OBSERVATIONAL STUDY

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Role of Omega-6 Fatty Acid Metabolism in Cardiac Surgery Postoperative Bleeding Risk

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OBJECTIVES: Cardiac surgery is frequently associated with significant postoperative bleeding. Platelet-dysfunction is the main cardiopulmonary bypass (CPB)-induced hemostatic defect. Not only the number of platelets decreases, but also the remaining are functionally impaired. Although lipid metabolism is crucial for platelet function, little is known regarding platelet metabolic changes associated with CPB-dysfunction. Our aim is to explore possible contribution of metabolic perturbations for platelet dysfunction after cardiac surgery.

DESIGN: Prospective cohort study.

SETTING: Tertiary academic cardiothoracic-surgery ICU.

PATIENTS: Thirty-three patients submitted to elective surgical aortic valve replacement.

INTERVENTIONS: Samples from patients were collected at three time points (preoperative, 6- and 24-hr postoperative). Untargeted metabolic analysis using high-performance liquid chromatography-tandem mass spectrometry was performed to compare patients with significant postoperative bleeding with patients without hemorrhage. Principal component analyses, Wilcoxon matched-pairs signed-rank tests, adjusted to FDR, and pairwise comparison were used to identify pathways of interest. Enrichment and pathway metabolomic complemented the analyses.

MEASUREMENTS AND MAIN RESULTS: We identified a platelet-related signature based on an overrepresentation of changes in known fatty acid metabolism pathways involved in platelet function. We observed that arachidonic acid (AA) levels and other metabolites from the pathway were reduced at 6 and 24 hours, independently from antiagreggation therapy and platelet count. Concentrations of preoperative AA were inversely correlated with postoperative chest tube blood loss but were not correlated with platelet count in the preoperative, at 6 or at 24 hours. Patients with significant postoperative blood-loss had considerably lower values of AA and higher transfusion rates. Values of postoperative interleukin-6 were strongly correlated with AA variability.

CONCLUSIONS AND RELEVANCE: Our observations suggest that an inflammatory-related perturbation of AA metabolism is a signature of cardiac surgery with CPB and that preoperative levels of AA may be more relevant than platelet count to anticipate and prevent postoperative blood loss in patients submitted to cardiac surgery with CPB.

KEY WORDS: cardiac surgery; inflammation; metabolism; platelet; postoperative bleeding

Excessive bleeding is a frequent complication after cardiac surgery with cardiopulmonary bypass (CPB), frequently requiring the use of allogenic blood products. Cardiac surgery accounts for 10–15% of transfusions in surgical patients in the United States yearly (1, 2), and more than half of patients receive transfusion of blood products during hospital stay (3). Use of transfusions is not

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KEY POINTS

- **Question:** What is the role of platelet-metabolic perturbation in cardiac surgery postoperative bleeding?
- **Findings:** In our study we have analyzed the metabolic perturbations of 33 patients submitted to aortic valve replacement, identifying a platelet-related signature based on an overrepresentation of changes in fatty acid metabolism pathways.
- **Meaning:** In patients submitted to cardiac surgery, the inflammatory perturbation of platelet fatty acid metabolism has a significant role in postoperative bleeding.

harmless, significantly impacting patients' outcome and increasing both morbidity and mortality, and healthcare costs (4). Furthermore, postoperative bleeding is also associated with a higher rate of reoperation, which is independently associated with poorer outcomes (5).

CPB has a distinctive and significant contribution for the disruption of hemostasis in cardiac surgery (6, 7). Platelet-function deficit is considered as the main CPB-induced hemostatic deficiency (8). CPB decreases the number of platelets by dilution, adhesion, destruction, and consumption (9), and this reduction is associated with excessive postoperative bleeding (9). Furthermore, remaining platelets are functionally impaired, but mechanisms remain unclear (10). Even patients with a normal platelet count show higher prevalence of platelet dysfunction of unknown-cause (11). Although circulating platelets appear structurally normal, recovering within minutes after CPB, bleeding times increase and remain prolonged for several hours (12). Flow cytometry in whole-blood after CPB revealed no significant changes in platelet surface receptors (8), and few advances have been made in the field recently.

While lipid metabolism is essential for platelet activity and function (13), little is known regarding platelet metabolic changes associated with CPB-dysfunction. To explore the possible contribution of metabolic perturbations for platelet dysfunction after cardiac surgery with CPB, we performed an untargeted metabolomic analysis using high-performance liquid chromatography-tandem mass spectrometry to identify specific metabolic signatures that contribute to platelet dysfunction after CPB.

MATERIALS AND METHODS

Study Population

The aortic valve replacement for inflammation (SVA-Study) registry is a study to characterize the inflammatory response after surgical aortic valve replacement (SAVR). Patients included were older than 18 years undergoing SAVR. Previous cardiac surgery, concomitant procedures, neoplasia, and use of corticosteroids were exclusion criteria. Blood was collected from 33 patients before surgery, and 6 and 24 hours after surgery. Plasma was prepared and stored at –80°C. Medical records were assessed to obtain clinical data. All patients provided written informed consent, and the study was approved by the Institutional Ethics Committee (Comissão Ética-Centro Hospitalar Lisboa Norte, Ref. N.º23/18, April 2018), in accordance with Declaration of Helsinki and following STROBE guidelines.

Classification of Hemorrhage

Postoperative chest tube output was quantified in the ICU hourly. Significant bleeding was considered when postoperative chest tube blood loss was above 600 mL within 12 hours, as defined by the International Initiative on Haemostasis Management in Cardiac Surgery (14). The need for transfusion (platelet concentrate, packed red-blood cells, fresh frozen plasma, and fibrinogen) was considered according to bleeding and/or to correct aggregation and coagulation deficiencies (6, 15).

Untargeted Metabolomics

After generation of blood plasma, proteins were removed by adding 400 uL of a methanol/ethanol mixture (4:1, v/v) to 100 ul of plasma, followed by vigorous vortex shaking for 5 minutes at room-temperature and centrifugating at 4000 × *g* for 10 minutes at 4°C. The supernatant was collected, transferred to an Eppendorf, shock frozen with liquid nitrogen, and stored at –80°C until analysis. Extracted samples were thawed on ice, centrifuged for 2 minutes at 15,000 × *g*, and diluted according to the different sample weight with 0.1% formic acid (reversed phase [RP]) or 50% acetonitrile

(ACN) (hydrophilic interaction chromatography [HILIC]). A total of 2.5 mL of each diluted sample was pooled and used as a quality control (QC) sample. Samples were randomly assigned into the autosampler, and metabolites were separated on a SeQuant ZIC-pHILIC HPLC column (Merck, 100 3 2.1 mm; 5 mm) or an RP-column (Waters, ACQUITY ® ultra - performance liquid chromatography high strength silica T3 150 3 2.1;1.8 mm) with a flow rate of 100 mL/min delivered through an Ultimate 3000 HPLC system (Thermo-Fisher Scientific). The gradient ramp-up time takes 25 minutes from 10% to 80% B in HILIC (A: ACN; B: 25 mM ammonium bicarbonate in water) and from 1% to 90% B in RP (A: 0.1% formic acid in water; B: 0.1% formic acid in ACN). Metabolites were ionized via electrospray ionization in polarity switching mode after HILIC separation and in positive polarity mode after RP separation. Sample spectra were acquired by data-dependent high-resolution tandem mass spectrometry on a Q-Exactive Focus (Thermo-Fisher Scientific). Ionization potential was set to +3.5/–3.0 kV, the sheet gas flow was set to 20, and an auxiliary gas flow of 5 was used. Samples were analyzed in a randomized fashion, and QC samples were additionally measured in confirmation mode to obtain additional MS/MS spectra for identification. Obtained datasets were processed by compound discoverer 3.0 (Thermo-Fisher Scientific). Compound annotation was conducted by searching the mzCloud database with a mass accuracy of 3 ppm for precursor masses and 10 ppm for fragment ion masses as well as ChemSpider with a mass accuracy of 3 ppm using BioCyc, Human Metabolome Database, Kyoto Encyclopedia of Genes and Genome, MassBank, and MetaboLights as databases.

Cytokine Measurement

Cytokine concentrations were determined using the human interleukin-6 (IL-6) kit (ELISA-MAX Deluxe Sets, BioLegend, San Diego, CA), according to manufacturer's protocol and measured on a Tecan spectrophotometer plate-reader.

Statistical Analysis

Principal component analysis (PCA) was performed with R (16), using the basic package "stats." Remaining statistical analysis was performed using GraphPad Prim 9.0 (GraphPad Software, San Diego, CA). Continuous variables are presented with median with interquartile range (IQR) and were analyzed using Wilcoxon matched-pair signed rank test for paired samples and Wilcoxon rank-sum test for nonpaired samples, adjusted to false discovery rate. Pairwise comparison was performed between pre- and postoperative samples. The Bonferroni correction was performed to reduce the chances of obtaining false-positive results (type I errors). Categorical variables are reported in percentage or frequency and were analyzed using chi-square test. For correlation, we used the Spearman rank-order correlation. Predictive models were performed using logistic regression. Enrichment and pathway metabolomic analysis was performed using the MetaboAnalyst 5.0 tool (17). The following symbols were used in figures to indicate statistical significance: ns: nonsignificant; $p < 0.05$(*); $p < 0.01$(**); $p < 0.001$(***); $p < 0.0001$(****).

RESULTS

Untargeted Metabolomics

Patients demographic and clinical characteristics are detailed in **Table 1**. No differences were observed between the two groups (significant bleeding and no significant bleeding) regarding age, gender, comorbidities, left ventricle function, and median Euroscore II. Untargeted metabolomics data for the complete population recorded 8,668 metabolic features for each plasma sample. To identify metabolites associated with an increased risk of postoperative hemorrhage, we employed multivariate analysis, mainly pattern recognition tools, such as PCA, between patients with or without significant postoperative hemorrhage. The PCA plot did not reveal a clear separation in the metabolic profile between both groups. We have then performed a pairwise hypothesis test comparing pre- and postoperative metabolite measurements at 24 hours. The pairwise hypothesis test identified statistically significant differences for 547 metabolites (p value < 0.001). Using the 547 significant metabolites, an enrichment and pathway analysis was performed. From the top 30 pathways identified in our analysis (**Supplementary Fig. 1, *A*** and ***B***, http://links.lww.com/CCX/B57), we identified a platelet-related signature, characterized by an overrepresentation of changes in one known fatty acid metabolism pathway (arachidonic acid [AA] pathway) involved in platelet function. Although it was not the

TABLE 1.
Surgery and Postoperative Data

Variable	All Patients	No Significant Bleeding	Significant Bleeding	*p*
n	33	22	11	
Age, yr, median (IQR)	73.5 (68.5–76.8)	75 (69.3–80.3)	71.5 (67.3–74)	0.115
Male sex, *n* (%)	16 (48.5)	11 (50)	5 (45.5)	> 0.999
Body mass index (kg/m^2)	27.8 (24.7–30.5)	27.5 (24.7–29.8)	28.4 (23.3–31.8)	0.849
Use of antiaggregation, *n* (%)				
Acetylsalicylic acid	15 (45.5)	10 (45.5)	5 (45.5)	> 0.999
Hypertension, *n* (%)	24 (72.7)	14 (63.6)	10 (90.9)	0.212
Diabetes mellitus, *n* (%)	11 (33.3)	5 (22.7)	6 (54.5)	0.117
Dyslipidemia, *n* (%)	7 (21.2)	4 (18.2)	3(27.3)	0.661
Chronic kidney disease, *n* (%)	4 (12.1)	3 (13.6)	1 (9.1)	> 0.999
Peripheral vascular disease, *n* (%)	3 (9.1)	2 (9.1)	1 (9.1)	> 0.999
Cerebrovascular disease, *n* (%)	0 (0)	0 (0)	0 (0)	–
Chronic lung disease, *n* (%)	5 (15.2)	4 (18.2)	1 (9.1)	0.643
Ischemic cardiopathy, *n* (%)	6 (18.2)	4 (18.2)	2 (18.2)	> 0.999
Previous cardiac surgery, *n* (%)	0 (0)	0 (0)	0 (0)	–
Preserved LV function, *n* (%)	33 (100)	22 (100)	11(100)	> 0.999
Moderate LV function 31–50%, *n* (%)	0 (0)	0 (0)	0 (0)	–
Poor LV function (21–30%), *n* (%)	0 (0)	0 (0)	0 (0)	–
Very poor LV function (<20%), *n* (%)	0 (0)	0 (0)	0 (0)	–
EuroSCORE II (IQR)	1.3 (1–1.83)	1.37 (1.01–2.1)	1.1 (0.94–1.85)	0.536
Cardiopulmonary bypass time, min (IQR)	36.5 (32.3–64)	36.5 (31.8–66)	38 (32.3–59.5)	> 0.999
Cross-clamp time, min (IQR)	27.5 (22.3–52.5)	26.5 (22.8–53.5)	31 (21–50)	0.865
SOFA 24 hr, median (IQR)	1 (0–4)	1 (0–3.5)	2 (1–4)	0.218
SOFA 0 at 24 hr (%)	11 (33)	10 (45.5)	1 (9.1)	0.037
Postoperative bleeding (mL)	400 (300–600)	300 (300–400)	600 (600–700)	< 0.0001
Necessity of transfusion	11 (33.3)	6 (27.3)	5 (45.5)	0.437
RBCs	8 (24.2)	4 (18.2)	2 (18.2)	> 0.999
Platelet concentrate	6 (18.2)	3 (13.6)	5 (45.5)	0.083
Fresh frozen plasma	5 (15.2)	2 (9.1)	1 (9.1)	> 0.999
Reoperation due to tamponade, *n* (%)	0 (0)	0 (0)	0 (0)	–
Acute kidney injury *n* (%)	14 (42.4)	9 (40.5)	5 (45.5)	0.803
Atrial fibrillation, *n* (%)	8 (24.2)	8 (36.4)	0 (0)	0.022
Haemodynamic support, *n* (%)	13 (39.4)	7 (31.8)	6 (54.5)	0.208
Mechanical ventilation >6 hr, *n* (%)	12 (36.4)	5 (22.7)	7 (63.6)	0.021
Neurologic complications, *n* (%)	3 (9.1)	0 (0)	3 (27.3)	0.01
Infection, *n* (%)	1 (3)	1 (4.5)	0 (0)	0.472
Wound infection, *n* (%)	0 (0)	0 (0)	0 (0)	–
ICU length of stay	2 (1–3)	2 (1–3)	2 (2–4)	0.352
Hospital length of stay	6 (5–7)	6 (5–7)	6 (5–7)	0.806
Discharge, *n* (%)				
Other hospital	4 (12.1)	2 (9.1)	2 (18.2)	0.451
Home	29 (87.9)	20 (90.9)	9 (81.8)	

EuroSCOREII = European System for Cardiac Operative Risk Evaluation II, IQR = interquartile range, LV = left ventricular, SOFA = Sequential Organ Failure Assessment.

Hemodynamic support: use of vasopressors to maintain adequate perfusion, without the need for mechanical support.

Neurologic complication: occurrence of stroke, delirium, or postoperative cognitive dysfunction.

most significant pathway identified in our metabolites' set, it was an excellent candidate for a pathway with impact on platelet dysfunction, since AA is an essential contributor to platelet's function. As our aim was to evaluate the contribution of metabolic perturbations for platelet dysfunction, we proceeded to assess changes in the AA metabolism. The list of the studied metabolites, associated with the identified pathway, is presented in **Supplementary Table 1** (http://links.lww.com/CCX/B57). Further identification of the other significant metabolites and/ or pathways was not completed.

We have evaluated the concentrations of the nine features associated with the AA pathway: AA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), conjugated linoleic acids (CLAs), platelet-activating factor (PAF) (as part of the upstream activation of AA metabolism), 8,11,14-eicosatrienoic acid (eicosanoids pathway), 12-Hydroxyeicosatetraenoic acid (12-HETE) and glutamic acid (lipoxygenase pathway), and thromboxane B2 (TxB2) (cyclooxygenase pathway) (**Supplementary Fig. 1, *C*** and ***D***, http://links.lww.com/CCX/B57).

Considering all patients with measurements for the three time-points, levels of AA in the preoperative were 5.93×10^8 (ion counts—all the values are ion counts [area] linearly proportional to the actual concentration of the metabolite) (IQR, 4.4×10^8 to 7.4×10^8). Although absolute measurements remained stable at 6 hours—5.6×10^8 (IQR, 4.3×10^8 to 7.1×10^8; $p = 0.55$), they were significantly decreased at 24 hours 4.17×10^8 (IQR, 3.08×10^8 to 5.5×10^8; $p < 0.0001$; **Supplementary Fig. 2*A***, http://links.lww.com/CCX/B57).

Revealing a similar pattern, levels of EPA (7.98×10^7; IQR, 5.15–12.67×10^7) and DHA (5.23×10^8; IQR, 4.5–8.06×10^8) remained stable at 6 hours (8.19×10^7; IQR, 4.92–10.8×10^7; $p = 0.247$ and 5.43×10^8; IQR, 3.57–7.34×10^8; $p = 0.505$, respectively) but significantly decreased at 24 hours (4.27×10^7; IQR, 2.95–6.86×10^7; $p < 0.0001$ and 3.57×10^8; IQR, 2.54–5.41×10^8, $p < 0.0001$, respectively; Supplementary Fig. 2*A*, http://links.lww.com/CCX/B57). CLA increased at 6 hours (3.1×10^8; IQR, 1.8–4.91×10^8; $p = 0.059$) and returned to near basal concentrations at 24 hours (2.3 x 10^8; IQR, 1.36–3.9×10^8; $p = 0.972$; Supplementary Fig. 2*A*, http://links.lww.com/CCX/B57).

AA metabolites derived from the lipoxygenase pathway, such as glutamic acid (20.59×10^8; IQR, 15.97–25.02) and 12-HETE (1.45×10^7; IQR, 0.92–1.98), had a significant reduction in the absolute measurements at 6 hours (13.37×10^8; IQR, 9.97–17.03×10^8; $p < 0.0001$ and 1.17 x 10^7; IQR, 0.77–1.54×10^7; $p < 0.0001$, respectively) and 24 hours (13.03×10^8; IQR, 8.58–16.56×10^8; $p < 0.001$, and 1.13×10^7; IQR, 0.88–1.63×10^7; $p < 0.0001$, respectively; Supplementary Fig. 2*A*, http://links.lww.com/CCX/B57).

PAF concentrations (16.99×10^8; IQR, 14.05–18.8×10^8) were significantly reduced at 6 hours (9.65×10^8; IQR, 7.56–11.37×10^8; $p < 0.0001$) and 24 hours (6.89×10^8; IQR, 5.86–8.16×10^8; $p < 0.0001$), compatible with the previously reported reduction of platelet activity after CPB. No significant changes were observed in TxB2 concentrations at 6 and 24 hours.

Interestingly, the concentrations of 8,11,14-eicosatrienoic acid (1.83×10^8; IQR, 1.37–2.32 x 10^8) were unchanged at 6 hours (2.06×10^8; IQR, 1.51–2.38×10^8; $p = 0.271$) but reduced at 24 hours (1.24×10^8; IQR, 1–1.53×10^8; $p < 0.001$).

Because two postoperative measurements were tested for the nine metabolites, a Bonferroni-adjusted significance level of 0.00278 was calculated to account for the increased possibility of type-I error.

To evaluate if the variation of the metabolites followed a similar trend than the absolute value, we then calculated the change in the metabolites measurements at 6 and 24 hours (percentage of change comparing to preoperatory levels; **Supplementary Fig. 2*B***, http://links.lww.com/CCX/B57; and **Supplementary Table 2**, http://links.lww.com/CCX/B57). Although a high percentage of patients had a pronounced reduction at 6 hours, median reduction of AA, EPA, and DHA was not significant at 6 hours. However, levels were significantly reduced at 24 hours. On the other hand, CLA had an increase at 6 hours with a nonsignificant variation at 24 hours. Glutamic acid and 12-HETE had a similar pattern of reduction both at 6 and 24 hours. Although PAF measurement was reduced at 6 hours, the reduction was even more pronounced at 24 hours. In contrast, TxB2 values were more reduced at 6 hours than at 24 hours; 8,11,14-eicosatrienoic acid showed a particular pattern, with a median increase of 19.4% (–15% to 44.8%), significantly decreasing at 24 hours.

Platelet Count and Morphology and Antiplatelet Therapy

In our population, platelet count decreased after surgery (**Fig. 1*A***), with a median –32.8% (–39.5% to

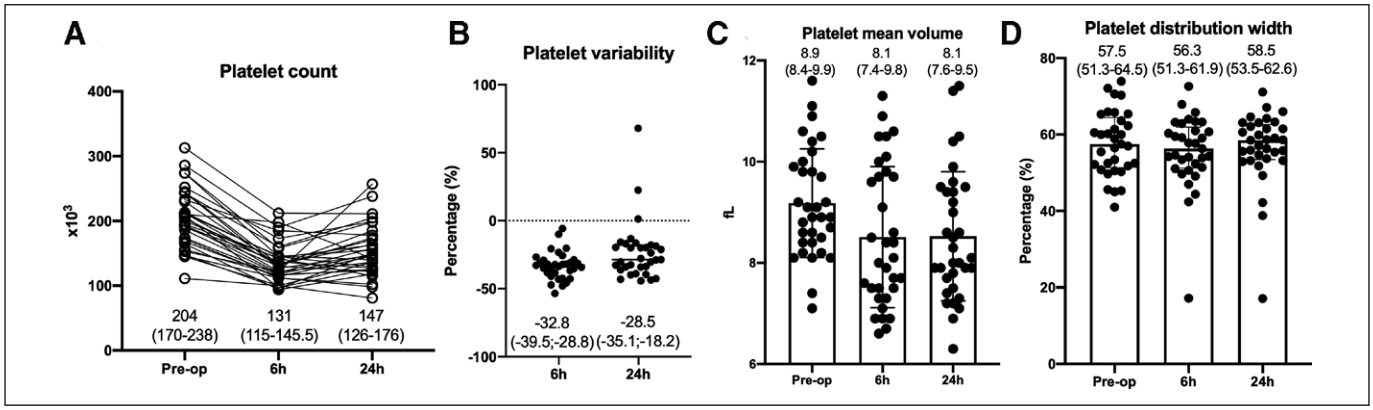

Figure 1. Platelet count (**A**), variability in percentage (**B**), platelet mean volume (**C**), and platelet distribution width (**D**) of preoperative and 6- and 24-hr postoperative patients. Values are presented in median with interquartile range.

–28.8%) at 6 hours and –28.5% (–35% to –18.2%) at 24 hours (**Fig. 1*B***). The reduction was consistent within all patients (Fig. 1*B*) and was not correlated with any comorbidity or the use of anticoagulation and/or antiaggregation (data not shown). Consistent with previous reports, mean platelet volume (MPV) was also reduced at 6 and 24 hours (**Fig. 1*C***). However, platelet distribution width (PDW) remained stable at all times (**Fig. 1*D***).

Importantly, the variation in the postoperative metabolite levels was not induced by the CPB reduction of platelet count, since platelet count at preoperative, 6 hours, and 24 hours was not correlated with metabolites measurement neither with the percentage of variation in the same time point (data not shown). The absolute value and the variation of the metabolites were also not correlated with the variation in the MPV and PDW (data not shown).

Considering that a substantial percentage of patients were medicated with platelet inhibitors (AAT), we assessed if preoperative AAT influenced preoperative levels and their variation. None of our patients was taking nonsteroidal anti-inflammatory drugs, and 45.5% (15 patients) were taking acetylsalicylic acid (ASA) (Table 1). Patients taking ASA had similar levels of the studied metabolites in the preoperative

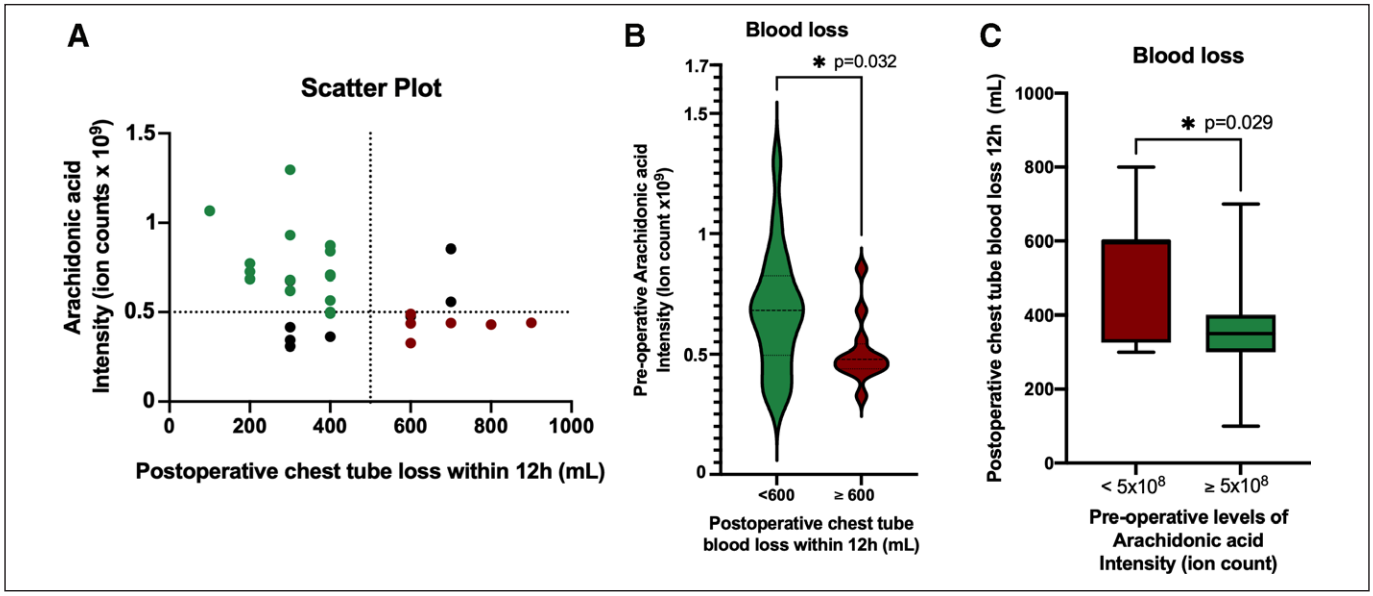

Figure 2. A, Correlation of postoperative bleeding (mL) within the first postoperative 12 hr and preoperative quantification of arachidonic acid. **B**, Preoperative arachidonic acid levels between patients with (*green*) or without (*red*) a significant postoperative bleeding (defined by postoperative chest tube blood loss of more than 600 mL within 12 hr). **C**, Median postoperative chest tube blood within 12 hr according to arachidonic acid quantification.

(**Supplementary Fig. 3**, http://links.lww.com/CCX/B57). No significant differences were observed in any of the analyzed compounds in absolute levels for each time point and for the percentage of reduction at 6 and 24 hours (**Supplementary Figs.** 3 and **4**, http://links.lww.com/CCX/B57).

Arachidonic Acid Signature and Postoperative Bleeding

Based on the AA metabolic signature observed, we tested whether preoperatory levels of AA were associated with postoperative bleeding. We indeed found that levels of preoperative AA were inversely correlated with postoperative chest tube blood loss at 24h (R = –0.3957; *p = 0.03) (**Fig. 2*A***). In contrast, the postoperative blood loss was not correlated with platelet count in the preoperative (R = –0.04; p = 0.815), at 6 hours (R = –0.098; p = 0.597) or at 24 hours (R = –0.06; p = 0.741). Considering patients with a significant postoperative blood loss (at least 600 mL in the first 12 hours after surgery), the preoperative levels of AA were considerably lower (4.8 x 10^8 vs 6.8 x 10^8; *p = 0.032) (**Fig. 2*B***). The subjects with low levels of preoperatory AA (first quartile of no significant bleeding) had increased risk of postoperative bleeding with a relative risk 2.8 (95% CI, 1.19–6.68) and odds ratio 7 (95% CI, 1.35–30.25) (*p = 0.045). Among individuals with preoperative levels of AA below 5×10^8, the postoperative blood loss (600 cc; IQR, 325–600 vs 350 cc; IQR, 300–400; *p = 0.029) was substantially higher (**Fig. 2*C***).

Furthermore, the transfusion rate was also higher in patients with reduced preoperative AA levels and with a postoperative reduction. In patients with preop AA levels below 5×10^8 ion count, 42% required postoperative transfusion, versus 22% in patients with higher levels (p = 0.418) (**Fig. 3*A***). Additionally, patients with reduced levels of AA at 6 hours also had a higher rate of transfusion, compared with those with unchanged or increased values (40% vs 28%; p = 0.489) (Fig. 3*A*). Regarding other outcomes in patients with postoperative bleeding, the necessity of hemodynamic support was higher, although not statistically significant, as well as mechanical ventilation for more than 6 hours and neurologic complications (Table 1). Interestingly, postoperative atrial fibrillation was more common in the group without significant bleeding.

AA was later evaluated for predictive accuracy for identifying patients with an increased risk of significant bleeding. From the receiver operating characteristic curve, AA was a predictor of postoperative significant bleeding with an AUC of 73.2% (**Fig. 3*B***).

As CPB is associated with a systemic inflammatory response, we also measured IL-6 levels (**Supplementary Fig. 5*A***, http://links.lww.com/CCX/B57) and correlated them with postoperative AA variability (**Fig. 3*C***). We observed a strong correlation between IL-6 levels and the AA variability (r = –0.602; ***p = 0.0004), suggesting a possible role of the inflammatory setting in AA metabolism perturbation. Curiously, IL-6 levels were lower in patients with significant postoperative bleeding (**Supplementary Fig. 5*B***, http://links.lww.com/CCX/B57).

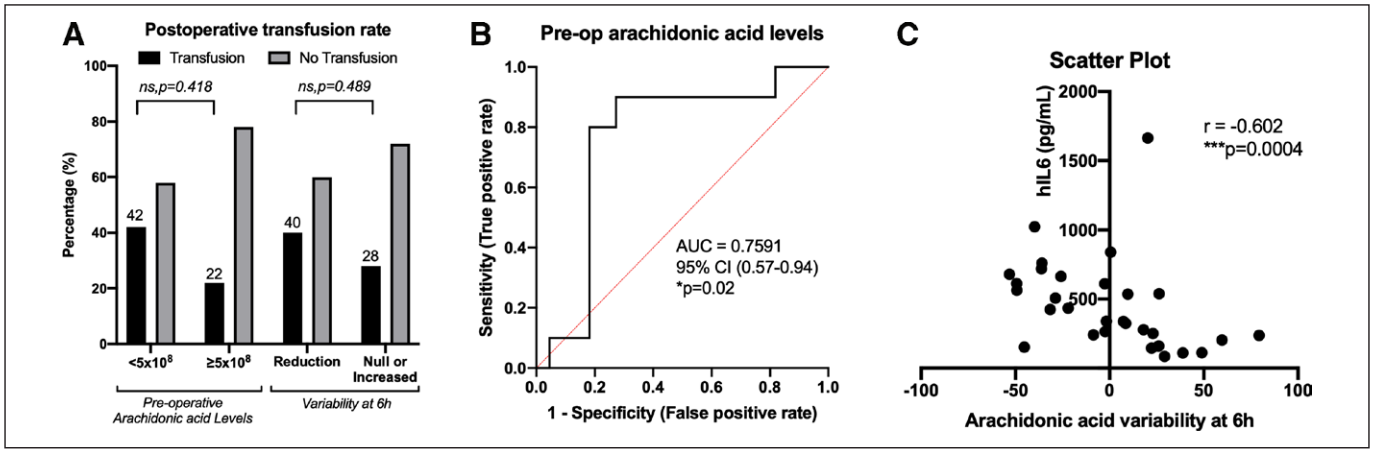

Figure 3. A, Postoperative transfusion rate considering preoperative arachidonic acid quantification and the reduction in percentage at postoperative 6 hr. **B**, Receiver operating characteristic curve based on the preoperative arachidonic acid measurements and represented by an area under the curve (AUC) of 0.73 indicating a satisfactory predictive ability. **C**, Correlation between human interleukin 6 levels and variability in arachidonic acid levels at 6 hr postoperative (percent change compared with preoperative levels).

DISCUSSION

Untargeted metabolomics of samples collected after cardiac surgery with CPB identified a significant platelet-associated signature, related to omega-6 fatty-acid oxidation. Our results suggest an association between AA metabolism perturbation and postoperative bleeding. To our knowledge, our study is the first platelet-metabolic analysis after cardiac surgery with CPB.

Although platelet transfusion is widely used to restore perioperative hemostasis, the decision is mainly empirical since there is no consensus regarding dose, trigger, and efficacy (18). Furthermore, platelet transfusions are associated with several complications, including increased use of vasoactive drugs, extended mechanical ventilation, prolonged stay in the ICU and hospital, increased risk of postoperative infection, and transfusion-related acute kidney injury (19, 12, 13). In fact, in our study, patients with postoperative significant bleeding had higher rates of hemodynamic support, mechanical ventilation for more than 6 hours, and neurologic complications. Additionally, platelet transfusion is associated with considerable healthcare costs (21). The effort is now centered on reducing the use of blood products to decrease transfusion-related complications and costs. Therefore, it is essential to understand how cardiac surgery affects platelet function and how we can establish new strategies to minimize it.

Interestingly, polyunsaturated fatty acids (PUFAs), such as omega-6 fatty-acids, have been associated with lower risk of cardiovascular events, especially due to anti-inflammatory proprieties (22, 23). PUFA may regulate platelet activation enhancing endothelial repair, but current evidence is limited. Additionally, it is well established that AA and its metabolites modulate platelet aggregation. AA-triggered aggregation is significantly decreased after CPB (1), but no previous studies correlated how perioperative levels of AA and its metabolism affect postoperative hemorrhage.

AA concentration influences both normal cellular functions and the development of platelet dysfunction. AA metabolites act as local hormones and/or signaling molecules in response to basal metabolism or upon regulation by immune response stimuli, such as the production of cytokines (24). CPB is associated with a significant systemic inflammatory response, with a complex and incompletely understood cross-talk between inflammation and coagulation (24, 25). It is known that endothelial dysfunction during CPB induces the production of cytokines and platelet-induction factors (24, 25). Some AA metabolites, such as 12-HETE, have an important role in immune-mediated platelet activation (23). Although not yet completely understood, 12-HETE potentiates dense granule secretion via nicotinamide adenine dinucleotide phosphate-oxidase activation (23, 24) and the activation of surface immunoreceptors (25, 26). Furthermore, platelets after CPB have decreased levels of 12-HETE and a depressed activation of lipoxygenase activity (27, 28), contributing to postoperative bleeding risk through an innate immunity-dependent perturbation of coagulation factors function (29, 30).

In fact, we have observed that IL-6 concentrations 6 hours after surgery are strongly correlated with the variability in AA levels in our patients, suggesting that inflammation plays an essential role in postoperative AA dysfunction. Interestingly, not all the pathways of AA-activation may have the same relevance in postoperative bleeding. We have seen that levels of TxB2 remain stable, consistent with previous studies that have also reported that TxB2 plasma levels 1 and 24 hours after the operation had returned to preoperative values (12). Furthermore, although concentrations of metabolites from both eicosanoids and lipoxygenase pathways were reduced at 6 and 24 hours, the reduction remains approximately the same at 6 and 24 hours, whereas the reduction of the metabolite representing lipoxygenase is much pronounced at 24 hours. Thus, although cyclooxygenase pathway may remain unchanged, eicosanoid and lipoxygenase pathways may be differently disrupted. Curiously, patients with significant postoperative bleeding had lower levels of IL-6, especially beginning 12-hour postoperative. It has been documented that AA can induce the release of IL-6 from inflammatory cells, inducing the production of acute-phase proteins to balance some of the detrimental effects of AA metabolites in shock (31). IL-6 augments platelet count and function, and the thrombotic potential of IL-6 is greater than its fibrinolytic effect in hemorrhage, especially in the early stages of bleeding (32). Our observation that IL-6 levels are lower in patients with significant hemorrhage is a possible consequence of the AA metabolism perturbation. Further studies are needed to elucidate how inflammation disturbs AA metabolism and to better dissect the molecular mechanisms involved.

There are some limitations in our study. Although measurements correlate changes between different time points for the same patient in order to minimize confounding factors, our study population is small, potentially causing a statistical analysis under power. Further studies are needed to validate these findings in a larger population and to address other pathways identified in our analysis, apart from acid AA metabolism.

CONCLUSIONS

In summary, we show an inflammatory-related perturbation of AA metabolism as a signature of cardiac surgery with CPB and that preoperative levels of AA may be more relevant than platelet count to anticipate and prevent postoperative blood loss in patients submitted to cardiac surgery with CPB, especially when the reduction after CPB is associated with higher transfusion rate. Platelet loss of function is partially responsible for postoperative bleeding, and although platelet transfusions are associated with less postoperative blood loss, they increase morbidity and mortality (20). AA supplementation at a dose equal to, or lower than, the dietary intake is safe and increases plasmatic levels of AA in a dose-dependent manner (33). Preoperative AA administration may be a safe and inexpensive option to reduce platelet dysfunction after cardiac surgery with CPB, with the potential to reduce postoperative bleeding and the need for transfusion and should be subsequently explored.

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