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Regular Article

Platelet activation increases in patients undergoing vascular surgery

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ABSTRACT

Background: Platelets are a major contributor to atherothrombosis and may contribute to the heightened risk of perioperative cardiovascular events. We sought to examine changes in platelet activity in subjects undergoing vascular surgery.
Methods: Platelet activity in 18 patients (median age 74, 45% female) undergoing non-emergent open vascular surgery was assessed by light transmission aggregometry in response to saline, epinephrine and adenosine-5 diphosphate (ADP), and by flow cytometric analysis of monocyte-platelet aggregation (MPA). Platelet activity was assessed preoperatively (T1), 1-hour into the operation (T2), 1-hour (T3), 24-hours (T4) and 48-hours post-operatively (T5). Data were compared using the Wilcoxon Signed Ranks Test. Continuous variables are summarized as medians and (interquartile, IQR) ranges.
Results: Spontaneous platelet aggregation increased transiently during the surgical period (T1-5.8% [2.4, 10.8], T2-13.5% [9.3, 26.5], T3-7.5% [3.3, 17], T4-10.0% [7.3, 16.3], T5-7.25% [4.5, 29.9], $P = 0.002$). Similar trends in perioperative platelet activity were noted for platelet aggregation in response to epinephrine ($P = 0.035$) and ADP ($P = 0.036$). Using flow cytometry, we found an increase in MPA during the perioperative period ($P = 0.047$), which was most significant between T1 and T3 ($P = 0.005$).
Conclusions: Platelet activity increases significantly during and following open vascular surgery. This data may help explain the pathophysiology of increased thrombotic risk during the perioperative period of vascular surgery.

Introduction

Subjects undergoing open vascular surgery are at significant risk for perioperative cardiovascular events [1]. Atherothrombosis is the underlying mechanism of adverse cardiovascular events, and is believed to play a major role in the heightened perioperative risk of cardiovascular complications [2–4]. Platelets are a major contributor to atherothrombosis and are a key target of intervention in the treatment and prevention of cardiovascular morbidity and mortality in both the operative and non-operative settings. Platelet activity is increased in subjects with peripheral artery disease and vascular surgery may increase platelet activity even further. To date, studies measuring platelet activity in the perioperative period have found conflicting results [5,6]. While several studies found that platelet aggregation is increased in the early postoperative period, these studies used a limited number of platelet markers [6–8].

Increased platelet activity is associated with significant morbidity and mortality [9–11]. Understanding the dynamic profile of platelet activity in the perioperative setting is crucial to understanding the increased risk of cardiovascular events in the perioperative period. We therefore designed a prospective pilot study to evaluate the changes in markers of platelet activity in subjects undergoing non-emergent open vascular surgery.

Methods

Participants

Between December 2010 and February 2011, 18 consecutive patients scheduled for open vascular surgery procedures were recruited for the study. Included operations consisted of carotid endarterectomy, open aneurysmal repair and lower extremity bypass repair. Subjects with known thrombocytopenia (platelet count <75), renal failure (creatinine clearance <30 ml/min), active cancer, active infection, predisposition to bleeding, any use of non-steroidal anti-inflammatory drug within 72 hours, or any antithrombotic therapy were excluded. All participants provided informed consent before participation in the study approved by the local institutional review board.

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Study Design

After informed consent was obtained, blood sampling was performed at set time points during the perioperative period; 1 hour preoperatively (Time point [T] 1), 1-hour into the operation (T2), 1-hour post-operatively (T3), ≈24-hours (1-day) postoperatively (T4), and ≈ 48-hours (2-days) postoperatively (T5). All samples collected were in the fasting state and samples from T4 and T5 were drawn in the early morning. All patients had an arterial line available for blood collection at T1 to T3. For samples from T4 and T5, blood collection was performed through an arterial line or through venipuncture using a 19-guage needle if the arterial line was already removed. The validity of combining arterial and venous sampling is supported by several studies, including a recent paper from our group that found very good agreement between arterial and venous samples with respect to markers of platelet activity [12]. For all samples, the first 2 ml was discarded and the remaining blood was collected into a vacutainer tube containing EDTA for complete blood count (CBC) and vacutainers containing 3.2% (0.105 moles/liter) sodium citrate (Becton Dickenson, Franklin Lakes, NJ) for platelet activity. The time between blood draw and aggregometry was uniform at each time point as samples were taken directly to the lab for analysis after collection.

Platelet and Hematological Parameters

Complete blood count was performed using a Sysmex (Mundelein, Illinois, USA) XE-2100 analyzer. Complete blood count was performed within 60 minutes of phlebotomy.

Light Transmission Aggregometry

Following phlebotomy, blood was centrifuged at 200 *g* for 10 minutes to obtain platelet rich plasma (PRP) [13]. Platelet poor plasma (PPP) was obtained by centrifuging PRP at 2500 *g* for 10 minutes. Light transmission aggregometry (LTA) was performed according to the manufacturer's specification using Helena (Beaumont, TX, USA) AggRAM light transmission aggregometer, reagents, cuvettes, and stir bars. As previously described, 225uL of PRP was incubated at 37 °C for 90 seconds, stir bar speed was 1200 rpm, and sample run time was 10 minutes after addition of 25uL agonist[13]. Platelet aggregation (%) was evaluated at 300 s, and at maximum aggregation. Aggregation was induced using final concentrations of 1 μM adenosine diphosphate (ADP), 1.5 mM arachidonic acid (AA), 0.4 μM epinephrine, and saline for spontaneous platelet aggregation. All aggregation studies were completed within 2 hours of phlebotomy, consistent with the recommendation that specimens be tested within 3–4 hours set forth by the College of American Pathologists.

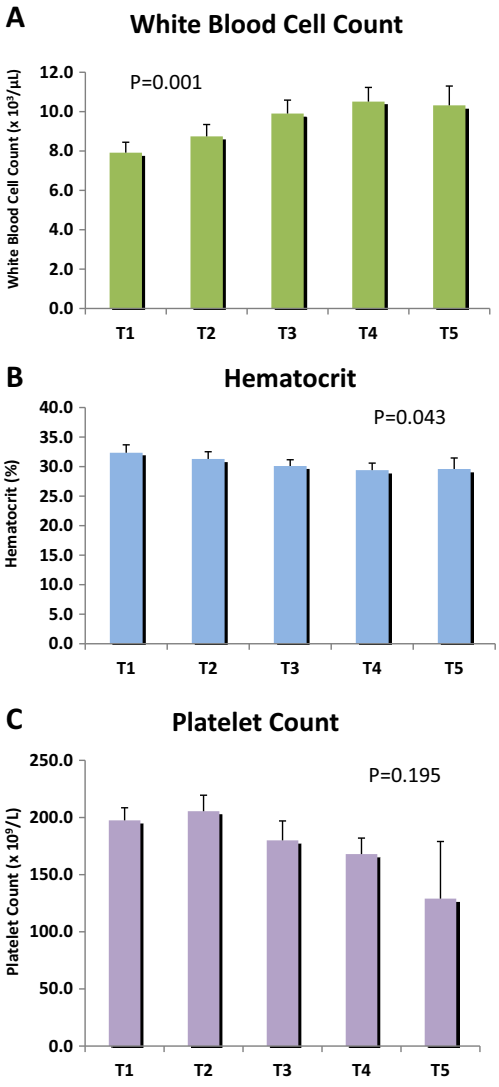

Fig. 1. Hematology analysis of the A) white blood cell count, B) hematocrit and C) platelet count during the perioperative period. White blood cell count gradually increased with each sample, hematocrit decreased over time, and platelet count was not significantly different over time, with the only significant difference occurring between T1 and T3.

Table 1
Preoperative Demographic and Clinical Data.

	All Patients (N = 18) No. (%)
Age, yrs, mean ± SD [range]	72.0 ± 17.0 [29–94]
Gender (male/female)	10/18 (55.6/44.4)
Surgery	
CEA	10 (55.6)
AAA	3 (16.7)
Bypass	5 (27.8)
Smoking status	
Never	7 (38.9)
Current	5 (27.8)
Former	6 (33.3)
Alcohol use (drinks)	
None	6 (33.3)
1-6 per week	10 (55.6)
1-2 per day	1 (5.6)
3-4 per day	1 (5.6)
Use within 72 hours of surgery	
Aspirin	11 (61.1)
Plavix	6 (33.3)
Statin	14 (77.8)
Pre-op Creatinine, mean ± SD	1.01 ± 0.36
Pre-op Glucose, mean ± SD	109 ± 40.5

CEA, carotid endarterectomy; AAA, abdominal aortic aneurysm open repair.

Impedance Aggregometry

Multiple electrode aggregometry (Multiplate™, Dynabite, Munich, Germany) was performed in whole blood and evaluated according to manufacturer's instructions. Aggregation was induced by (final concentrations) 500 μM thrombin receptor-activating peptide 32 μM (TRAP) and by normal saline for spontaneous aggregation (SPA). Tests elapsed for 6 min and reported as area under the curve (AUC), maximum aggregation units (AU), and velocity.

Flow Cytometry

As previously described [14], monocyte platelet aggregation (MPA) was measured via whole blood flow cytometry using the Accuri 6 system. Immediately following phlebotomy, whole blood was fixed