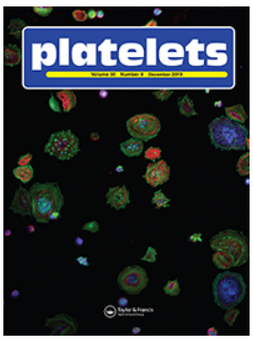

Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: https://www.tandfonline.com/loi/iplt20

Increased platelet reactivity and platelet–leukocyte aggregation after elective coronary bypass surgery

Torbjörn Ivert, Magnus Dalén, Charlotte Ander, Ragnhild Stålesen, Marie Lordkipanidzé & Paul Hjemdahl

To cite this article: Torbjörn Ivert, Magnus Dalén, Charlotte Ander, Ragnhild Stålesen, Marie Lordkipanidzé & Paul Hjemdahl (2019) Increased platelet reactivity and platelet–leukocyte aggregation after elective coronary bypass surgery, Platelets, 30:8, 975-981, DOI: 10.1080/09537104.2018.1542122

To link to this article: https://doi.org/10.1080/09537104.2018.1542122

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

platelets

Platelets, 2019; 30(8): 975–981
 DOI: https://doi.org/10.1080/09537104.2018.1542122

Taylor & Francis
Taylor & Francis Group

Check for updates

Increased platelet reactivity and platelet–leukocyte aggregation after elective coronary bypass surgery

Torbjörn Ivert[1], Magnus Dalén[1], Charlotte Ander[2], Ragnhild Stålesen[2], Marie Lordkipanidzé[3], & Paul Hjemdahl[2]

[1]Heart and Vascular Theme, Karolinska University Hospital and Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, [2]Department of Clinical Pharmacology, Karolinska University Hospital and Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, and [3]Faculté de pharmacie, Université de Montréal,and Research center, Montreal Heart Institute, Montréal, Québec, Canada

Abstract

Inflammatory mechanisms are activated, and thrombotic complications occur during the initial months after coronary artery bypass grafting (CABG). Therefore, changes over time of platelet activation and platelet–leukocyte interactions after CABG are of interest. Whole-blood flow cytometry was performed before, and 4–6 days, one month, and three months after elective CABG in 54 men with stable coronary artery disease treated with acetylsalicylic acid (ASA). Single platelets and platelet–leukocyte aggregates (PLAs) among monocytes (P-Mon), neutrophils (P-Neu), and lymphocytes (P-Lym) were studied without and with stimulation by submaximal concentrations of ADP, thrombin, and the thromboxane analog U46619. White blood cell counts were increased during the initial postoperative course, and platelet counts were increased after one month. Platelet P-selectin expression was significantly enhanced at one month when stimulated by thrombin and U46619 and at three months with ADP and thrombin. All PLAs subtypes were increased at one month without stimulation *in vitro*. P-Mon and P-Neu stimulated by ADP, thrombin, or U46619 were significantly increased one month after the operation but decreased compared to baseline at three months. Agonist stimulated P-Lyms were increased at one month and remained increased at three months after ADP stimulation. There was significant platelet activation and formation of PLAs unstimulated and after agonist stimulation by ADP, thrombin, and a thromboxane analog after CABG in patients with stable coronary artery disease irrespective of ASA treatment. Changes observed up to three months after CABG support further studies of the clinical implications of protracted increases in platelet activation and platelet–leukocyte interactions.

Keywords

Coronary bypass, platelet activation, platelet aggregates

History

Received 25 June 2018
Revised 14 October 2018
Accepted 22 October 2018
Published online 13 November 2018

Introduction

During recent years, it has become increasingly clear that platelets and platelet–leukocyte interactions are involved in the complex pathophysiology of atherosclerosis and the terminology athero-thrombosis is frequently used in this context [1–8]. Cardiopulmonary bypass activates inflammatory mechanisms and several aspects of hemostasis [9,10]. We have previously reported on biphasic pro-thrombotic and inflammatory responses after coronary artery bypass grafting (CABG) in a study focusing on the first week after surgery [11]. Thromboembolic events may occur during the first month after cardiac surgery and early vein graft failure is related to thrombosis and high platelet reactivity [12,13]. Thus, more intense antiplatelet therapy than acetylsalicylic acid (ASA) alone may be required to improve the prognosis of patients with stable coronary artery disease undergoing CABG [14,15].

We have previously reported on the efficacy of different ASA dosages following CABG and found that 75 mg twice daily or 160 mg once daily inhibited thromboxane-dependent platelet activation more effectively than 75 mg once daily [16]. The present study describes platelet reactivity and circulating platelet–leukocyte aggregates (PLAs) irrespective of ASA dosage following elective CABG in patients without indication for dual antiplatelet therapy. Flow cytometry of single platelets or PLAs is insensitive to ASA treatment as such measurements are performed under static conditions that do not promote thromboxane generation [17–19]. The present aim was to investigate changes in thromboxane independent platelet activation and platelet–leukocyte interaction including responses to agonist stimulation during the recovery phase, at one and three months after elective CABG in patients with stable coronary artery disease.

Patients and Methods

Subjects

We included patients with stable angina pectoris scheduled to undergo elective CABG. Exlusion criteria were intake of any other platelet inhibitor than ASA during the last seven days

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/iplt.

Correspondence: Torbjörn Ivert, Heart and Vascular Theme, Karolinska University Hospital, Stockholm SE-171 76, Sweden. E-mail: torbjorn.ivert@ki.se

prior to surgery, known bleeding disorder or kidney failure, preoperative platelet count outside of the range 100,000–450,000/μL, or a hemoglobin level below 8 g/L. We screened 75 subjects of whom 54 were included in the final analysis; 20 patients were excluded because of incomplete follow-up with blood samples and one patient with insulin-treated diabetes mellitus and advanced three-vessel disease died suddenly two months after the operation. Results in 42 of the patients have been previously reported regarding the serum thromboxane B2 effect of the ASA doses 75 mg twice daily, 160 mg once daily, and 75 mg once daily [16]. Twelve patients with complete cytometric data and included in the present analyses were excluded in the previous study due to faulty compliance with the prescribed ASA dosage and study procedures.

All patients were treated with ASA (Trombyl®, Pfizer Health AB, Strängnäs, Sweden) 75 mg OD before the operation and they received ASA 75 mg OD or BID or 160 mg OD at discharge from the hospital. The study was approved by the Regional Ethical Review Board in Stockholm (2011/1074–31/1) and by the Swedish Medical Products Agency (EudraCT 2011–002233-19). The study is registered with ClinicalTrials.gov (NCT02482857). A signed consent form was obtained from all participants.

Study Design

All operations were performed through a standard sternotomy with the aid of standard cardiopulmonary bypass using a non-pulsatile centrifugal pump and a membrane oxygenator. The extracorporeal system was primed with Ringer's acetate. Cold blood cardioplegia was used for myocardial protection at a core temperature of 36°C while the aorta was cross-clamped. Heparin was given before cardiopulmonary bypass (3 mg/kg) to maintain activated clotting time >480 s.

Blood Collection

Blood samples were collected for platelet function testing on admittance before the operation, repeated 4–6 days after surgery before hospital discharge, and at follow-up after one and three to four months. Blood was obtained from an antecubital vein using vacutainer tubes containing EDTA for hematological analyses and sodium citrate for flow cytometry. Complete blood cell counts and mean platelet volume were assessed in samples anticoagulated with EDTA, within 20 min of sampling to minimize platelet swelling, using a MICROS 60 cell counter (ABX Diagnostics, Montpellier, France).

Flow Cytometric Analyses

Venous blood was collected by venipuncture without stasis, using siliconized vacutainer tubes containing 1/10 volume of 3.8% trisodium citrate (Becton Dickinson, Meylan, France).

Five μL aliquots of blood were added to 45 μL HEPES-buffered saline (150 mmol/l NaCl, 5 mmol/l KCl, 1 mmol/l MgSO4, 10 mmol/l HEPES, pH 7.4) containing appropriately diluted antibodies as well as agonists or vehicle within 5 min of collection. Samples were incubated at room temperature and static conditions in the dark for 20 min, and then diluted and mildly fixed with 0.5% formaldehyde saline. Agonists (final concentrations) used to activate platelets and stimulate PLA formation *in vitro* were as follows: 1 μM adenosine diphosphate (ADP), 0.04 U/mL human alpha-thrombin, or 0.3 μM of the thromboxane analog U46619. We chose these submaximal agonist concentrations based on our previous experience to be able to detect bidirectional changes in platelet sensitivity to agonist stimulation, and to employ several agonists to elucidate possible effects via different activation pathways.

Whole blood flow cytometric measurements of platelet P-selectin expression and PLAs have been described previously [20,21]. Platelets were gated by size and light scatter and verified as CD42b positive in ≥99% of events. Total leukocytes were gated as CD45 positive cells using a PE-labeled anti-CD45 MAb (clone J33; Immunotech, Marseille, France) and neutrophils and lymphocytes were determined by size and granularity. Monocytes were further identified using a PC-5 labeled MAb against CD14 (clone RMO52; Beckman Coulter, Miami, FL, USA).

Platelet P-selectin expression data are reported as percentages of P-selectin positive cells in the platelet population. PLAs are presented as percentages of platelet-conjugated leukocytes among leukocyte subtypes, that is, monocytes (P-Mon), neutrophils (P-Neu), and lymphocytes (P-Lym).

Statistics

Descriptive statistics with arithmetic means with standard deviations (SD) or 95% confidence intervals (CI) and graphical methods were used to characterize the data. In cases of skewed distributions medians with interquartile ranges (IQR) are presented. A paired *t* test or in case of skewed distributions the Wilcoxon test were used to analyze changes from baseline to postoperative time points. Repeated measures analysis of variance and the non-parametric Kruskal–Wallis one-way analysis of variance were applied to compare multiple measurements. Spearman rank order correlations were used to examine P-selectin expression in relation to PLAs. Two-tailed *p*-values ≤5% were considered statistically significant. STATSTICA 13 (StatSoft, Dell) was used to analyze data.

Results

Preoperative characteristics of the 54 patients are shown in Table 1. The European System for Cardiac Operative Risk Evaluation (EuroSCORE) II was used to estimate the risk of surgery [22]. All patients received an internal mammary graft,

Table I. Characteristics of the 54 patients at recruitment.

	Median	IQR
Age (years)	*68*	*59–72*
Logistic EuroSCORE II %*	1.9	0.5–12.1
	Mean	*SD*
BMI (kg/m^2)	27	3
Blood pressure (mm Hg)		
Systolic	138	19
Diastolic	80	8
Hemoglobin (g/L)	142	10
eGFR (mL/min)	90	28
	n	*%*
Male sex	54	100
History of smoking	34	59
Hypertension	43	80
Hyperlipidemia	54	100
Diabetes mellitus	14	26
Medication		
Beta blocker	46	85
ACE inhibitor	27	50
Statin	49	91

SD: standard deviation; IQS = interquartile range; ACE: angiotensin-converting enzyme; eGFR: estimated glomerular filtration rate using the Cockroft and Gault equation [35].

*Mortality risk according to European System for Cardiac Operative Risk Evaluation Score II [22].

and both mammary arteries were used in 19 patients. Mean cardiopulmonary bypass time was 91 min, and an average of 2.9 grafts was inserted. Median blood loss was 580 (interquartile range 450–750) ml. Eight of the 54 included patients (15%) reported minor bleedings such as slight nose bleeds, mouth bleeding associated with tooth brushing, or bruises at the clinical follow-up after three months. There were no major bleeds or gastrointestinal complications. None of the patients reported recurrent angina during the three months of follow-up.

Hematological Analyses

There was a marked drop in average postoperative hemoglobin level that was normalized at three months after the operation (Figure 1a). White blood cell counts were increased during the initial postoperative course, and platelet counts were slightly reduced after the operation but significantly elevated by 32% after one month without significant changes of mean platelet volumes (Figure 1b-d).

Flow Cytometric Data

The patients were treated with different low dosages of ASA after the operation [16]. Flow cytometric results did not differ between the three treatment groups, and data could thus be pooled for all patients (Supplementary Tables I and II).

Platelet Activation

Single platelet surface P-selectin expression was significantly enhanced at one month when stimulated by thrombin and the thromboxane analog U46619 (by 8% and 23%, respectively), and at three months when stimulated by ADP and thrombin (by 10% and 8%, respectively) (Figure 2). Mean levels without agonist stimulation were reduced by 12% from preoperative levels at three months (from 2.5 ± 1.1% to 2.2 ± 1.1%).

Platelet–Leukocyte Aggregation

The percentages of PLAs separated into subsets are shown in Figure 3. Baseline unstimulated levels of PLAs were low and

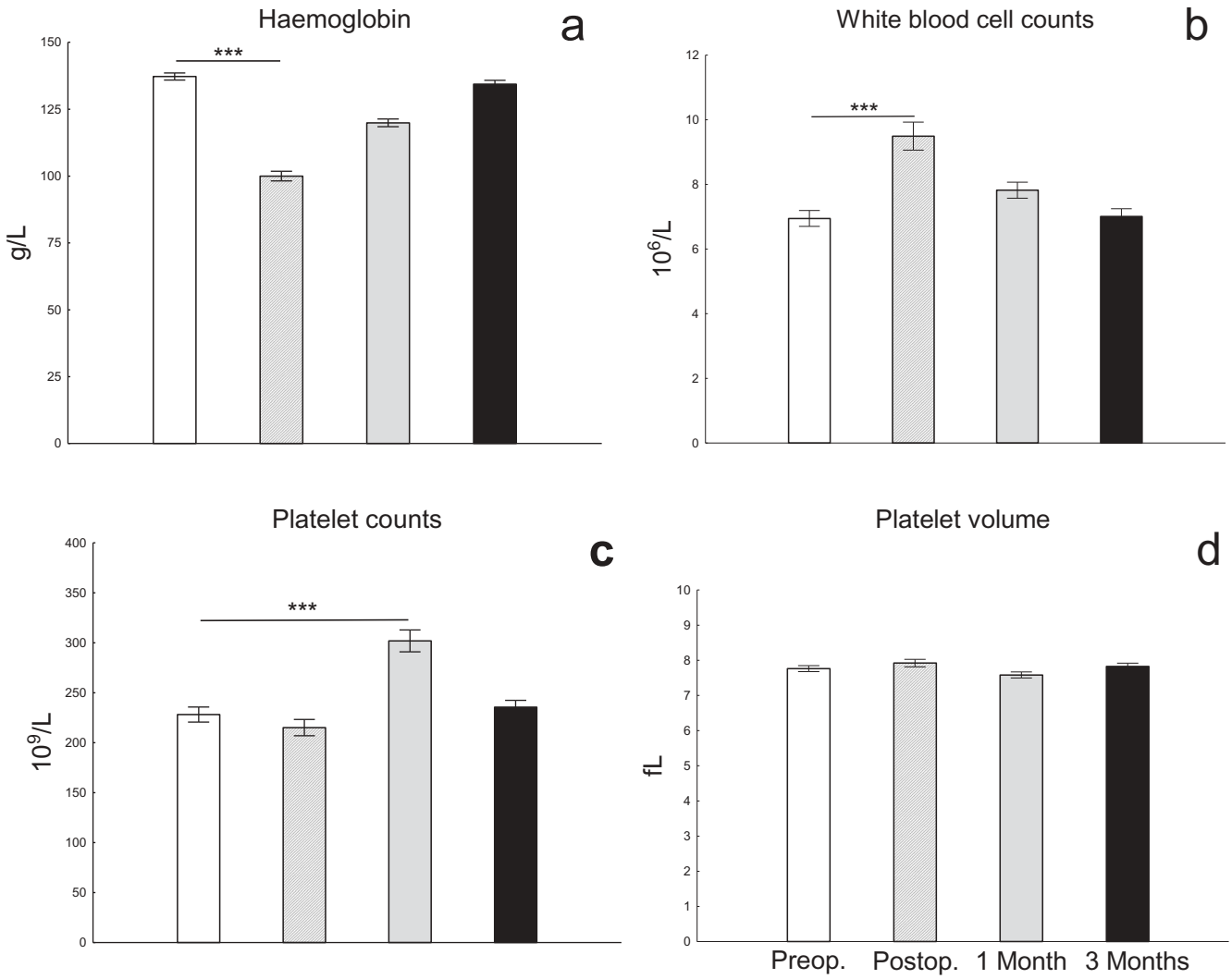

Figure 1. Mean levels with 95% confidence limits of (a) hemoglobin, (b) white blood cell counts, (c) platelet counts, and (d) platelet volumes in 54 patients before and after CABG. Dashed columns indicate results 4–6 days after surgery, shaded columns at one months and black columns at three months. ***$p < 0.001$ for difference between postoperative and preoperative levels are indicated.

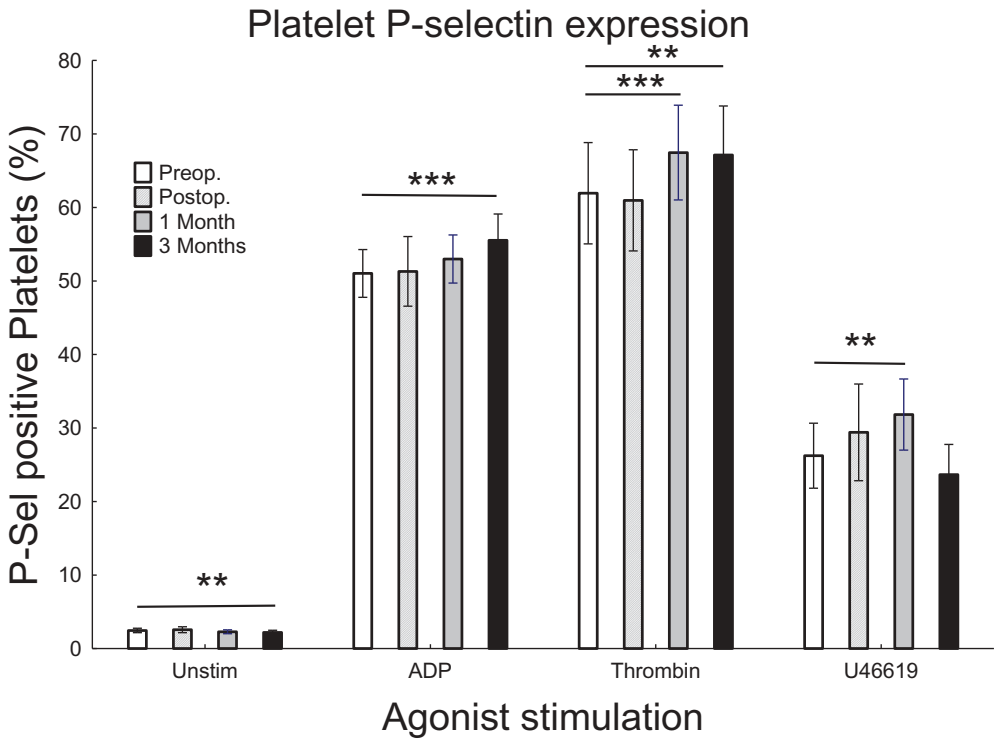

Figure 2. Mean percentages with 95% confidence limits for platelet P-selectin positive cells in the platelet population in 54 patients before and after CABG. Unstimulated basal levels and levels after agonist stimulation by adenosine diphosphate (ADP), thrombin, and the thromboxane A_2 analog U46619 are shown. Dashed columns indicate results 4–6 days after surgery, shaded columns at one month, and black columns at three months. $^{**}p < 0.01$ and $^{***}p < 0.001$ for difference between postoperative and preoperative levels are indicated.

reflect circulating PLAs when using our methodology [20]. Agonist stimulation increased P-Mon and P-Neu to more than 50% but to less than 10% for P-Lym. In unstimulated samples, all subsets of PLAs were increased one month after CABG. The percentages of CD14 positive P-Mons and P-Neus formed by *in vitro* stimulation with ADP, thrombin, or U46619 were significantly increased one month after the operation but reduced compared to baseline at three months. Agonist stimulated P-Lyms were increased at one month and remained increased at three months after ADP stimulation.

Correlations

There were several significant correlations at one and three months between agonist stimulated levels of platelet P-selectin expression and the corresponding agonist stimulated PLA formation *in vitro* (Supplementary Table III).

Discussion

In the present study, we found mildly increased platelet responsiveness to agonist stimulation using whole blood flow cytometry up to three months after elective CABG despite ASA use. Circulating PLAs of all subtypes (platelet-conjugated monocytes, neutrophils, and lymphocytes) were elevated one month after surgery, and agonist stimulated PLA formation *ex vivo* was also enhanced at this time point. Agonist stimulated P-Mon and P-Neu formation was reduced at three months compared to baseline whereas P-Lym formation was not. There was no pattern indicating selectivity for any signaling pathway as platelet and PLA responsiveness was elevated with stimulation by ADP, thrombin, and a thromboxane analog. We have previously shown that CABG causes early (within one week) activation of circulating platelets and leukocytes, increased numbers of circulating PLAs, and marked activation of coagulation and inflammatory responses [11]. We presently show that platelet and PLA responses are protracted and in some cases not normalized even after three months.

Only patients with stable coronary artery disease who underwent elective CABG were included in this study as patients treated for acute coronary syndromes require dual antiplatelet treatment after the operation [23]. ASA was not discontinued before the operation, as the associated bleeding risk is small and withdrawal would leave the patient unprotected against acute ischemic events [21,24,25]. Minor bleeding that in some cases might have been associated with ASA treatment was reported by 15% of the patients during the follow-up. Flow cytometric assays under static conditions are independent of thromboxane formation due to platelet aggregation, and can be used to investigate platelets regardless of ASA therapy [17–19]. In agreement with this, we found no differences between groups treated with different ASA dosages and could pool all patients.

CABG performed with the aid of cardiopulmonary bypass caused an early drop in hemoglobin levels reflecting blood loss and hemodilution. Leukocyte counts were the highest 4–6 days after the operation reflecting inflammatory responses to cardiopulmonary bypass [9]. These changes were normalized three months after the operation. Platelet counts were markedly elevated one month after the operation but mean platelet volume that reflects platelet turnover and platelet reactivity (large, newly formed platelets being more reactive) did not change [26].

The postoperative responses to CABG with cardiopulmonary bypass were more pronounced for PLAs than for single platelets. It is of interest that P-Mons may be a more sensitive marker for platelet activation *in vivo* than P-selectin expression on single platelets [27] presumably since circulating platelets shed the exposed P-selectin *in vivo* [28]. This may explain why the P-selectin expression of unstimulated (circulating) platelets did

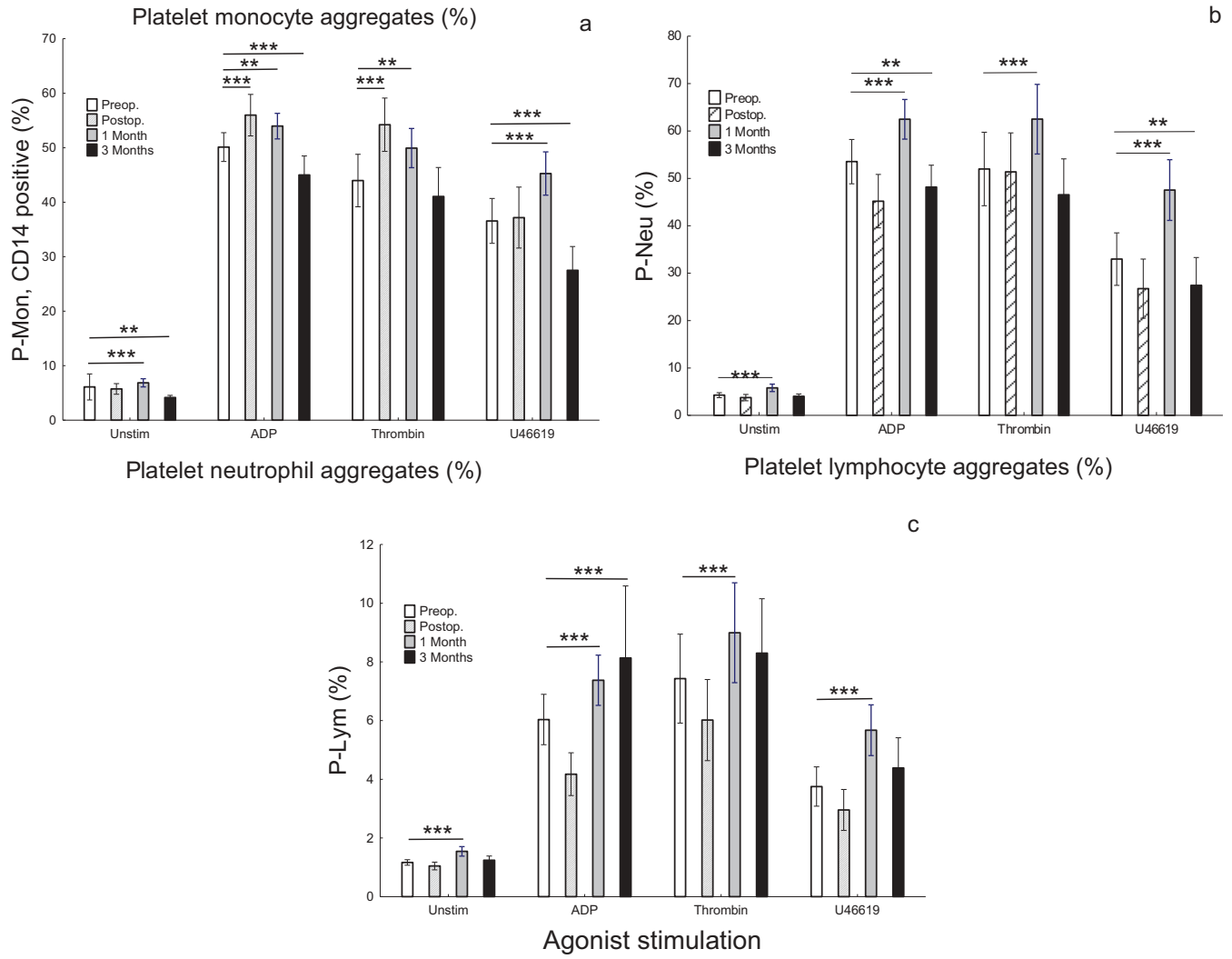

Figure 3. Mean percentages with 95% confidence limits of (a) CD14 positive platelet–monocyte aggregates (P-Mon), (b) platelet–neutrophil aggregates (P-neu), and (c) platelet–lymphocyte aggregates (P-lym) in 54 patients before and after CABG. Unstimulated levels and levels after agonist stimulation by ADP, thrombin, and the thromboxane A_2 analog U46619 are shown. Dashed columns indicate results 4–6 days after surgery, shaded columns at one month, and black columns at three months. $^{**}p < 0.01$ and $^{***}p < 0.001$ for difference between postoperative and preoperative levels are indicated.

not change, whereas circulating P-Mons were increased at one month. Increased platelet reactivity to agonist stimulation *ex vivo* was found up to three months after surgery, and larger responses were detected among PLAs than among single platelets in the present study. Of interest is that agonist stimulated platelet P-selectin expression correlated with the corresponding formation of PLAs at one and three months, as shown in Supplementary Table III. Thus, platelet sensitization appears to be of importance for the increased reactivity of PLAs after CABG surgery.

Platelets that are adhered to the endothelium and platelets binding to leukocytes are involved in the recruitment of leukocytes to the vessel wall and the initiation of vascular inflammation and atherosclerosis [4,6–8,29]. Binding via P-selectin glycoprotein ligand-1 is crucial in the interaction between platelets and the microvascular endothelium as well as in interactions with leukocytes and the formation of PLAs [29,30]. Neutrophils can be activated by high mobility group box 1 protein expressed in platelets, and neutrophil extracellular traps play a key role in interaction with platelets [31]. Platelet binding to monocytes increases their adhesiveness and transendothelial migration, and platelet binding to neutrophils promotes transmigration and their recruitment to inflammatory sites [4,29,30]. Recent work has shown a crucial importance of platelets in guiding neutrophils and inflammatory monocytes to their exit points in the microvasculature [32]. The present data showing increased circulating P-Mons and P-Neus, as well as increased responses to agonist stimulation *ex vivo* one month after the CABG operation, may be interpreted as reflecting inflammatory and prothrombotic challenges to the vasculature. The P-Mon and P-Neu responses were lower than before the operation after three months, indicating wound healing and subsiding inflammation, whereas signs of mildly increased platelet reactivity persisted. Clearance of activated platelets and neutrophils from the circulation and the associated decrease in platelet–leukocyte aggregates may have contributed to this finding [28,33]. Platelets participate in the regulation of CD4 positive T-cells with implications for immune responses and vascular inflammation [8,34]. Our findings of increased platelet binding to lymphocytes and increased agonist stimulated formation of P-Lyms one month after the CABG procedure are of interest in this context.

Activated platelets and formation of PLAs may increase the risk of thrombosis after CABG. The present data support the need for efficient antiplatelet therapy during the first three months after

the operation. Dual antiplatelet therapy may be an option for a limited period of time also among stable patients undergoing elective CABG.

Limitations

We were not able to include all screened patients as we required a follow-up examination of surviving patients three months after the operation, and good-quality blood samples for flow cytometry with minimal *in vitro* artefacts at all time points. The patients were treated with different low doses of ASA after the operation [16] but we do not believe this has confounded our findings since flow cytometry of single platelets or PLAs is not influenced by the thromboxane pathway of platelet activation [17–19] and the flow cytometric data reported here did not vary between the ASA treatment groups.

Conclusions

We conclude that elective CABG in ASA-treated patients with stable coronary artery disease evoked mild signs of platelet activation up to three months after the operation. Increased platelet–leukocyte interactions that may facilitate vascular inflammation and thrombosis were found especially one month after the operation. Our findings support further studies of the clinical implications of protracted platelet activation after CABG.

Supplementary data

Supplementary data for this article can be accessed here.

Acknowledgment

The authors are grateful for the expert technical assistance of Maud Daleskog.

Funding

The Swedish Research Council/Medicine, the Swedish Heart-Lung Foundation, Karolinska Institutet and the Stockholm County Council.

Conflict of interest

None of the authors have any conflict of interest to report in relation to this work.

ORCID

Marie Lordkipanidzé http://orcid.org/0000-0001-7418-6766

References

1. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999;138:S419–420.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–1695. doi:10.1056/NEJMra043430.
3. Martins Pda C, Zwaginga JJ. Leukocyte-platelet aggregates: new particles reflecting and effecting cardiovascular disease. Thromb Haemost 2005;94:1120–1121.
4. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. Blood Rev 2007;21:99–111. doi:10.1016/j.blre.2006.06.001.
5. Totani L, Evangelista V. Platelet-leukocyte interactions in cardiovascular disease and beyond. Arterioscler Thromb Vasc Biol 2010;30:2357–2361. doi:10.1161/ATVBAHA.110.207480.
6. Massberg S, Brand K, Gruner S, Page S, Muller E, Muller I, Bergmeier W, Richter T, Lorenz M, Konrad I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med 2002;196:887–896.
7. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378–3384. doi:10.1172/JCI27196.
8. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. Blood 2014;123:2759–2767. doi:10.1182/blood-2013-11-462432.
9. Warren OJ, Smith AJ, Alexiou C, Rogers PL, Jawad N, Vincent C, Darzi AW, Athanasiou T. The inflammatory response to cardiopulmonary bypass: part 1–mechanisms of pathogenesis. J Cardiothorac Vasc Anesth 2009;23:223–231. doi:10.1053/j.jvca.2008.08.007.
10. Sniecinski RM, Chandler WL. Activation of the hemostatic system during cardiopulmonary bypass. Anesth Analg 2011;113:1319–1333. doi:10.1213/ANE.0b013e3182354b7e.
11. Li N, Astudillo R, Ivert T, Hjemdahl P. Biphasic pro-thrombotic and inflammatory responses after coronary artery bypass surgery. J Thromb Haemost 2003;1:470–476.
12. Parang P, Arora R. Coronary vein graft disease: pathogenesis and prevention. Can J Cardiol 2009;25:e57–62.
13. Gluckman TJ, McLean RC, Schulman SP, Kickler TS, Shapiro EP, Conte JV, McNicholas KW, Segal JB, Rade JJ. Effects of aspirin responsiveness and platelet reactivity on early vein graft thrombosis after coronary artery bypass graft surgery. J Am Coll Cardiol 2011;57:1069–1077. doi:10.1016/j.jacc.2010.08.650.
14. Verma S, Goodman SG, Mehta SR, Latter DA, Ruel M, Gupta M, Yanagawa B, Al-Omran M, Gupta N, Teoh H, et al. Should dual antiplatelet therapy be used in patients following coronary artery bypass surgery? A meta-analysis of randomized controlled trials. BMC Surg 2015;15:112. doi:10.1186/s12893-015-0096-z.
15. van Diepen S, Fuster V, Verma S, Hamza TH, Siami FS, Goodman SG, Farkouh ME. Dual antiplatelet therapy versus aspirin monotherapy in diabetics with multivessel disease undergoing CABG: FREEDOM insights. J Am Coll Cardiol 2017;69:119–127. doi:10.1016/j.jacc.2016.10.043.
16. Ivert T, Dalen M, Ander C, Stalesen R, Nasman P, Lordkipanidze M, Hjemdahl P. Platelet function one and three months after coronary bypass surgery in relation to once or twice daily dosing of acetylsalicylic acid. Thromb Res 2017;149:64–69. doi:10.1016/j.thromres.2016.11.018.
17. Rinder CS, Student LA, Bonan JL, Rinder HM, Smith BR. Aspirin does not inhibit adenosine diphosphate-induced platelet alpha-granule release. Blood 1993;82:505–512.
18. Chronos NA, Wilson DJ, Janes SL, Hutton RA, Buller NP, Goodall AH. Aspirin does not affect the flow cytometric detection of fibrinogen binding to, or release of alpha-granules or lysosomes from, human platelets. Clin Sci (Lond) 1994;87:575–580.
19. Li N, Hu H, Hjemdahl P. Aspirin treatment does not attenuate platelet or leukocyte activation as monitored by whole blood flow cytometry. Thromb Res 2003;111:165–170.
20. Li N, Goodall AH, Hjemdahl P. Efficient flow cytometric assay for platelet-leukocyte aggregates in whole blood using fluorescence signal triggering. Cytometry 1999;35:154–161.
21. Li N, Wallen NH, Hjemdahl P. Evidence for prothrombotic effects of exercise and limited protection by aspirin. Circulation 1999;100:1374–1379.
22. Nashef SA, Sharples LD, Roques F, Lockowandt U. EuroSCORE II and the art and science of risk modelling. Eur J Cardiothorac Surg 2013;43:695–696. doi:10.1093/ejcts/ezs468.
23. m. Authors/Task Force, Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Juni P, et al. 2014 ESC/EACTS guidelines on myocardial revascularization: the task force on myocardial revascularization of the european society of cardiology (ESC) and the European association for cardio-thoracic surgery (EACTS)developed with the special contribution of the European association of percutaneous cardiovascular interventions (EAPCI). Eur Heart J 2014;35:2541–2619. doi:10.1093/eurheartj/ehu278.
24. Lordkipanidze M, Diodati JG, Pharand C. Possibility of a rebound phenomenon following antiplatelet therapy withdrawal: a look at the clinical and pharmacological evidence. Pharmacol Ther 2009;123:178–186. doi:10.1016/j.pharmthera.2009.03.019.
25. Beving H, Zhao C, Albage A, Ivert T. Abnormally high platelet activity after discontinuation of acetylsalicylic acid treatment. Blood Coagul Fibrinolysis 1996;7:80–84.
26. Lordkipanidze M. Platelet turnover in atherothrombotic disease. Curr Pharm Des 2012;18:5328–5343.
27. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation 2001;104:1533–1537.

28. Maugeri N, Rovere-Querini P, Evangelista V, Covino C, Capobianco A, Bertilaccio MT, Piccoli A, Totani L, Cianflone D, Maseri A, et al. Neutrophils phagocytose activated platelets in vivo: a phosphatidylserine, P-selectin, and {beta}2 integrin-dependent cell clearance program. Blood 2009;113:5254–5265. doi:10.1182/blood-2008-09-180794.
29. Da Costa Martins PA, van Gils JM, Mol A, Hordijk PL, Zwaginga JJ. Platelet binding to monocytes increases the adhesive properties of monocytes by up-regulating the expression and functionality of beta1 and beta2 integrins. J Leukoc Biol 2006;79:499–507. doi:10.1189/jlb.0605318.
30. Lam FW, Burns AR, Smith CW, Rumbaut RE. Platelets enhance neutrophil transendothelial migration via P-selectin glycoprotein ligand-1. Am J Physiol Heart Circ Physiol 2011;300:H468–475. doi:10.1152/ajpheart.00491.2010.
31. Maugeri N, Campana L, Gavina M, Covino C, De Metrio M, Panciroli C, Maiuri L, Maseri A, D'Angelo A, Bianchi ME, et al. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. J Thromb Haemost 2014;12:2074–2088. doi:10.1111/jth.12710.
32. Zuchtriegel G, Uhl B, Puhr-Westerheide D, Pornbacher M, Lauber K, Krombach F, Reichel CA. Platelets guide leukocytes to their sites of extravasation. PLoS Biol 2016;14:e1002459. doi:10.1371/journal.pbio.1002459.
33. Ma R, Xie R, Yu C, Si Y, Wu X, Zhao L, Yao Z, Fang S, Chen H, Novakovic V, et al. Phosphatidylserine-mediated platelet clearance by endothelium decreases platelet aggregates and procoagulant activity in sepsis. Sci Rep 2017;7:4978. doi:10.1038/s41598-017-04773-8.
34. Li N. CD4+ T cells in atherosclerosis: regulation by platelets. Thromb Haemost 2013;109:980–990. doi:10.1160/TH12-11-0819.
35. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41. doi:10.1159/000180580.