Prospective observational study of the effect of dual antiplatelet therapy with tranexamic acid treatment on platelet function and bleeding after cardiac surgery

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R4 REVISED VERSION

Postoperative Bleeding induced by dual antiplatelet therapy, aspirin and clopidogrel, with standardized acid tranexamic treatment and preoperative platelet activity assessment. A Propensity Score Analysis.

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Short running title: Bleeding with aspirin and clopidogrel in cardiac surgery

Abstract (237 words)

Background: The bleeding impact of dual antiplatelet therapy (DAPT), aspirin and clopidogrel, maintained until coronary artery bypass graft surgery (CABG) is still a matter of debate. In all studies, the lack of preoperative antiplatelet activity measurement and the heterogeneity of antifibrinolytic protocols make the conclusions questionable. The aim of this study was to determine, after preoperative antiplatelet activity measurement, if the maintenance of DAPT until CABG increases bleeding in patients treated with tranexamic acid (TA).

Methods: This prospective study included 150 consecutive patients, 89 treated with aspirin and 61 treated with DAPT undergoing a first-time planned on-pump CABG with TA treatment. Antiplatelet activity was measured with platelet aggregation tests and quantification of VASP phosphorylation. Postoperative bleeding at 24 hours was recorded and propensity score analysis was performed.

Results: Based on VASP assay, 54% of patients showed high on-clopidogrel platelet activity inhibition. Postoperative bleeding at 24 hours increased by 22% in DAPT group compared with the aspirin group (680 [95%CI: 360-1665] vs 558 [95%CI: 267-1269] mL, P<0.01), consistent with an increase in blood transfusion (21% vs 7%, P=0.01) and a trend to higher mediastinitis incidence (15% vs 4%, P=0.05). Bleeding was correlated with the extent of clopidogrel antiplatelet effect, the best correlation being obtained with VASP assay.

Conclusions: When DAPT is maintained until the day of CABG in patients treated with TA, postoperative bleeding at 24 hours increases consistently in parallel to preoperative antiplatelet activity induced by clopidogrel.

MESH Keywords: antifibrinolytic agents, blood loss, clopidogrel, coronary artery bypass, mediastinitis.

ClinicalTrial.gov: NCT01216150

Total (Text + reference+ Figure Legends): 4 754 words

Introduction

The benefit of clopidogrel combined with aspirin (DAPT) is well-established in the treatment of both acute coronary syndrome and ischemic cardiomyopathy.[1] Aspirin maintenance during surgery does not increase the postoperative bleeding in coronary artery bypass grafting (CABG) patients,[2] but the continuing use of DAPT until CABG is still a matter of debate. Numerous studies or meta-analyses have tried to address this question[1 3-6] and guidelines have been published as a result.[6-8] Nevertheless, the conclusions are questionable because of major methodological concerns. The most important bias is the lack of preoperative platelet activity measurement even though a large inter-individual variability in response to clopidogrel is well known.[9-11] In only one retrospective study, the authors assessed platelet activity. Unfortunately authors mixed patients undergoing off- or on-pump CABG[9] while cardio-pulmonary bypass (CPB) is known to impact postoperative bleeding.[12] In addition, while intra-operative treatment with anti-fibrinolytic agents has been shown to reduce perioperative bleeding in cardiac surgery,[13 14] most of the published data refer to studies in which neither antifibrinolytic agents nor standardized infusion protocols were used, increasing the heterogeneity of studied populations.[9 15 16] With aprotinin, the postoperative bleeding was not increased when DAPT was maintained until the day of surgery.[14] Since the withdrawal of aprotinin from the market, tranexamic acid (TA) is currently recommended in cardiac surgery despite being less efficacious than aprotinin.[7] In addition, TA may have a partial reverse effect of platelet activity inhibition induced by DAPT.[17]

After a systematic preoperative platelet activity measurement in each patient to dissociate clopidogrel good-responder and poor-responder, the present study was designed to compare the postoperative bleeding at 24 hours after maintenance of DAPT vs aspirin in

patients undergoing on-pump elective primary CABG and receiving TA infusion, an antifibrinolytic agent being liable to restore platelet activity in DAPT group.

METHODS

Patients

The ICARE study (ClinicalTrial.gov: NCT01216150) is a prospective observational trial which has been conducted at a large academic hospital from December 2009 to November 2010. Because of the numerous biases discussed above and because it is still matter of debate, the maintenance of DAPT until the day of surgery was usual in our institution. For this reason, we made the choice to perform an observational study in which we assessed the postoperative bleeding impact of DAPT by propensity score analysis using boosted regression trees. No additional blood sample was performed for this study. Biological measurements performed in this study are routine clinical standardized for all patients undergoing cardiac surgery in our institution. Because preoperative antiplatelet activity was measured in some residual blood sample (less than 5 ml at all), waived informed consent was approved by the ethical committee from our institution. However, oral and written information was given to the patients.

During the study period, all patients undergoing isolated first-time CABG were enrolled.[14] Patients admitted for emergency procedure were excluded. We excluded patients in whom mechanical support or an intra-aortic balloon pump was required since their antithrombotic therapy management differs during the postoperative period. Finally, we excluded all patients who did not receive aspirin until surgery, those who had been preoperatively exposed to antiplatelet glycoprotein IIb/IIIa inhibitors, and those with a history of hematological disease that may interfere with coagulation or platelet functions. Patients treated with DAPT and in whom clopidogrel was stopped more than 10 days before surgery were included in the aspirin group. In case of clopidogrel discontinuation less than 10 days before the surgery day, patient was excluded to be absolutely sure to rule out a potential

residual effect of clopidogrel. Low molecular weight heparin (LMWH) was systematically discontinued 12 hours before surgery.

Intra-operative management

All patients enrolled in this study were pre-treated by TA (Exacyl®, Sanofi-Aventis, Paris, France) intravenous bolus infusion at 10 mg kg-1 for 20 min just after induction of anaesthesia, followed by a continuous intravenous infusion of 2 mg kg-1 h-1 until the end of surgery according to the published following standardized protocol.[18] In case of renal dysfunction, continuous intravenous infusion was adapted as follows: 1.5 mg kg-1 h-1 for serum creatinine range of 140-290 µmol L-1, 1 mg kg-1 h-1 for serum creatinine 291-580 µmol L-1, 0.5 mg kg-1 h-1 for serum creatinine > 581 µmol L-1.

As previously described,[14] before aortic cannulation, an initial loading dose of heparin was directly administrated by the surgeon into the right atrium to obtain a whole-blood-activated clotting time > 400 sec measured using a micro-coagulation analyzer, (HemochronJr II, Inter- national Technidyne Corporation, Edison, NJ). After discontinuation of the cardiopulmonary bypass, heparin was neutralized by protamine sulfate (0.008–0.01 mg UI-1 of total heparin dose intra-operatively used). Intraoperative cell salvage was used systematically (Electa, Dideco, Mirandola, Italy).

Postoperative anti-thrombotic therapy

Early postoperative antithrombotic therapy consisted of an intravenous bolus of 100 mg aspirin given 6 hours after the arrival in the intensive care unit (ICU).[14] Initial prophylactic anticoagulation was started 6 hours after the end of the surgical procedure with non-fractioned heparin continuously infused at 100 UI kg-1 day-1, switched for once daily administration of

enoxaparin 40 mg the day after surgery. Despite transfusion was a decision left to the clinical team, written guidelines are used in our department. In case of persistent bleeding only, platelet transfusion was administered in first line in case of persistent bleeding without any blood clot formation in operating room and/or when platelet count was less than 80,000 µL-1. No preventive transfusion was given. Adapted from the American recommendations of the Society of Thoracic Surgeons and the Society of Cardiovascular Anaesthesiologists,[19] red blood cells transfusion was recommended in our institution in case of hemoglobin less than 7g dL-1, fresh frozen plasma transfusion in case of serious bleeding with prothrombin time less than 50%, and fibrinogen for plasma concentration less than 2g dL-1.

Preoperative platelet reactivity assessment

The preoperative level of platelet function inhibition was systematically measured in each patient. Platelet aggregation tests and flow cytometric assays with vasodilator-stimulated phosphoprotein (VASP) were systematically performed.[20] [21] Aggregation studies were performed within 3 hours after blood collection and test results were known retrospectively, at the end of the study.[20] As previously described, platelet aggregation test was performed in citrated platelet-rich plasma adjusted to 250.10^9 platelets L-1 and analyzed on a TA-8V optical platelet aggregometer (Soderel Medical, Heillecourt, France). The following agonists were used to induce platelet aggregation: Arachidonic acid (1.5 mmol L-1, Helena Biosciences, England) to explore the aspirin effect, Horm collagen (1 µg mL-1, Nycomed Switzerland) and TRAP6 (20 µmol L-1, DiagnosticaStago, Asnières, France) to explore global platelet function, and Adenosine Diphosphate (ADP) (10 and 20 µmol L-1, Biopool, Sweden) to explore the clopidogrel effect. We recorded the maximal platelet aggregation peak (MPA) and residual platelet aggregation after 6 min (RPA). The laboratory ADP-induced MPA cut-off for distinguishing clopidogrel good- and poor-responders was 50%.[20] To determine the VASP phosphorylation state of whole blood, we used a standardized flow

cytometric assay (Becton Dickinson, Le Pont de Claix, France). The platelet reactivity index (PRI, %) was calculated from the median fluorescence intensity of samples. Patients were considered as "clopidogrel poor-responders" to clopidogrel 75mg-maintenance dose when PRI value was above 60%.[20 22 23] The laboratory arachidonic acid-induced MPA cut-off were classified as poor-responders to aspirin, when using the cut off MPA > 20%.[24] Platelet secretion was tested by their capacity to expose surface P-selectin (CD62P) after activation with the thrombin receptor peptide agonist (TRAP), as previously described.[21]

Endpoints

The primary endpoint was the chest tubes blood output during the first 24 postoperative hours. For sample size calculation, we estimated at 400 [200] mL (expressed as mean [SD]) the postoperative bleeding at 24 hours after CABG with aspirin.[14] Excessive bleeding was defined by an increment of at least 25% of this value.[14] Secondary endpoints were the rate of surgical re-exploration for excessive bleeding, transfusion requirement, prolonged postoperative mechanical ventilation (>10 h), and prolonged ICU length of stay (>72 h), overall hospital length of stay, hospital free days at 30 days defined as the number of days spent out of the hospital during the first 30 postoperative days, mediastinitis (surgical-site infection with positive bacteriological culture) and 30-day mortality defined as the number of patients who died during the first 30 postoperative days.

Statistical analysis

Assuming an alpha risk of 0.05 and a beta risk of 0.20 and because mean amount of postoperative bleeding after CABG has been previously measured at 400 [200] mL in the aspirin group,[14] and assuming, according to our own experience, that 60% of patients are treated with aspirin and 40% of them are treated with DAPT, we estimated that at least 134 patients should be included in our study, 80 in the aspirin group and 54 in the DAPT group.

Lastly, allowing for estimating patients lost to follow up and some technical problems after sample collection in platelet function analysis, the final sample was estimated at 150 patients during 40 weeks.

Data are expressed as mean [SD] or as median [95% confidence interval] in non - normally distributed variables or number (%). Comparison between groups was performed using the Student t test or analysis of variance and the Newman-Keuls *post-hoc* test in normally distributed variables, and the Mann Whitney test for multiple comparisons and the Kruskal-Wallis test in non-normally distributed variables. Comparisons of proportions were performed using the Fisher exact method. Correlation between a predictor variable and a response variable was studied with a linear regression analysis.

The effect of DAPT on postoperative bleeding at 24 hours was assessed by propensity scores using boosted regression trees, as reported previously.[23] Patients were assigned to a propensity score that reflects the probability that they would receive dual antiplatelet therapy. Standardized differences were used to assess the balance between groups. An absolute standardized difference (ASD) above 20% was considered to represent meaningful imbalance. Due to the exploratory nature of the study, we have made no adjustment for multiple testing.

All P values are two-tailed, and $P< 0.05$ was considered to denote significant differences. Statistical analysis was performed with R (version 2.10.0, The R Foundation for Statistical Computing, http://www.r-project.org/foundation/).

Results

We included 89 (59%) patients in the aspirin group and 61 (41%) patients in the DAPT group (Fig. 1). After propensity score-matched analysis, preoperative clinical variables did not show any significant differences between groups except for a lower prevalence of hypertension, and prior non-STEMI in aspirin group compared with the DAPT group, respectively (Table 1). In addition, patients treated with beta-blockers and nitrate were significantly more frequent in aspirin group than in DAPT group (Table 1). No patient was treated with LMWH during a period of 12 hours before surgery. Preoperative biological characteristics were similar between groups (data not shown). Especially, platelet count, hemoglobin and fibrinogen were not significantly different between groups (data not shown).

Regarding arachidonic acid-induced platelet aggregation, 11 (12%) patients in the aspirin group and 2 (3%) patients in the DAPT group were classified as poor-responders to aspirin (Fig. 2A).

Using VASP assay, the most specific test to monitor $P2Y_{12}$ inhibition, 28 patients (46%) were considered as clopidogrel poor-responders (Fig. 2C). As expected, the MPA ADP10 µM significantly differed between groups (Fig. 2B) (Table 2).

Collagen and TRAP-induced aggregations significantly differed between the two groups, reflecting a higher efficiency of platelet inhibition with DAPT compared to aspirin (Table 2). This effect was observed for both MPA and RPA for collagen and for RPA for TRAP, reflecting a lower stability of the platelet aggregates. In line, after activation with TRAP, P-selectin expression was lower in the DAPT than in the aspirin group, (48 [95%CI: 20-86] vs 62 [95%CI: 32-107], P<0.01).

Intra-operative period

Without any difference between aspirin and DAPT group, both left and right mammary artery grafts were used in 96% and 95% respectively, left mammary artery graft was used alone in 4% and 5% respectively, while venous graft was used in association with left mammary artery graft in only 3% and 2% respectively. Whereas the initial bolus of heparin was similar between groups, total dose of heparin requested for maintaining ACT > 400 s was higher in the aspirin group (Table 3). After heparin inhibition, both total dose of administered protamine sulfate and final ACT were similar between groups. No significant difference was noted in bypass time and cross-clamping time (Table 3).

Volume of blood collected in the cell saver and retransfused to the patient at the end of the surgery was not significantly different between groups (Table 3). Intra-operative transfusion of platelet units (*apheresis* or random donor units) was significantly more frequent in DAPT group compared with the aspirin group (Table 4). Using VASP assay as reference, intraoperative transfusion of platelet units was not significantly different between clopidogrel good- and poor-responders (30% vs 25%, P=0.71). In contrast, RBC transfusion was not significantly different between groups. No patient was treated with LMWH during a period of 12 hours before surgery or GPIIb/IIIa inhibitors and preoperative medications were similar between groups (data not shown).

Postoperative period and main outcome

At 12 hours after surgery, the median chest tube output was 40% higher in the DAPT group than in the aspirin group (450 [95%CI: 165-1305] mL vs 320 [95%CI: 130-890] mL, P<0.01). Accordingly, the hemoglobin was significantly lower in the DAPT group compared to aspirin group. Using the VASP test as reference, the median chest tube output was significantly increased in the clopidogrel good-responder group compared with aspirin group (510 [95%CI: 266-1395] mL vs 320 [95%CI: 130-890] mL respectively, P<0.01) and did not

significantly differ between the clopidogrel poor-responders and aspirin groups (353 [95%CI: 155-1143] mL vs 320 [95%CI: 130-890] mL, NS).

At 24 hours after surgery, the median cumulative chest tube output was 22% higher in DAPT group than in the aspirin group (680 [95%CI: 360-1665] vs 558 [95%CI: 267-1269] mL, P<0.01). Using the VASP test, the median chest tube output at 24 hours increased significantly both in the clopidogrel good-responder and poor-responder groups compared with aspirin group (800 [95%CI: 437-1802] mL and 550 [95%CI: 340-1527] mL vs 558 [95%CI: 267-1269] respectively, P<0.01). In addition, the median chest tube output increased significantly in the clopidogrel good-responder compared with the clopidogrel poor-responder patients (P<0.01). Whereas the hemoglobin value was not significantly different between groups, postoperative red blood cells units transfusion was significantly increased in the DAPT group compared with the aspirin group (Table 4).

Despite a relatively low correlation between preoperative antiplatelet reactivity (platelet aggregation and VASP) and cumulative postoperative bleeding at 24 hours, VASP assay was the test with the best correlation between platelet reactivity and bleeding (Fig. 3).

In parallel with the increased transfusion, the postoperative mediastinitis incidence trends consistently to increase in the DAPT group (4% vs 15% respectively, P=0.05). We did not find any significant difference between groups in term of prolonged mechanical ventilation (>10 hours), cumulative postoperative ICU length stay, total length of hospitalization stay, hospital free days and 30-days mortality (Table 4).

Discussion

In this prospective study, we have shown that taking into account the variability of responsiveness to clopidogrel assessed with VASP assay, the magnitude of the postoperative bleeding increased significantly more in clopidogrel good-responder than in clopidogrel poor-responder. In contrast with results obtained with aprotinin,[14] DAPT maintained until the day of surgery increased postoperative bleeding in CABG patients treated with TA.

The DAPT causes a synergistic inhibition of thromboxane A2 and ADP-dependent activation pathways. If a higher platelet inhibition until the day of the surgery may increase the perioperative bleeding, the blood transfusion requirement and/or the re-operation rate, the withdrawal of clopidogrel has shown to be deleterious.[26] Numerous studies and meta-analyses have investigated the impact of DAPT on postoperative bleeding when clopidogrel was stopped 5 days before the surgery.[1 4-7] Unfortunately, the answer is still far from being clear mainly because of several methodological biases such as inhomogeneous discontinuation of clopidogrel "within 5 days" before surgery,[27] heterogeneity in type of surgery, elective or urgent,[16 27 28] on-pump or off-pump,[9 15 16 27 29] complex or redo.[16] All these patients were mixed in the same investigated group although these parameters are known to have different effects on postoperative bleeding.[12] Anyway, two major biases have to be considered. First, and this is probably the most important, none of these studies measured the preoperative platelet activity inhibition. Indeed, clopidogrel treatment results in a wide variability of inhibition of platelet function.[10] In our study, antiplatelet treatment was maintained until the day of surgery, no patient was treated with un-fractionated heparin or GPIIb/IIIa inhibitors, all patients included underwent planned and first-time on-pump CABG surgery and on-clopidogrel antiplatelet activity was systematically measured preoperatively with VASP assay. Therefore, in agreement with the literature,[9-11 30] we found 46% of clopidogrel poor-responder patients. The magnitude of postoperative bleeding increased with the inhibitory

pharmacodynamic response to clopidogrel, as shown with the increase of bleeding between aspirin group and the clopidogrel poor-responder and clopidogrel good-responder groups, respectively. These results highlight the risk of wrong conclusions in previous meta-analysis[1] [5-6] and Guidelines[7] [8] in part because of the lack of platelet function measurement.

The second major bias is the large heterogeneity or the lack of intraoperative antifibrinolytic pre-treatment for both patients from the same groups and trials.[15] [16] [27] [29] Indeed, antifibrinolytic agents have some different mechanisms of action. In this line, aprotinin, a serine protease inhibitor, neutralizes free plasmin which induces the clot lysis. In addition to its antifibrinolytic effect, TA may have a partial reverse effect of platelet aggregation dysfunction due to DAPT.[17] A large meta-analysis including 1620 patients had shown the crucial role playing by antifibrinolytic treatment for decreasing the postoperative bleeding in cardiac surgery.[13] Previously, we investigated the impact of aprotinin treatment, infused during the intra-operative period, on postoperative bleeding with aspirin compared with DAPT maintained until the day of CABG surgery. We concluded that postoperative bleeding at 24 hours was not different between groups.[14] In the present study performed with TA, an antifibrinolytic known to be less efficient than aprotinin[31] irrespective of previous DAPT exposure, postoperative bleeding at 24 hours increased by 22% in the DAPT group compared with the aspirin group, especially in the clopidogrel good-responder patients. Initially, during intraoperative period, RBC transfusion was similar between groups while platelet transfusion increased significantly in DAPT group. Intraoperative platelet transfusion was recommended in first line when platelet count was less than 80,000 µL-1 and/or in case of persistent bleeding without any blood clot. The restrictive guidelines for RBC transfusion, recommended when hemoglobin was less than 7 g dL-1, could explain this delayed increase for RBC transfusion. At 24 hours, hemoglobin is stable and similar between groups, maintained at the same level by red blood cells and platelet transfusions in DAPT group.

Unfortunately, the increase of blood transfusion requirement is known to be correlated to the incidence of mediastinitis.[32] Here, even if the difference is not statistically significant perhaps because of not enough power (P=0.05), we revealed a 4-fold (375%) increase in the incidence of mediastinitis in the DAPT group compared with aspirin group.

Some limitations have to be taken into account in relation to this study. This trial, although conducted prospectively was an observational work. Hidden biases cannot be completely ruled out, although the propensity score adjustment technique used in this study limited this possibility, we must recognize that the sample size of this study might not have been large enough to control for the complex interaction of confounders which may occur in this clinical setting. However, recognized predictors of bleeding were balanced after propensity score analysis. In addition, platelet and red blood cells transfusion was a decision left to the clinical team, other source of bias. This point could explain the poor correlation between preoperative platelet inhibition results and postoperative bleeding. Nevertheless, as described above, written guidelines are used in the clinical routine in the department. Moreover, main outcome was bleeding at 24 hours, not the transfusion.

In conclusion, in contrast with results obtained in a previous study with aprotinin, the use of TA is associated with an increased postoperative bleeding which is all the more important given that the platelet activity is consistently inhibited by clopidogrel. The consequences are increased red blood cells transfusion and a trend to increased mediastinitis in the DAPT group compared with the aspirin group. Our findings show the double requirement to take into account both the inter-individual response to clopidogrel and the type of antifibrinolytic agent used during cardiac surgery before performing any guidelines in the field.

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Declaration of interest

The authors **have not disclosed** any potential conflict of interest

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Authors' contributions: Pr. Riou, Pr Gaussem, Pr Ouattara and Pr. Amour conceived the study, performed the data analysis and their interpretation and wrote the manuscript. Dr Garnier contributed to the conception and design, acquisition data and analysis, contributed to increase the quality of the manuscript were involved in the experiments. Dr Szymezak, Dr Helley, Dr Bertil performed the biochemical analysis, contributed to interpretation of results and reviewed the manuscript. Dr. Le Manach contributed to the design of the study, did the statistics and reviewed the manuscript. All authors read, corrected, and approved the final manuscript. Pr Amour and Dr Garnier attest to the integrity of the original data and analysis

reported in the manuscript. Pr Amour is the archival Doctor. All authors meet all four conditions to comply with ICMJE recommendations. No conflict of interest exists.

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Table 1 Baseline characteristics of patients

		Before Propensity Score Adjustment				After Propensity Score Adjustment			
		aspirin	DAPT	ASD	P	aspirin	DAPT	ASD	P
Demographical data									
Age	*Years*	66.5 [37.3-87.9]	62.7 [30.8-85.4]	32.1	0.04	63.7 [41.6-80.1]	62.7 [40.6-79.4]	15.6	0.35
Male	*Nb (%)*	77 (87%)	47 (77%)	22.3	0.15	74.8 (84%)	47 (77%)	16.5	0.32
BMI	*(kg m-2)*	26.6 [20.5-36.0]	26.3 [21.0-32.7]	14.3	0.43	26.4 [20.8-34.0]	26.3 [21.0-32.7]	4.33	0.82
Co-morbidities	*Nb (%)*								
Arterial hypertension		69 (78%)	52 (85%)	21.6	0.23	69 (77%)	52 (85%)	22.8	0.22
Peripheral vascular disease		13 (15%)	13 (21%)	13.5	0.39	16 (18%)	13 (21%)	8.7	0.61
Stable angina		42 (47%)	32 (52%)	10.5	0.53	42 (47%)	32 (52%)	10.3	0.55
Prior non-STEMI		18 (20%)	19 (31%)	23.4	0.14	19 (21%)	19 (31%)	21.4	0.19
Prior STEMI		32 (36%)	26 (43%)	16.6	0.31	34 (38%)	26 (43%)	13	0.44
COPD		8 (9%)	6 (10%)	2.8	0.86	10 (11%)	6 (10%)	3.3	0.86
Diabetes mellitus		35 (39%)	19 (31%)	17.5	0.30	33 (37%)	19 (31%)	12.1	0.48
Renal insuffisiency (clearance <60 mL min-1)		19 (21%)	15 (25%)	8.2	0.61	20 (22%)	15 (25%)	5.5	0.75
Cardiographic data									
LVEF	*%*	60 [37-73]	60 [40-78]	9.2	0.59	60 [39-76]	60 [40-78]	11	0.55
Coronary arteries stenosis	*Nb*	3 [1-3]	3 [1-3]	9.3	0.57	3 [1-3]	3 [1-3]	13.1	0.41
Left main coronary artery stenosis	*Nb (%)*	28 (31)	20 (33%)	2.8	0.87	26 (29%)	20 (33%)	7.9	0.64
Tritroncular patients	*Nb (%)*	67 (75%)	42 (69%)	13.8	0.39	67 (75%)	42 (69%)	12.1	0.47
LogisticEUROscore		2.3 [0.9-6.8]	2.1 [0.9-9.8]	8.3	0.57	2.2 [0.9-7.8]	2.1 [0.9-9.8]	3.4	0.86
Preoperative medications	*Nb (%)*								
Beta-blockers		81 (91%)	48 (79%)	29.8	0.04	79 (89%)	48 (79%)	24.7	0.12
ACE inhibitors		54 (61%)	36 (59%)	3.3	0.84	51 (57%)	36 (59%)	3.2	0.85
Calcium antagonists		28 (31%)	19 (31%)	0.7	0.97	26 (29%)	19 (31%)	4.8	0.77
Nitrates		19 (21%)	6 (10%)	38.3	0.05	18 (20%)	6 (10%)	32.7	0.09
Statins		83 (93%)	55 (90%)	10.3	0.51	82 (92%)	55 (90%)	6.1	0.72
Low molecular weight heparin		17 (19%)	17 (28%)	19.4	0.22	17 (19%)	17 (28%)	19.6	0.22

Data are presented as median with [IC_{95}]; STEMI: ST segment elevation myocardial infarction; COPD: chronic obstructive pulmonary disease; LVEF: left ventricular ejection fraction; ACE: angiotensin conversion enzyme; ASD: absolute standardized difference. ASD above 20% was considered to represent meaningful imbalance; $P< 0.05$: was considered to denote significant differences.

Table 2 : Platelet function baseline values and correlation with postoperative bleeding

		Platelet Function Baseline Values			Correlation with Postoperative Bleeding	
		aspirin (n=89)	DAPT (n=61)	P value	R value	P value
Agregations						
Arachidonic acid	*MPA*	12 [4-27]	9 [1-18]	<0.01	-0.15	0.07
	RPA	10 [2-24]	8 [0-16]	<0.01	-0.16	0.05
ADP 10 µmol L-1	*MPA*	62 [42-73]	41 [14-55]	<0.01	-0.23	<0.01
	RPA	55 [27-72]	22 [0-46]	<0.01	-0.28	<0.01
ADP 20 µmol L-1	*MPA*	66 [52-78]	50 [20-63]	<0.01	-0.27	<0.01
	RPA	62 [38-77]	32 [0-53]	<0.01	-0.31	<0.01
Flow cytometry						
VASP assay (PRI)		/	57 [12-77]		-0.33	0.01

Data are presented as median with [IC_{95}]; ADP : adenosine diphosphate ;VASP : Vasodilator-Stimulated Phosphoprotein assay; PRI: platelet reactivity index ; MPA: maximal platelet aggregation peak; RPA: residual platelet aggregation after 6 minutes; R value: correlation coefficient value; P< 0.05: was considered to denote significant differences.

Table 3: Intra-operative characteristics

		Before Propensity Score Adjustment				After Propensity Score Adjustment			
		aspirin	DAPT	ASD	P	aspirin	DAPT	ASD	P
Anticoagulation									
Total heparin dose	*(UI kg-1)*	313 [274-392]	310 [272-353]	44.1	0.02	313 [274-392]	310 [272-353]	50.0	0.02
Protamine	*(% of total heparin dose)*	90.9 [71.9-100]	91.6 [72.7-100]	17.5	0.30	90.9 [71.9-100]	91.6 [72.7-100]	19.8	0.26
Extracorporeal circulation times									
Aortic clamping time	*(min)*	57 [36-92]	57 [29-92]	18.2	0.27	57 [36-92]	57 [29-92]	24.8	0.18
CPB time	*(min)*	66 [44-100]	67 [37-102]	14.4	0.39	66 [44-100]	67 [37-102]	16.5	0.33
Volume of cell savage used	*(mL)*	385 [231-708]	395 [250-679]	4.7	0.77	385 [231-708]	395 [250-679]	11.1	0.17

Data are presented as median with [IC_{95}]; CPB: cardiopulmonary bypass; ASD: absolute standardized difference. ASD above 20% was considered to represent meaningful imbalance; $P< 0.05$: was considered to denote significant differences.

Table 4 Transfusion data and clinical outcomes

		Before Propensity Score Adjustment				After Propensity Score Adjustment			
		aspirin	DAPT	ASD	P	Aspirin	DAPT	ASD	P
Intra-operative transfusion									
Patients transfused with RBC	*Nb (%)*	12 (13%)	16 (26%)	28.7	0.06	14 (16%)	16 (26%)	22.6	0.17
Patients transfused with platelets	*Nb (%)*	3 (3%)	17 (28%)	54.2	<0.01	3 (3%)	17 (28%)	55.6	<0.01
First 24th hours postoperative transfusion									
Patients transfused with RBC	*Nb (%)*	7 (8%)	13 (21%)	32.6	0.03	6 (7%)	13 (21%)	35.6	0.01
Patients transfused with platelets	*Nb (%)*	8 (9%)	10 (16%)	19.8	0.20	6 (7%)	10 (16%)	24.9	0.09
Patients transfused with plasma	*Nb (%)*	3 (3%)	6 (10%)	21.5	0.13	3 (3%)	6 (10%)	22.2	0.12
First 24th hours surgical re-exploration *	*Nb (%)*	1 (1%)	4 (7%)						
Clinical outcomes									
Prolonged mechanical ventilation (>6h)	*Nb (%)*	27 (30%)	19 (31%)	2,0	0.99	27 (30%)	19 (31%)	3.0	0.85
Mediastinitis	*Nb (%)*	4 (4%)	9 (15%)	28.7	0.04	4 (5%)	9 (15%)	28.3	0.05
Hospital free days (at 30 days)	*(Days)*	18 [0-24]	17 [2-24]	11.2	0.67	17 [1-24]	17 [2-24]	15.2	0.85

***** *No statistical test done because populations are <5 in each group*

Data are presented as median with [IC_{95}]; RBC: red blood cell; ASD: absolute standardized difference. ASD above 20% was considered to represent meaningful imbalance; P< 0.05: was considered to denote significant differences.

Figure legends

Figure 1: Study flowchart.

CABG: coronary artery bypass graft; IABP: intra-aortic balloon pump

Figure 2: Comparison of antiplatelet therapy effect of aspirin (Panel A with arachidonic acid maximal aggregation; cut off at 20%) and clopidogrel [panel B (with ADP10 µM maximal aggregation; cut off at 50%) and panel C (platelet reactivity index (PRI, %) with vasodilator-stimulated phosphoprotein (VASP) assay; cut off at 60%) in patients treated preoperatively with aspirin (n= 89) and DAPT (n= 61).

Figure 3: Correlation between cumulative postoperative bleeding at 24 hours and arachidonic acid residual aggregation (%) (Panel A, n=150), ADP10 µM residual aggregation (%) (Panel B, n=150) and platelet reactivity index (PRI, %) with vasodilator-stimulated phosphoprotein (VASP) assay Panel c, (n=61)

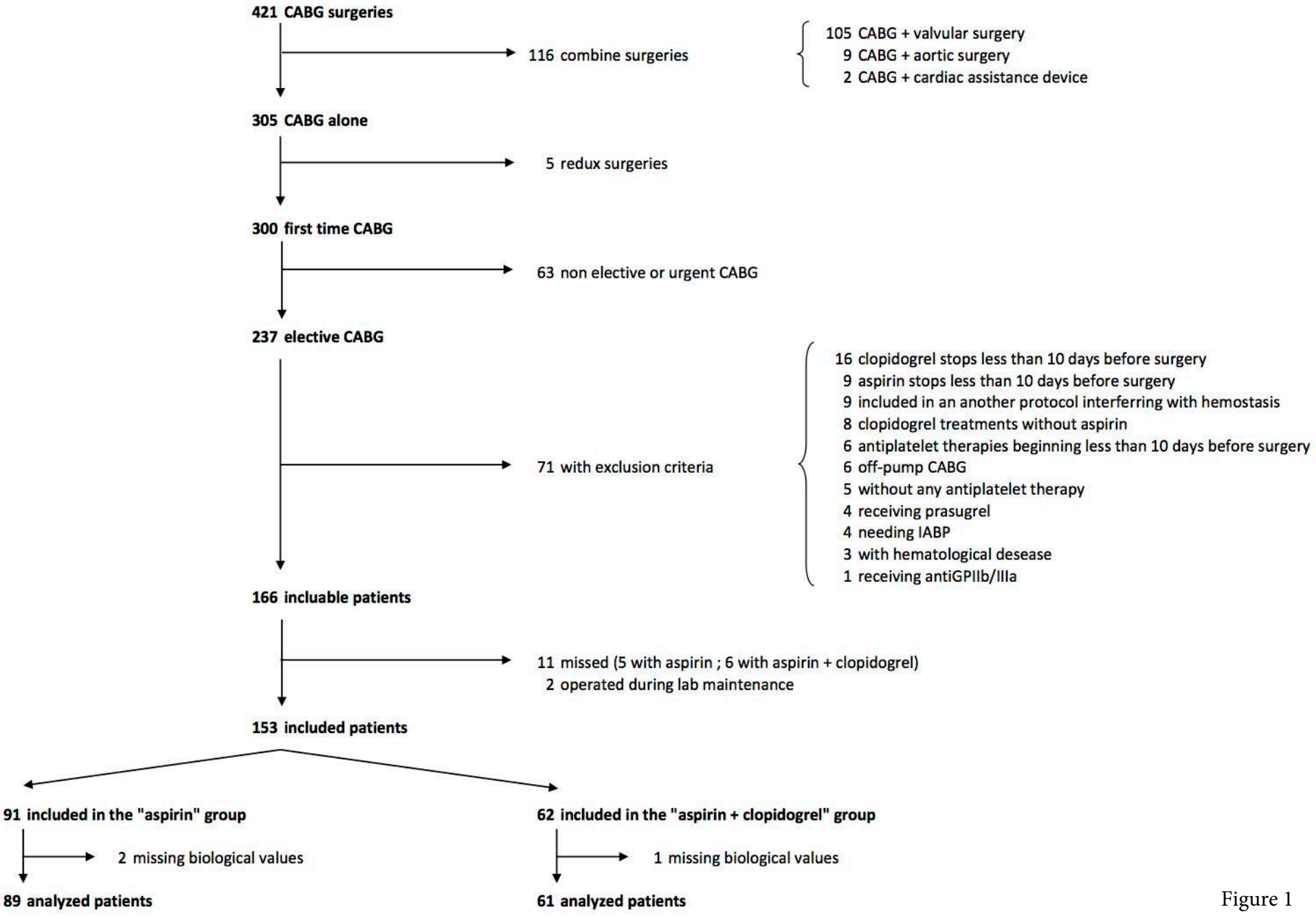
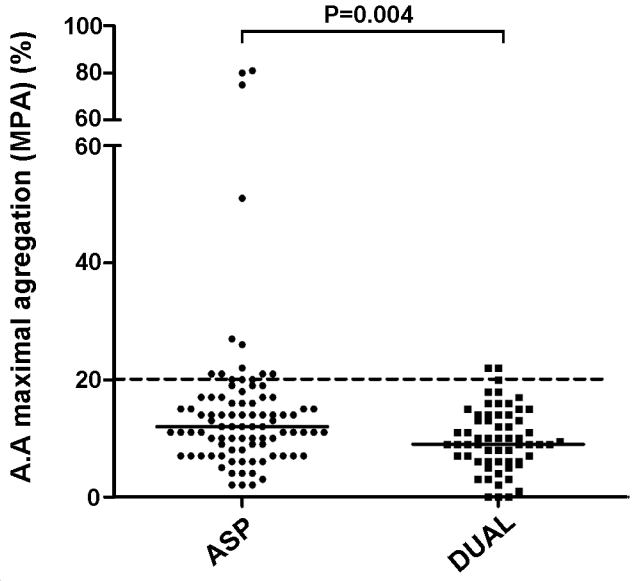
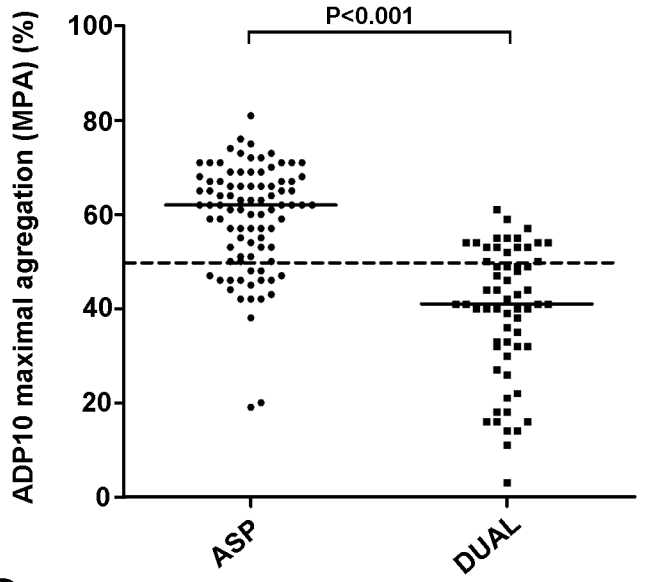

421 CABG surgeries
116 combine surgeries
105 CABG + valvular surgery
9 CABG + aortic surgery
2 CABG + cardiac assistance device
305 CABG alone
5 redux surgeries
300 first time CABG
63 non elective or urgent CABG
237 elective CABG
71 with exclusion criteria
16 clopidogrel stops less than 10 days before surgery
9 aspirin stops less than 10 days before surgery
9 included in an another protocol interferring with hemostasis
8 clopidogrel treatments without aspirin
6 antiplatelet therapies beginning less than 10 days before surgery
6 off-pump CABG
5 without any antiplatelet therapy
4 receiving prasugrel
4 needing IABP
3 with hematological desease
1 receiving antiGPIIb/IIIa
166 incluable patients
11 missed (5 with aspirin ; 6 with aspirin + clopidogrel)
2 operated during lab maintenance
153 included patients
91 included in the "aspirin" group
2 missing biological values
89 analyzed patients
62 included in the "aspirin + clopidogrel" group
1 missing biological values
61 analyzed patients

Figure 1


P=0.004
A.A maximal agregation (MPA) (%)
100
80
60
60
40
20
0
ASP
DUAL
A


P<0.001
ADP10 maximal agregation (MPA) (%)
100
80
60
40
20
0
ASP
DUAL
B

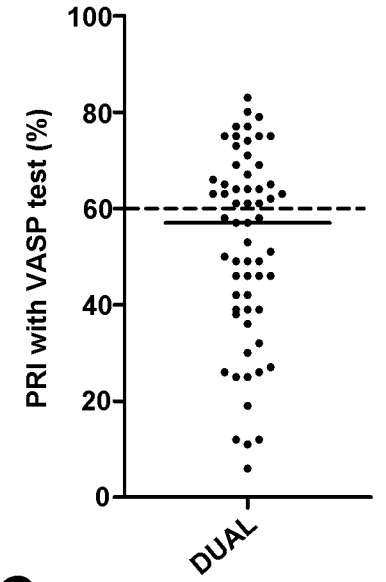
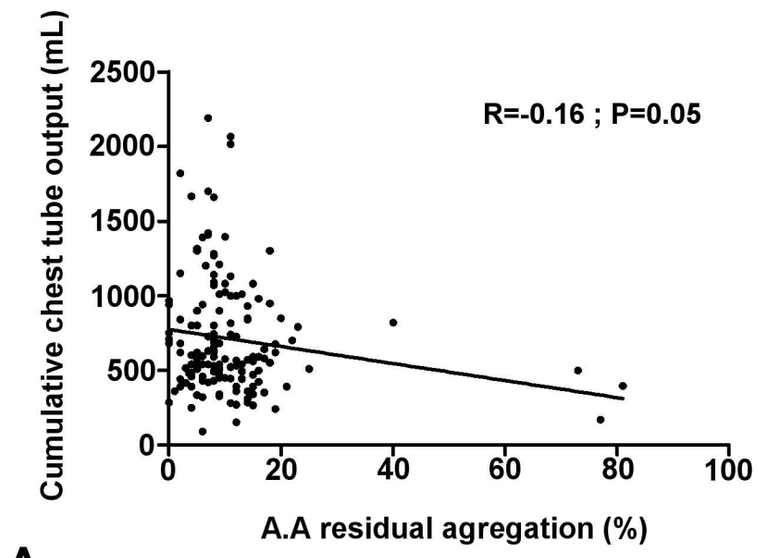
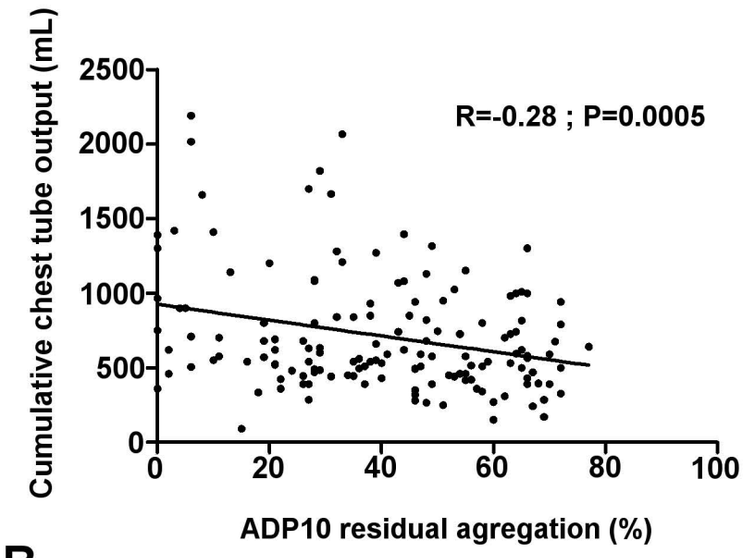

PRI with VASP test (%)
100
80
60
40
20
0
DUAL
C

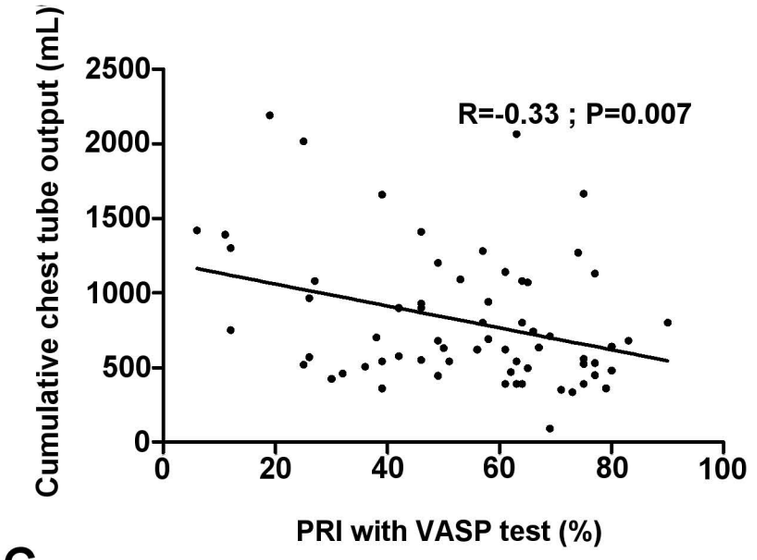
Figure 2


Cumulative chest tube output (mL)
2500
2000
1500
1000
500
0
R=-0.16 ; P=0.05
0
20
40
60
80
100
A.A residual agregation (%)

A


Cumulative chest tube output (mL)
2500
2000
1500
1000
500
0
R=-0.28 ; P=0.0005
0
20
40
60
80
100
ADP10 residual agregation (%)

B


Cumulative chest tube output (mL)
2500
2000
1500
1000
500
0
R=-0.33 ; P=0.007
0
20
40
60
80
100
PRI with VASP test (%)

C

Figure 3