Presurgical levels of circulating cell-derived microparticles discriminate between patients with and without transfusion in coronary artery bypass graft surgery

Wenche Jy, PhD,[a] Orlando Gómez-Marín, MSc, PhD,[b] Tomas A. Salerno, MD,[c] Anthony L. Panos, MD,[c] Donald Williams, MD,[c] Lawrence L. Horstman, BS,[a] and Yeon S. Ahn, MD[a]

Objectives: Improved understanding of presurgical risk factors for transfusions will lead to reduction in their number and related complications. The goal of this study is to identify these factors in coronary artery bypass graft (CABG) surgery.

Methods: Presented herein are results of analyses of data from an ongoing study of transfusion in CABG surgery. Of 122 patients, 81 received transfusion (Tx) and 41 did not (NoTx). In addition to routine tests, presurgical levels of microparticles from platelets (PMPs), red cells (RMPs), and other lineages were assayed.

Results: The Tx and NoTx groups were similar with respect to most presurgical variables but differed in distribution of gender, blood type, diabetes prevalence, activated partial thromboplastin time (aPTT), hemoglobin (HGB), and microparticle levels. Stepwise multiple logistic regression was used to evaluate presurgical variables and to develop a model to assess risk factors for transfusion. $CD41^+$ PMP and $CD235^+$ RMP levels were found to be the main risk factors for transfusion. The Model's discriminating ability was assessed using receiver operating characteristic curve analysis, which showed that the area under the model curve ($\pm$ standard error) was 0.86 ± 0.04 (95% confidence interval, 0.77-0.94). According to the model, patients with higher presurgical levels of circulating $CD41^+$ PMP, $CD235a^+$ RMP, and HGB, as well as a shorter aPTT, are less likely to receive transfusion(s).

Conclusions: Presurgical levels of $CD41^+$ PMPs and $CD235a^+$ RMPs are the main risk factors for transfusion in CABG, followed by HGB and aPTT. (J Thorac Cardiovasc Surg 2015;149:305-11)

See related commentary on pages 312-3.

Blood transfusion saves many lives but is associated with higher incidence of adverse outcomes compared to no transfusion.[1,2] The adverse effects of transfusion include higher incidence of postsurgical infections, longer hospital stay, poorer surgical outcomes, and higher mortality.[3-7] There is a growing interest in how blood transfusion should be better managed.[1,7]

A number of risk factors for transfusion during surgery have been identified, including older age, female gender, weight, renal insufficiency, abnormal left ventricle ejection fraction, emergency surgery, longer cardiopulmonary bypass (CPB) time, and low presurgical hemoglobin levels.[8-11] In addition, several parameters of coagulation and platelet status have been shown to be associated with surgical transfusion.[12-15]

Cell-derived microparticles are small membranous vesicles of size <1.0 μm, released during cell activation and apoptosis. Evidence is accumulating that circulating microparticles play an important role in hemostasis.[16] They are capable of accelerating blood coagulation and enhancing platelet aggregation and adhesion.[16-18] Clinical studies have revealed that patients who are deficient in microparticle generation are prone to bleeding episodes.[17,19] Patients with immune thrombocytopenic purpura who had high levels of circulating microparticles were protected against bleeding compared to those with lower levels of microparticles and similarly low platelet counts.[20] The role of microparticles as a risk factor for transfusion has not been previously reported.

The major aim of the present analysis is to assess the presurgical levels of microparticles as well as clinical and sociodemographic characteristics as risk factors for transfusion during and/or after coronary artery bypass graft (CABG) surgery.

From the Wallace H Coulter Platelet Laboratory,[a] Division of Hematology and Oncology, Department of Medicine, Departments of Public Health Sciences, Medicine, and Pediatrics,[b] and Division of Cardiothoracic Surgery,[c] Department of Surgery, Miller School of Medicine, University of Miami, Miami, Fla.

This work was supported by grants from the National Institutes of Health, National Heart, Lung and Blood Institute (1R01HL098031), and the Wallace H. Coulter Foundation.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication March 24, 2014; revisions received Aug 29, 2014; accepted for publication Oct 6, 2014.

Address for reprints: Wenche Jy, PhD, 1600 NW 10th Ave. R-36A, Miller School of Medicine, University of Miami, Miami, FL 33136 (E-mail: wjy@med.miami.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2014.10.042

PM

Abbreviations and Acronyms

AUC = area under the curve
aPTT = activated partial thromboplastin time
CABG = coronary artery bypass graft
CPB = cardiopulmonary bypass
Cy5 = Cyanine 5
EMP = endothelium-derived microparticle
FITC = fluorescein isothiocyanate
HGB = hemoglobin
LMP = leukocyte-derived microparticle
MP = microparticle
PE = phycoerythrin
PMP = platelet-derived microparticle
PT = prothrombin time
QC = quality control
RBC = red blood cell
RMP = red cell–derived microparticle
ROC = receiver operating characteristic
TEG = thromboelastography

MATERIALS AND METHODS

Patient Population

We present the results of analyses of data obtained from an ongoing randomized clinical trial on transfusion practice in patients undergoing CABG surgery (NCT01185600). The main hypothesis of the original trial is that transfusion of washed packed cells results in improved surgical outcomes and lower levels of proinflammatory biomarkers compared to transfusion of unwashed packed cells. At presurgery, participants are randomized to 1 of these 2 groups, but some of them end up not needing a transfusion. As a part of the study, cell-derived microparticles are assayed for all participants. Results presented in this manuscript are limited to presurgical data used to compare patients who received transfusion(s) versus those who did not.

Inclusion/exclusion criteria. All patients admitted to the University of Miami medical center for CABG surgery are screened as potential study participants. The exclusion criteria include (1) age <20 years, (2) pregnancy, (3) refusal to accept blood transfusion, (4) combined CABG and other procedures such as valve replacement, (5) emergency surgery, and (6) known bleeding disorders. Informed written consent is obtained from each eligible patient who agrees to participate in the study.

Transfusion criteria. Each participant is classified as either a high-risk or non-high-risk patient. The former class includes patients with renal failure, or on clopidogrel (Plavix) within 5 days prior to surgery, or with ejection fraction <20%. The transfusion criteria are as follows: (1) for the high-risk group, hematocrit ≤28%; (2) for the non-high-risk group, hematocrit ≤25%; or (3) for any patient with active blood loss and unstable vital signs (eg, falling blood pressure, tachycardia, or hypoxemia), the surgical team uses clinical judgment for transfusion.

Preoperative medications. Coumadin and clopidogrel (Plavix) are discontinued at least 5 days prior to surgery. Aspirin is continued without interruption according to protocol. Those who did not stop Plavix within 5 days are considered in the high-risk group for bleeding (see above transfusion criteria). Heparin is discontinued 5 hours before surgery and resumed 24 hours after surgery with prophylactic subcutaneous daily injection. For the present analyses, no special consideration was given to numerous other drugs taken for various comorbidities, but all are recorded in the database.

A total of 122 patients undergoing CABG have been recruited for the study. Of them, 81 received red blood cell (RBC) transfusion(s) during and/or after surgery (Tx Group), whereas the remaining 41 did not receive any transfusion (NoTx Group). The present report is concerned with the assessment of presurgical characteristics as risk factors for transfusion in patients undergoing CABG surgery.

Laboratory Studies

Routine complete blood count, platelet, coagulation tests, and blood chemistry are performed before surgery and as needed postoperatively. Special studies performed are listed below.

Blood Sampling and Handling

At 1 hour prior to surgery, using a 21-gauge needle, venous blood samples are drawn into plastic vacuum tubes containing sodium citrate (Vacuettes; Greiner Bio-One, Monroe, NC). To minimize tissue factor contamination, the first tube is used for routine lab tests and subsequent blood samples are used for microparticle assays. Samples are centrifuged at $1800 \times g$ for 15 minutes within 1 hour of collection to minimize release of microparticles ex vivo. All samples are maintained at room temperature. After centrifugation, supernatants containing the microparticles (platelet-poor plasma) are removed for flow cytometric assays, within 4 hours after blood drawing.

Materials for Flow Cytometry

The sources of monoclonal antibodies and markers are as follows. Beckman-Coulter (Brea, Calif): anti-CD41–fluorescein isothiocyanate (FITC) (catalog no. IM0649U), anti-CD42B-FITC (catalog no. IM0648U), anti-CD45-phycoerythrin (PE) (catalog no. IM2078U), and CD235a-PE (catalog no. IM2211U); Becton-Dickinson (Franklin Lakes, NJ): anti-CD11B-PE-Cyanine 5 (Cy5) (catalog no. 555389) and CD62E-PE-Cy5 (catalog no. 550040); e-Bioscience (San Diego, Calif): anti-CD144-PE (catalog no. 12-1449-80); Sekisui Diagnostics (Stamford, Conn): anti-tissue factor-FITC (catalog no. 4508CJ); and Sigma-Aldrich (St Louis, Mo): annexin V–FITC (catalog no. 072M4060).

Microparticle Assays by Flow Cytometry

Microparticle species and subtypes are assayed by flow cytometric methods, briefly as follows. For labeling of microparticles, 20 μL of platelet-poor plasma are incubated with 4 μL of monoclonal antibodies specific for each cell lineage. The monoclonal antibodies used are anti-CD235a, for red cell–derived microparticles (RMPs); anti-CD41-FITC or CD31-PE plus CD42-FITC ($CD31^+/CD42^+$), for 2 phenotypes of platelet-derived microparticles (PMPs); anti-CD144-PE, CD62E-PE-Cy5, or CD31-PE plus CD42-FITC ($CD31^+/CD42^-$), for 2 phenotypes of endothelium-derived microparticles (EMPs); anti-CD45-PE or CD11b-PE-Cy5, for leukocyte-derived microparticles (LMPs); anti–tissue factor (TF)-FITC for TF^+ microparticles. Annexin V^+-derived microparticles are labeled by 2 μL of annexin V–FITC (Sigma) plus 2.4 μL of 40 mM $CaCl_2$. Next, the samples are gently shaken (60 rpm) for 20 minutes to ensure optimal antibody binding and then diluted with 500 μL of 0.9% NaCl plus 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4. Additional details on flow cytometric procedures are described in Jy et al.[18]

Thromboelastography (TEG)

For TEG assays, all blood samples are tested not more than 3 hours after drawing. A total of 330 μL of whole blood is added to a well containing 20 μL of 200 mM $CaCl_2$ to initiate coagulation. The TEG parameters of interest in this study are as follows: *R*, lag time to initial fibrin formation; *K*, time to amplitude of 20 mm; *A*, angle, reflecting the initial rate; MA, maximum amplitude, reflecting platelet function; and coagulation index, which is a composite global measure, calculated by the following formula:

$$\text{Coagulation index} = -0.2454R + 0.0184K + 0.1655\text{MA} - 0.0241A - 5.022.$$

PM