The effect of fresh versus standard blood transfusion on microvascular endothelial function

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Background The duration of red blood cell (RBC) storage may have a negative impact on endothelial nitric oxide bioavailability. We tested the hypothesis that transfused fresh blood will have a more favorable effect on microvascular endothelial function as compared to older standard issue blood.

Methods Participants requiring chronic RBC transfusions were enrolled in a crossover design study to receive fresh (<7 days of storage) or standard (up to 42 days of storage) blood on 2 separate visits. Endothelial function was assessed by reactive hyperemia peripheral arterial tonometry that was measured before and after transfusions. For each participant, the difference between endothelial function pretransfusion and posttransfusion was assessed in relation to blood storage time.

Results Twenty-one patients (71 ± 16 years, 52% females) were enrolled. Mean age of fresh blood was 5.5 days (±1.0), and that of standard blood was 24.5 days (±7.9 days). The pretransfusion hemoglobin was 83.1 ± 2.5 g/L; and posttransfusion, 98.9 ± 2.6 g/L. An average of 2 U of packed RBCs was transfused. Microvascular endothelial function decreased more frequently after transfusion of standard blood compared to fresh blood. Standard issue blood transfusion was associated with decrease in reactive hyperemia peripheral arterial tonometry index (–0.25 ± 0.63) compared to fresh blood (+0.03 ± 0.49); $P = .026$.

Conclusion Transfusions of standard issue blood are associated with less favorable effect on microvascular endothelial function as compared to fresh blood. (Am Heart J 2016;181:156-61.)

Blood transfusion laboratories play a central role in ensuring an adequate and safe supply of blood products needed for various medical conditions.[1] Red blood cells (RBCs) are usually processed as a concentrated preparation, packed RBC (pRBC), from whole blood by removing the plasma. Packed RBC transfusion is a common therapeutic intervention worldwide, and it is the number 1 discharge code diagnosis of admitted patients in the United States[2] with approximately 85 million units of RBCs transfused annually.[3] Storage of RBC products for up to 42 days has greatly facilitated the ability of transfusion services to meet the ever-growing demands for transfusion.[4] Current evidence indicates that storage of RBCs in nutrient media for up to 42 days maintains adequate RBC 2,3-diphosphoglycerate and ATP levels and a 24-hour posttransfusion survival of at least 75% of transfused RBCs in the circulation.[5]

Prolonged storage of RBCs ex vivo, however, is associated with a number of well-described changes to RBC and the storage medium collectively referred to as the storage lesion which have been shown to have negative impact in animal models.[6]

During storage, RBCs undergo progressive structural and functional changes that reduce oxygen-transport capacity and viability, leading to hemolysis and release of microparticles, free hemoglobin, heme, and iron into the circulation.[7,8] These degradation products can actively scavenge nitric oxide (NO) via deoxygenation reaction, leading to reduced NO and subsequent vasoconstriction and endothelial dysfunction.[9] There is an ongoing debate surrounding the relationship between length of storage of RBCs and outcomes of transfused patients.[10-13]

Several recent randomized controlled trials did not detect a difference in outcomes between patients transfused with fresh and older stored blood.[12,14,15] However, with so many variables that may affect clinical outcomes, these

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studies raise as many questions as answers. It is not clear what mechanism(s) primarily underlie the RBC storage lesion and whether any of these alterations lead to potential adverse clinical outcomes that can be measured such as an effect on vascular function.

Studies in diabetic mice with endothelial dysfunction demonstrate that transfusing pRBCs stored for 14 days induces systemic vasoconstriction and inflammation as compared to fresh pRBCs stored for no longer than 48 hours.[16] The preclinical relationship between blood transfusion and associated endothelial derived factors could have potential clinical implications.[17] The aim of this study was to test the hypothesis that transfused standard issue RBCs have a negative impact on microvascular endothelial function compared to fresh RBCs.

Methods

This is an observational, case cross-over, prospective, single-center study of patients attending the London Health Sciences Centre's IV Therapy Clinic.

The study was approved by the Research Ethics Board of Western University, and all participants provided written informed consent before enrollment. The study is registered at www.clinicaltrials.gov (identifier NCT02161042).

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the manuscript, and its final contents.

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Patients

Participants older than 18 years requiring chronic recurrent pRBC transfusions were enrolled to receive "fresh," defined as 7 days or less pRBC, and "standard issue" blood with storage duration up to 42 days on "first in, first out basis," on a second separate visit date. The experimental conditions were standardized at the 2 visits. All tests were performed in a relaxed calm atmosphere. Patients received the same background medications around the time of the 2 visits. They were instructed to refrain from smoking, alcohol, caffeine, and food rich in polyphenols for at least 12 hours before the study. Medications that could affect vascular reactivity were held. Each visit date lasted approximately 5 hours. Patients were allowed to have a very light breakfast at home as well as water and snack while receiving the blood transfusion. The first EndoPAT test at each visit was performed in the morning. After blood transfusion and a rest period, the second EndoPAT test at each visit was performed in the early afternoon. Participants were blinded to the type of blood transfused. The first 10 patients (group A) received standard issue blood on their first visit and fresh blood on the second visit. This sequence was reversed for the next 8 patients (group B). The last 3 patients were included in group A and received standard issue blood first (Figure 1). This order was not randomly assigned. Participants with latex allergy, life expectancy of <6 months, history and evidence of drug or alcohol abuse in the last 12 months, and any medical condition subjecting the participants to be at a higher risk for transfusion were excluded.

Endothelial function assessment

A fingertip pulse amplitude tonometry (peripheral arterial tonometry [PAT]) was used before and after blood transfusion to assess the peripheral vasodilator response, using the EndoPAT device (Itamar Medical, Inc, Caesarea, Israel). The EndoPAT has been validated as a method of microvascular endothelial function assessment in various studies.[17,18] The device uses unique biosensors placed on the tip of the index finger of each hand to measure pressure. Endothelial function is measured via a reactive hyperemia-peripheral arterial tonometry index (Rh-PAT index). The protocol for measurement of reactive hyperemia involved a baseline measurement for 5 minutes. On the test arm (nondominant arm), a blood pressure cuff was inflated 60 mm Hg above the baseline for 5 minutes. After deflation of the cuff, the PAT tracing was recorded for 5 minutes. The ratio of measured PAT signals in comparison to baseline measurements was calculated through a proprietary computer algorithm automatically normalizing for baseline signal and indexed to the contralateral arm. The calculated ratio, the Rh-PAT index, reflects the degree of endothelial function. Endothelial function was assessed at baseline and immediately after the transfusion of blood, using the EndoPAT to calculate the Rh-PAT index as described. The arm opposite to the test arm was used for blood transfusions.

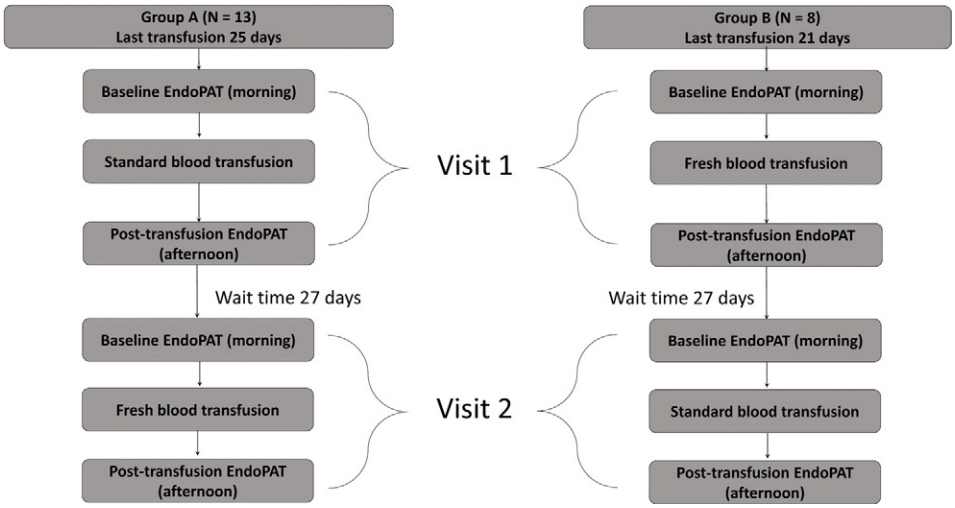
Statistical analysis

Continuous variables are summarized by mean and SD or median (25th, 75th percentiles) if not normally distributed, and categorical variables are presented as counts/percentages. Comparisons between continuous variables were performed using the Student t test, paired t test, or Wilcoxon rank sum test when appropriate. Categorical variables were compared with the Pearson χ^2 test. Pearson correlation coefficients were used to quantify the univariate linear relationship between variables. P values are 2 tailed, and statistical significance was defined as $P < .05$.

Results

During the study period, 21 participants were enrolled and had transfusion of fresh and standard blood on 2 separate occasions with concomitant assessment of endothelial function. The mean age of participants was 71 ± 16 years, and 52% were females. All patients were transfusion dependent. The median duration of transfusion dependence with 25th to 75th percentiles was 1,578 days (221-3,510 days). The number of prior transfusions was 39 (18-181). The last transfusion was received 24.4 ± 11.8 days before study participation. The time interval between visits 1 and 2 was

Figure 1



Study flow diagram. All EndoPAT studies were standardized. Days reported are median.

Table I. Baseline characteristics and associated medical conditions

Characteristic	n = 21
Sex (female) (%)	11 (52%)
Age (y)	71 ± 16
Weight (kg)	73 ± 16.6
Height (m)	1.66 ± 0.10
Pretransfusion Hb (g/L)	83.1 ± 2.4
Posttransfusion Hb (g/L)	98.8 ± 2.6
Biochemical profile	
Creatinine (μmol/L)	85.5 ± 30.8
Fasting glucose (mmol/L)	7.1 ± 3.2
WBC (10^9/L)	6.9 ± 7.17
Cholesterol (mmol/L)	3.6 ± 1.0
Triglycerides (mmol/L)	1.6 ± 1.3
HDL (mmol/L)	1.0 ± 0.4
LDL (mmol/L)	1.8 ± 0.9
Total cholesterol:HDL ratio	3.9 ± 1.4
Associated medical conditions	
Stable angina	6 (28%)
Prior myocardial infarction	2 (9.5%)
Heart failure	4 (19%)
Hypertension	9 (43%)
Hypercholesterolemia	7 (33%)
Smoking	
Former	7 (33%)
Current	4 (19%)
Never	10(48%)
Diabetes	6 (29%)
Previous CVA/TIA	3 (14%)

Hb, Hemoglobin; *WBC*, white blood cell; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *CVA/TIA*, cerebrovascular accident/transient ischemic attack.

27 days (21-56 days). There was no significant difference between groups in any of these parameters. Concomitant medical conditions included diabetes (29%), hypertension (43%), and hypercholesteremia (33%). The demographic and clinical characteristics of all the patients are described in Table I. Myelodysplastic syndrome (MDS) was the most frequent (57%) indication for blood transfusion (Table II). Medications used are described in Table III.

Mean age of fresh pRBCs was 5.5 ± 1.04 days, and that of standard pRBCs was 24.5 ± 7.9 days. The average pretransfusion hemoglobin was 83.09 ± 2.46 g/L, and posttransfusion value was 98.86 ± 2.64 g/L. An average of 2 U of pRBCs were transfused. There was a similar increase in hemoglobin levels by 14.0 ± 11.4 g/L after fresh blood transfusion and by 14.5 ± 8.4 g/L after standard issue blood transfusion (P = .89).

At baseline, patient heart rate was 71 ± 13 beats/min. Systolic and diastolic blood pressures were 119 ± 19 mm Hg and 61 ± 8 mm Hg, respectively. Posttransfusion heart rate was 69 ± 11 beats/min. Systolic and diastolic blood pressures were 121 ± 22 mm Hg and 63 ± 9 mm Hg, respectively. Hemodynamics were very similar between the first and second visits.

Baseline Rh-PAT index for the first visit (before standard or fresh transfusion) was 2.27 ± 0.74; and at the second visit, 2.28 ± 0.58 (P = .69).

Baseline Rh-PAT index before standard blood transfusion was 2.41 ± 0.68 and decreased posttransfusion to 2.16 ± 0.75 (P = .08). The Rh-PAT index at baseline before fresh blood transfusion was 2.18 ± 0.55; and post–fresh blood transfusion, 2.21 ± 0.7 (P = .78). There was no significant difference between Rh-PAT index at the baseline visit for standard transfusion and the baseline visit for fresh transfusion (P = .34).

Baseline EndoPAT values were 2.1 ± 0.22 for patients with MDS and 2.48 ± 0.23 for patients with other hematologic conditions (P = .21).

The baseline EndoPAT measurements were significantly correlated with body mass index (BMI) (P = .018). There

Table II. Different indications for transfusion

Indications of blood transfusion	n = 21
MDS	12
Myelofibrosis	1
Red cell aplasia	1
B-cell lymphoproliferative disorders including chronic lymphocytic leukemia	2
Chronic liver disease	1
Diamond-Blackfan anemia	1
Microcytic anemia	3

Table III. Concomitant medications

Medications	%
Aspirin	9.5
Warfarin	14.3
β-Blocker	19
ACE inhibitor or ARB	33.3
Spironolactone	9.5
Lipid-lowering agent	28.6
Insulin	7.1
Oral hypoglycemic agent	28.6
Ca-channel agonists	23.8
Glyceryl trinitrate	23.8

ACE, Angiotensin-converting enzyme; *ARB*, angiotensin receptor blocker.

was no significant correlation with the presence of other risk factors.

Microvascular endothelial function decreased more often after transfusion of standard pRBCs, with a decrease in Rh-PAT index (−0.25 ± 0.63) compared to minimal change on average with fresh pRBCs (+0.03 ± 0.49) (P = .026) (Figure 2).

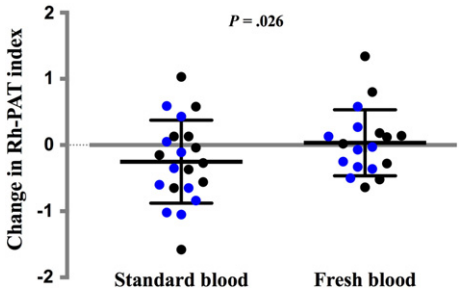
Discussion

The present study demonstrates that transfusion of standard blood may have a negative acute impact on microvascular endothelial function as compared to the transfusion of fresh blood.

The negative effect on Rh-PAT index as seen in our study is likely due to physiological changes in pRBC related to storage time.[13] Reductions in NO availability after transfusion of stored blood has been described.[4]

Standard issue blood transfusion leads to shedding of microparticles that actively scavenge NO due to deoxygenation reaction and leads to endothelial dysfunction. Indeed, a randomized trial of fresh (<14 days) versus standard issue blood (>21 days) demonstrated that NO-mediated vasodilation was reduced after transfusion of old stored blood as compared to fresh blood.[19] Our study extends this observation by its cross-over design. Endothelial dysfunction is associated with decreased NO availability and can cause vasospasm, vasoconstriction, thrombus formation, and abnormal vascular proliferation

Figure 2



Change in endothelial function. Absolute change in Rh-PAT values posttransfusion compared to baseline. Blue indicates patients with MDS; black, all other patients.

leading to increased atherogenesis and enhancing the cardiovascular risks.[20]

We have not tested circulating biomarkers to further assess endothelial function. Measurement of circulating levels of nitrites and nitrosylated proteins could help in estimation of endothelial generation of NO but may not always represent endothelial NO production. Inflammatory cytokines and adhesion molecules such as E-selectin might have also been measured in the circulation.[21]

Patients with atherosclerosis often demonstrate evidence of oxidative stress and endothelial dysfunction.[22] It is unclear what the effect of blood transfusion is on such interaction and if a change in certain biomarker level is a cause or a result of endothelial dysfunction. The degradation products formed in pRBC can scavenge NO which may result in endothelial dysfunction.[9]

Endothelial dysfunction is an independent predictor of cardiovascular events.[17,23] There are different methods for assessment of endothelial function. It can be performed in the coronary arteries[24] or noninvasively in the peripheral circulation.[17,25] Two common noninvasive methods that are used are the brachial flow-mediated dilatation (FMD) which assesses conduit vessel endothelial function[25] and the EndoPAT which reflects more the microcirculation.[17] These 2 tests have some degree of correlation with coronary endothelial function but measure different aspects of the vascular system, and results of both tests have been shown to correlate with clinical outcome.[26] Although FMD is a well-accepted method for assessment of endothelial function, it has limitations and is not well standardized.[26,27] The results of FMD are affected by probe position, technique for image acquisition, arterial edge detection, and software used.[25,26,28] We chose to use the EndoPAT for this study being less operator dependent, more standardized compared to brachial FMD and highly reproducible.[29] The EndoPAT results correlated with BMI but not with other risk factors. This finding is in accordance with previous studies demonstrating that the EndoPAT results are associated with metabolic risk factors such as diabetes mellitus and BMI

and less with traditional risk factors.[26,30,31] The EndoPAT results seem to provide additive information beyond traditional risk factors and thus useful in risk assessment beyond Framingham risk score.[17,31]

Blood transfusions may have an impact on endothelial dysfunction and potentially lead to worse cardiovascular outcomes. The participants in our study required repeat blood transfusions and therefore are potentially at risk due to recurrent blood transfusion effects on endothelial dysfunction.

We assessed microvascular endothelial function once following each transfusion, and therefore, our study does not provide information regarding the duration of the effect on endothelial function. Although abnormal EndoPAT results are a predictor of worse outcomes,[17,32] studies using EndoPAT often chose a single cutoff separating patients into dichotomous groups with normal or abnormal endothelial function. It is unknown if the small transient change in Rh-PAT with blood transfusion observed in our patients is clinically important. Although it is unknown if the effect of a single blood transfusion on endothelial function in participants with cardiac disease is harmful, a small-scale randomized trial showed that a liberal approach of blood transfusions was associated with increased mortality, recurrent myocardial infarction, and heart failure as compared to more restrictive use of blood transfusions in patients with acute myocardial infarction.[33] The effect of blood transfusion in patients presenting with myocardial infarction will be further assessed in the MINT trial (NCT02619136).

In a cardiac surgery cohort, blood transfusion is associated with more infections and ischemic postoperative morbidity, hospital stays, increased early and late mortality, and hospital costs.[34] A negative impact was also observed in a review of 9 studies that included patients from cardiac surgery, trauma, and intensive care.[11] A study from Cleveland clinic demonstrated that administration of standard blood (15-42 days of storage) as compared to fresher units (14 days of storage) to patients undergoing heart surgery was associated with acute negative effect including increase in in-hospital mortality and renal failure as well as increase in 1-year mortality.[35] Our study extends those observations and demonstrates acute negative impact on microvascular endothelial function which may in part account for some of the morbidity ascribed to transfusion of older stored blood in cardiac patients.

Numerous observational studies have shown conflicting results and at least 6 randomized controlled trials underway or recently completed have shown no difference in clinical outcomes due to RBC storage duration.[12,14,15] Specifically, 3 large multicenter randomized controlled trials in the critically ill,[15] cardiac patients,[12] and premature infants[14] did not identify any advantage in clinical outcomes of fresh versus older stored RBC products. It remains uncertain which of the myriad of RBC product changes during storage is important or whether any of the observed ex vivo changes has any measureable adverse clinical outcomes. Given the complexity of the RBC storage lesion and potential host disease variabilities in the population studied, even large randomized trials may be limited in their ability to provide a firm conclusion.[36]

Limitations

Our study was small in size, and therefore, the results may be impacted by intervariability and intravariability of the EndoPAT test. We limited the study to patients with hematology disorders. Therefore, it cannot be generalized to patients with other conditions including patients with cardiovascular disorders requiring blood transfusions. The long-term impact of repeat blood transfusions has not been studied.

The measurements obtained by the EndoPAT reflect the microcirculation, and therefore, the results of our study do not reflect effect of blood transfusion on conduit vessel endothelial function.

In conclusion, transfusion of standard issue blood product had a negative acute impact on microvascular endothelial function.

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References

1. Hess JR. Conventional blood banking and blood component storage regulation: opportunities for improvement. Blood Transfus 2010;8(Suppl 3):s9-15.
2. Pfuntner A, Wier LM, Stocks C. *Most frequent procedures performed in U.S. hospitals, 2010. Healthcare Cost and Utilization Project. 2013.*
3. Carson JL, Grossman BJ, Kleinman S, et al. Red blood cell transfusion: a clinical practice guideline from the AABB*. Ann Intern Med 2012;157(1):49-58.
4. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci U S A 2007;104(43): 17063-8.
5. Roback JD, Combs MR, Grossman BJ, Hillyer CD. *AABB technical manual. 16th ed. Maryland, USA: American Association of Blood Banks. 2008.*
6. Hess JR. Measures of stored red blood cell quality. Vox Sang 2014;107(1):1-9.
7. Donadee C, Raat NJ, Kanias T, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011;124(4):465-76.
8. Berra L, Coppadoro A, Yu B, et al. Transfusion of stored autologous blood does not alter reactive hyperemia index in healthy volunteers. Anesthesiology 2012;117:56-63.
9. Reiter CD, Wang X, Tanus-Santos JE, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nat Med 2002;8(12): 1383-9.

10. Edgren G, Kamper-Jorgensen M, Eloranta S, et al. Duration of red blood cell storage and survival of transfused patients (CME). Transfusion 2010;50(6):1185-95.
11. Tinmouth A, Fergusson D, Yee IC, et al. Clinical consequences of red cell storage in the critically ill. Transfusion 2006;46(11):2014-27.
12. Steiner ME, Ness PM, Assmann SF, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med 2015;372(15):1419-29.
13. Chin-Yee I, Arya N, d'Almeida MS. The red cell storage lesion and its implication for transfusion. Transfus Sci 1997;18(3):447-58.
14. Fergusson DA, Hebert P, Hogan DL, et al. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial. JAMA 2012;308(14):1443-51.
15. Lacroix J, Hebert PC, Fergusson DA, et al. Age of transfused blood in critically ill adults. N Engl J Med 2015;372(15):1410-8.
16. Yu B, Lei C, Baron DM, et al. Diabetes augments and inhaled nitric oxide prevents the adverse hemodynamic effects of transfusing syngeneic stored blood in mice. Transfusion 2012;52(7):1410-22.
17. Rubinshtein R, Kuvin JT, Soffler M, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J 2010;31(9):1142-8.
18. Kuvin JT, Mammen A, Mooney P, et al. Assessment of peripheral vascular endothelial function in the ambulatory setting. Vasc Med 2007;12(1):13-6.
19. Neuman R, Hayek S, Rahman A, et al. Effects of storage-aged red blood cell transfusions on endothelial function in hospitalized patients. Transfusion 2014.
20. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101(16):1899-906.
21. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007;115(10): 1285-95.
22. Lavi S, Yang EH, Prasad A, et al. The interaction between coronary endothelial dysfunction, local oxidative stress, and endogenous nitric oxide in humans. Hypertension 2008;51(1):127-33.
23. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 2003;23(2):168-75.
24. Lavi S, Prasad A, Yang EH, et al. Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease. Circulation 2007;115(20):2621-7.
25. Lavi S, Thorpe K, Luca MC, et al. Inhibition of sPLA2 and endothelial function: a substudy of the SPIDER-PCI trial. Can J Cardiol 2012;28(2):215-21.
26. Flammer AJ, Anderson T, Celermajer DS, et al. The assessment of endothelial function: from research into clinical practice. Circulation 2012;126(6):753-67.
27. Bots ML, Westerink J, Rabelink TJ, et al. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. Eur Heart J 2005;26(4):363-8.
28. Donald AE, Charakida M, Falaschetti E, et al. Determinants of vascular phenotype in a large childhood population: the Avon Longitudinal Study of Parents and Children (ALSPAC). Eur Heart J 2010;31(12):1502-10.
29. Selamet Tierney ES, Newburger JW, Gauvreau K, et al. Endothelial pulse amplitude testing: feasibility and reproducibility in adolescents. J Pediatr 2009;154(6):901-5.
30. Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation 2008;117(19):2467-74.
31. Hamburg NM, Palmisano J, Larson MG, et al. Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. Hypertension 2011;57(3):390-6.
32. Akiyama E, Sugiyama S, Matsuzawa Y, et al. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. J Am Coll Cardiol 2012;60(18):1778-86.
33. Cooper HA, Rao SV, Greenberg MD, et al. Conservative versus liberal red cell transfusion in acute myocardial infarction (the CRIT Randomized Pilot Study). Am J Cardiol 2011;108(8):1108-11.
34. Murphy GJ, Reeves BC, Rogers CA, et al. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation 2007;116(22):2544.
35. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008;358(12): 1229-39.
36. Chin-Yee IH, Chin-Yee BH, Pereira A. Clinical trials and the age of blood: ABLE but still wanting. Transfus Med 2015;25(5):349-50.