (Fig. 1). Systemic Hct levels decreased in Group A from $32.5 \pm 3.6\%$ to $26.3 \pm 2.3\%$ and in Group B from $33.6 \pm 4.6\%$ to $27.6 \pm 3.3\%$ in all patients at the before transfusion period when compared to after induction of anesthesia period ($p > 0.05$) (Table 2). Mean arterial pressure decreased in Group A from 94.5 ± 17 mmHg to 83.6 ± 11.2 mmHg and in Group B from 82.7 ± 19.3 mmHg to 74.3 ± 14.7 mmHg ($p > 0.05$). Changes in heart rate were in Group A from 79.1 ± 16.3 to 77.5 ± 14.8 bpm and in Group B from 78.4 ± 13.8 to 79.2 ± 17.5 bpm ($p > 0.05$) (Table 2). There were no differences in Hb, Hct, MAP and heart rate values between the two groups of major surgery patients ($p > 0.05$) (Table 2).

At the microcirculatory level, FCD decreased from $14.9 \pm 0.5$ to $10.7 \pm 1.3 \text{ mm capillary/mm}^2\text{ tissue}$ in Group A ($p < 0.001$) and from $15 \pm 1.4$ to $10.9 \pm 1.9 \text{ mm capillary/mm}^2\text{ tissue}$ in Group B ($p < 0.001$) during surgery at the before transfusion period (Fig. 2). Alterations in MFI in Group A were in both small sized vessels from $2.65 \pm 0.2 \text{ AU}$ to $2.39 \pm 0.38 \text{ AU}$ ($p < 0.01$) and medium sized vessels from $2.94 \pm 0.08 \text{ AU}$ to $2.85 \pm 0.15 \text{ AU}$ ($p > 0.05$). Alterations in MFI in Group B were in both small vessels from $2.88 \pm 0.1 \text{ AU}$ to $2.72 \pm 0.23 \text{ AU}$ ($p < 0.05$) and medium sized vessels from $2.96 \pm 0.07 \text{ AU}$ to $2.90 \pm 0.12 \text{ AU}$ ($p > 0.05$) (Figs. 3a and 3b).

3.3. Effect of RBC transfusion on hemodynamic and microcirculatory parameters

At the macrocirculatory level, in both Group A and B, RBC transfusion caused a significant increase in systemic hemoglobin concentrations. Systemic Hb content in-

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<td>Patient characteristics and transfusion data.</td>
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<td>RBC volume/patient (ml)</td>
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<td>All data, except the gender and diagnosis, are presented as mean ± SD. ns: Non-significance.</td>
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creased in Group A from 8.1 ± 1.1 to 9.8 ± 1.2 g dL\(^{-1}\) (\(p < 0.001\)) and in Group B from 8.7 ± 1.8 to 10.9 ± 1.4 g dL\(^{-1}\) (\(p < 0.001\)). Hb values were different between the two groups after the transfusion (\(p < 0.05\)) (Fig. 1). Hematocrit increased in Group A from 26.3 ± 2.3% to 31.4 ± 2.6% and in Group B from 27.6 ± 3.3% to 33.3 ± 2.9% (\(p > 0.05\)) (Table 2). After RBC transfusion, MAP increased from 83.6 ± 11.2 to 84.8 ± 13.1 mmHg in Group A (\(p > 0.05\)) and in Group B from 74.3 ± 14.7 to 81.7 ± 15.4 mmHg (\(p > 0.05\)). Heart rate increased from 77.5 ± 14.8 to 82 ± 12 bpm in Group A (\(p > 0.05\)) and decreased from 79.2 ± 17.5 to 77.5 ± 16.4 bpm in Group B (\(p > 0.05\)). There were no differences between the two groups with regards to Hct, heart rate and MAP values (\(p > 0.05\)) (Table 2).

At the microcirculatory level, RBC transfusion resulted in increased FCD from 10.7 ± 1.3 to 14.3 ± 2 mm capillary/mm\(^2\) tissue (\(p < 0.001\)) in Group A, while FCD was changed from 10.7 ± 1.3 to 10.9 ± 1.04 mm capillary/mm\(^2\) tissue in Group B (\(p > 0.05\)). FCD values were different between the two groups after the transfusion (\(p < 0.001\)) (Fig. 2). Alterations in MFI following RBC transfusion in Group A were in both small blood vessels from 2.39 ± 0.38 AU to 2.42 ± 0.25 AU (\(p > 0.05\)) and medium sized blood vessels from 2.85 ± 0.15 AU to 2.82 ± 0.15 AU (\(p > 0.05\)). Alterations in MFI in Group B were in both small sized blood vessels from 2.52 ± 0.20 AU to 2.72 ± 0.23 AU (\(p > 0.05\)) and medium sized blood vessels from 2.90 ± 0.12 AU to 2.84 ± 0.20 AU (\(p > 0.05\)). There was no difference between the two groups in MFI values after the transfusion (\(p > 0.05\)) (Figs. 3a and 3b).

4. Discussion
The principal finding of our study was that non-leukoreduced blood transfusions alter microcirculatory hemodynamics as observed in the sublingual area in patients during major surgery. Even though systemic Hb concentrations increased after non-leukoreduced RBC transfusions in both groups of patients, sublingual FCD was improved 30 min after the completion of fresh non-leukoreduced blood transfusions. Aged non-leukoreduced RBC transfusions did not correct sublingual FCD. RBCs stored for less than 7 days was superior to aged packed RBCs in restoring sublingual microcirculation. In short, aged RBC transfusions (stored for >21 days) did not improve microcirculatory capillary density as anticipated.

When the RBCs volume of the blood is reduced, the oxygen-carrying capacity of blood decreases and the oxygen transport to the tissues might get impaired. It has been showed that systemic oxygen delivery and oxygenation of the skeletal muscle and internal organs can be protected until a systemic hematocrit of around 20% or acute blood loss of up to 30% of circulating volume can often be treated [24]. Therefore, most intensivists and anesthesiologists manipulated the oxygen transport by using fluid, isotropic and vasoactive treatments rather than blood transfusion in both critically ill and perioperative patients. Reliance on hemoglobin alone is not valid in this setting. The adequacy of oxygen delivery must be assessed in individual patients, particularly in patients with limited cardiac reserve. Anticipated degree and rate of blood loss, the effect of body temperature or drugs/anesthetics on oxygen consumption,
mixed venous \( O_2 \) levels and \( O_2 \) extraction ratios may be helpful in assessing tissue oxygenation during perioperative period. American Society of Anesthesiologists Task Force have been proposed: transfusion is rarely indicated when the hemoglobin level is above 10 g dL\(^{-1}\) and is almost always indicated in patients when the hemoglobin level is less than 6 g dL\(^{-1}\). Patients may be transfused at a hemoglobin \( >8 \) g dL\(^{-1}\), when there are signs of inadequate oxygen delivery including; tachycardia, hypotension, myocardial ischemia and hypoxemia [24,28,29].

There were no differences in MAP and heart rate values but, FCD decreased in both groups of major surgery patients before the blood transfusion in our study. Because, microvascular oxygen delivery cannot be predicted from global haemodynamic measurements and adequate tissue perfusion i.e. microcirculation rather than maintenance of macrocirculation parameters (arterial pressure, HR, central venous pressure, CO, PCWP, etc.) [26,30,31]. Therefore, functional capillaries have been accorded increasing importance. In the view of these evidences, we evaluated the effects of non-leukoreduced red blood cell transfusions on the microcirculation in mixed surgical patients using OPS with microcirculation parameters.

However, most investigators showed that anaemia is associated with adverse outcome at the admission of trauma, most of studies have been reported an association between increased risk of mortality, morbidity and perioperative transfusions, with a large proportion of deaths occurring within 30 days in cardiac surgery patients [32]. Moreover, in another a large clinical study it was showed that a more restrictive RBC transfusions strategy [33]. We selected patients undergoing major surgery because these procedures are typically associated with both an increased mortality rate and increased likelihood of multiple RBCs transfusions. In the present study, our pre-transfusion mean Hb concentrations 8.3 g dL\(^{-1}\) which is similar when compared to the indicated universal blood transfusion threshold because in the CRIT study [34], the mean pre-transfusion Hb concentration was found to be 8.6 g dL\(^{-1}\) and it is similar to the TRICC [33] and ABC studies [35]. During this study, our post-transfusion Hb concentration ranged from 8.1 g dL\(^{-1}\) to 9.8 g dL\(^{-1}\) for fresh blood and from 8.7 g dL\(^{-1}\) to 10.9 g dL\(^{-1}\) for old blood.

Under standard conditions, RBC units can be stored for up to 42 days at 1–6 °C [36]. Blood units are used for transfusion an average on day 16 of storage in EU [37] or on day 21 in United States [34]. In our university hospital where this study was performed whole blood is collected into citrate–phosphate–dextrose anticoagulant containers and separated by centrifugation into red cell, platelet, and plasma components. Non-leukoreduced red cells are ultimately transferred into a pack containing an additive solution and stored at 4 ± 2 °C. The currently used packaging additive solution is trehalose–adenine–glucose–mannitol (TAGM), which, in Turkey, is licensed for the storage of RBCs for up to 35 days.

Trehalose is a non-reducing sugar and crucial for stabilizing biomembranes. Crowe et al. [38–41] have been studied the beneficial effects of trehalose in preserving biological material. Many research groups investigated various techniques with trehalose. Satpathy et al. [42] developed one method to introducing trehalose into human RBCs. They have demonstrated that trehalose exerted osmotic protection on RBCs and also incubation of RBCs in a hypertonic trehalose solution results can be removed by washing and resuspending the RBCs in an iso-osmotic medium. These results provide an important step in long-term preservation of RBCs with trehalose. Tozok et al. [43] investigated the effects of trehalose-loading and freeze-drying RBCs on hemolysis, and RBC structure and functionality. They have been demonstrated that hemolysis was minimized by freeze-drying RBCs in lyophilization with trehalose and freeze-dried RBCs had similar effects of superoxide dismutase, catalase, ATP, and a similar hemoglobin structure to fresh RBCs. But they had lower levels of 2,3-diphosphoglycerate (2,3-DPG). Kanias and Acker [44] showed contradictory results regarding the process of trehalose-loading. They demonstrated that trehalose is correlated with high osmotic pressure, which had minor effects during incubation at 4 °C, but seemed to have exacerbated the severity of cellular injury at 37 °C, as measured
by higher levels of hemolysis, methemoglobin and lipid peroxidation. In our daily practice, during preparation of blood, RBCs were tested for osmotic fragility by our institute blood bank because of these effects of TGAM solution. Therefore, we thought that using RBCs in TGAM additive solution could not be influenced our study results.

Fig. 2. Changes in FCD values between time points.

Fig. 3a. Changes in MFI values in small-sized vessels between time points.
We compared blood stored for more than 21 days against blood stored for less than 7 days in our study; retrospective analysis has determined that a number of units older than 21 days is an independent risk factor for multiple organ failure [45].

There are conflicting data on whether transfusion of old or fresh RBCs increases tissue oxygenation because accumulating evidence is showing that there can be a negative relationship between the storage time and RBCs function. Van Bommel et al. [46], using a rat haemorrhagic shock model, evaluated the effect of RBC storage lesion on oxygen delivery. They have found that transfusion of RBCs stored for 28 days did not restore the microcirculatory oxygenation. Chin-Yee et al. [47] have also observed that old (7 days storage) RBCs adhered to the vessel walls to significantly greater degree than the fresher cells (less than 24 h of storage). Raat et al. [13] used human blood for transfusion and they showed that old blood (5–6 weeks' storage) resulted in a 25% decrease in rats intestinal microvascular oxygenation after transfusion, and it was not observed with fresh blood (2–6 days). Collins and Stechenberg [48] have studied the effect of the exchange transfusion of 90% of the original RBC mass in rats with blood stored 1 day or 14–20 days to final Hct of 36% (normal), 28% (moderate anemia) and 17% (severe anemia). They have found that survival being lower only for the rats transfused with old RBCs at low Hct. In our study, although there were no differences both pre-transfusion and post-transfusion Hct levels in the between two groups, in the fresh blood group, blood transfusion increased FCD and FCD remained unaffected in the old blood group. And also, we found that Hb values were different between the two groups after the transfusion. In the old blood group, while post-transfusion Hb levels were higher than the other group, FCD was not affected. Windsant et al. [49] have demonstrated that prolonged storage significantly increased free Hb concentrations transiently and nitric oxide (NO) consumption. Development of hemolysis during storage might contribute to release of free hemoglobin and a nitric oxide (NO) scavenger. This may impair microcirculatory perfusion after transfusion with old blood. Therefore, in our study, FCD could not affected from blood transfusion in the old blood group.

We know that decreased or remained FCD is related with lowered perfusion pressure and observed with ischemia–reperfusion injury which has the complication of oxidative stress [50]. On the other hand, Van der Lindan et al. [51] demonstrated that fresh blood transfusion and increased blood flow (pump flow) are equally effective in restoring tissue oxygenation in anaesthetized dogs undergoing cardiopulmonary bypass. These experimental studies conclude that fresh blood is a better resuscitation fluid than older [52,53]. But, unfortunately, experimental studies in animal species have interspecies differences in RBC metabolism and structure, therefore they do not fully reproduce clinical conditions or replicate human physiology.

Some of the prospective and retrospective studies that evaluate the effect of RBC storage on patient outcomes in cardiac surgery, trauma, and critical care [54–57]. These studies found mixed results with some showing worse outcomes in patients who received older RBCs, whereas others showed no difference. Kiraly et al. [58] have been examined the effect of the age of the blood transfused on the tis-
sue oxygenation using near infrared spectroscopy in thirty-two critically injured trauma patients. In this study, they defined “old blood” as blood stored for 21 days or greater and “new blood” as blood stored for less than 21 days. They found that older RBCs decreased in peripheral tissue oxygenation. On the other hand, Weiskopf et al. [59] showed that erythrocytes stored for 3 weeks have similar effects as erythrocytes stored for 3.5 h in reversing the neurocognitive deficit of acute anemia in nine healthy volunteers donated. In addition most of the studies on blood banking-induced alterations in RBC flow properties have shown that routine cold storage is associated with reduction in RBC deformability [60], increased RBC flow, elevation of their adherence to endothelial cells [61,62] and increased aggregatability [63]. Actually, if patients had lower hematocrit, it may have improved microvascular flow. In our study; while microvascular flow index (MFI) remained unaffected in both groups, blood transfusion increased FCD in only the fresh blood group. In addition, there were no differences between the Hct values for both groups. According to these results; RBC flow properties did not effect from storage in our study.

We used RBCs that were prepared with the routine storage procedures of our Faculty of University Blood Bank, specifically non-leukoreduced. Most studies have shown that removal of white blood cells reduces storage-induced damage to RBCs [64–68]. The presence of leukocytes in transfused blood may play an important role in quality of transfused erythrocytes and responsible for the transmission of blood-borne infections. It is generally accepted that universal leukocyte depletion include a reduction in the incidence of non-hemolytic transfusion reactions, immunosuppression [69], and mortality. On the other hand, Weinberg et al. [57], have been demonstrated that a mortality association with older blood (>14 days) despite universal leukoreduction and leukoreduction on mortality is doubtful. Nathens et al. [70] compared 1864 injured patients to receive leukoreduced versus standard non-leukoreduced transfusions during the hospitalization. They have found that no difference in mortality or infectious morbidity in the 268 patients. But, they did not evaluate the age of transfused blood in their study design. Similarly, Englehart et al. [71] showed that the use of leukoreduction blood does not improve outcome in trauma patients. In our study, we used non-leukoreduced blood transfusion in our patients. To our study results, we thought that the age of blood could be more important than the presence of leukocytes in RBCs on microcirculation.

Our study has its limitations. First, we investigated a relatively small number of patients so that we may have missed minor differences attributable to measurements. Second, we included patients who underwent different kind of surgery that may have different microcirculation properties. Lastly, we did not correlate microcirculation parameters with any clinical outcome parameters like long-term survival or morbidity and mortality rate in our study.

In summary, we showed that the microvascular effects of RBC transfusions are quite variable and are correlated with the age of the transfused RBCs in major surgical patients. These effects cannot be predicted from systemic hemodynamics or systemic blood parameters like Hb and Hct variables. The risks and benefits of RBC transfusion should be assessed for every patient before transfusion during major operation. These results will need to be tested in selected populations under same kind of major surgery that have similar microcirculation characteristics.

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