

# Effect of two doses of aspirin on thromboxane biosynthesis and platelet function in patients undergoing coronary surgery

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## Summary

Early post-operative aspirin improves survival in patients undergoing coronary artery bypass graft (CABG). However, most patients do not benefit of aspirin after CABG, still remaining at risk of thrombotic events due to insufficient platelet inhibition, specifically via the thromboxane (TX) pathway. We evaluated the effect of two aspirin doses (100 or 325 mg daily, enteric coated formulations) on platelet function and TX biosynthesis in patients after CABG and assessed whether the incidence of residual platelet reactivity could be reduced by the higher dose. Fifty-six patients undergoing CABG were randomly assigned to 100 or 325 mg aspirin daily for five days in a prospective single-centre study. Treatment effect was assessed by measuring either platelet function (light-transmission aggregometry and point-of-care PFA-100<sup>®</sup>) or TX biosynthesis in collagen-stimulated platelets, serum,

urine, and in lipopolysaccharide (LPS)-cultured whole blood (WB). An insufficient TX inhibition was observed with 100 mg aspirin but not with the higher dose. The different effect of the two doses was, however, highlighted by either TX (platelet- or serum-derived) or by PFA-100<sup>®</sup> but not by the other assays. In conclusion, early after CABG, the incidence of residual platelet activity was lower in patients who received 325 mg aspirin. Moreover, evidence was provided that different methods yield different results in the detection of aspirin resistance, rendering them not interchangeable.

## Keywords

Aspirin, coronary surgery, platelet pharmacology, platelet aggregation, thromboxane

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## Introduction

Meta-analyses of clinical trials indicate that aspirin treatment of patients with cardiovascular disease can reduce adverse events by 25–44% (1). The anti-platelet effect of aspirin is due to the irreversible inhibition of platelet cyclooxygenase (COX)-1, the enzyme catalysing the conversion of arachidonic acid to thromboxane (TX)<sub>2</sub>, which is rapidly inactivated to TXB<sub>2</sub>. An almost complete suppression (>90%) of platelet COX-1 activity is required to prevent TX formation and subsequent platelet aggregation/activation (2, 3). This assumption, however, has been recently questioned in a study carried out with a modified Born method of platelet aggregation (4).

Several studies have reported that aspirin sometimes fails to completely inhibit platelet function in patients with cardiovascular disease (5). The term aspirin resistance is presently used to describe the inability of aspirin to reduce TX production or to produce a typical effect on one or more in vitro-tests of platelet function (6).

Aspirin resistance may be due to several reasons. Among them, alternative “upstream” pathways of platelet activation, such as stimulation of receptors for ADP, collagen or thrombin, or aspirin-insensitive TX biosynthesis (e.g. *via* monocyte COX-2) have been proposed (6). Acquired or genetic factors could also impair the inhibitory effect of aspirin on platelets (e.g. increased platelet turnover or polymorphisms involving platelet-associated proteins) (7). Moreover, it should be emphasised that the assessment of aspirin resistance is not simple because it is highly assay-dependent (8, 9), and a general agreement on standardised, reproducible and specific tests to detect this phenomenon is still lacking (10–12).

Patients after coronary artery bypass graft (CABG) are at high risk of adverse cardiovascular events, despite aspirin treatment. Occlusion of the bypass, either evident or silent, is one of the most threatening complications. A high incidence of resistance, evidenced as an incomplete inhibition of TX biosynthesis and platelet aggregation, has been reported in patients after CABG who received 100 mg aspirin (13, 14). From a therapeutic standpoint, it is im-

portant to establish whether aspirin resistance, detected in this clinical setting, can be overcome by increasing the dose.

To this aim, we evaluated the effect of two aspirin doses (100 or 325 mg, enteric coated formulations), administered to patients who underwent CABG, on platelet function and TX biosynthesis by using different tests. A group of patients with vascular risk factors (VRF patients) without indication of CABG and a group of healthy subjects (HS) who both received 100 mg aspirin for 5 days were enrolled as controls.

## Methods

### Patients and controls

This prospective study, which complies with the Declaration of Helsinki, was conducted at Centro Cardiologico Monzino IRCCS after approval from the Institutional Review Board. Sixty candidates to CABG surgery were consecutively enrolled following the AHA/ACC guidelines and 56 gave informed consent.

Pre-operative inclusion criteria were ejection fraction  $>30\%$  and left ventricular end-diastolic pressure  $<20$  mmHg. Patients aged over 80 years, suffering from renal or liver disease or taking drugs affecting coagulation or fibrinolysis within 10 days prior to surgery were excluded. Intra- and post-operative exclusion criteria were excessive bleeding ( $>1,000$  ml/24 h) or re-exploration for bleeding, peri-operative myocardial infarction, stroke or renal failure requiring dialysis. Patients admitted for an emergency procedure following failed percutaneous transluminal coronary angioplasty and off-pump coronary surgery were excluded.

Healthy subjects (HS,  $n = 10$ , 80% males, mean age  $48 \pm 8$  years) were recruited among the hospital staff. Patients with vascular risk factors (VRF,  $n = 9$ , 78% males, mean age  $56 \pm 2$  years; 56% hypertensives, 11% diabetics, 100% dyslipidaemics) were enrolled from those attending the clinic for global control of cardiovascular risk at Centro Cardiologico Monzino IRCCS.

### Intervention

At baseline ( $T_0$ ), before surgery, patients were without anti-platelet drugs for at least 3–5 days. Anaesthesia and CABG surgery were as previously described (15). The day after surgery, patients were randomised by a computer-generated list to receive either 100 or 325 mg enteric coated aspirin (Cardioaspirin 100<sup>®</sup> or Aspirina 03<sup>®</sup>, Bayer, Milan, Italy) once daily for 5 days. No additional antithrombotic drugs or NSAIDs, except heparin, were administered. Paracetamol or tramadol were used for post-operative pain. Patients, investigators and the staff involved were blinded to randomisation and treatment schedule. HS and VRF patients received a morning dose of 100 mg aspirin for 5 days. The treatment effect was assessed in patients 3 and 5 days after surgery ( $T_1$  and  $T_2$ , respectively) and at day 5 ( $T_2$ ) in HS and VRF patients.

The following determinations were performed: (i) platelet aggregation induced by collagen and ADP in platelet-rich plasma (PRP) by light-transmission aggregometry; (ii) TXB<sub>2</sub> levels in PRP stimulated with collagen, in serum, and in whole blood (WB) incubated with bacterial lipopolysaccharide (LPS); (iii) 11-dehydrothromboxane B<sub>2</sub> (11-dh TXB<sub>2</sub>) in urine; (iv) platelet activation in WB by platelet function analyser (PFA-100<sup>®</sup>, Dade-Behring, Milan, Italy).

### Sample collection

Blood was drawn from the antecubital vein in 3.5 ml evacuated tubes containing 3.2% sodium citrate or without anticoagulant. Sampling was done in the morning, 5–24 h before surgery ( $T_0$ ) and 16 to 21 h post-aspirin administration (both for  $T_1$  and for  $T_2$ ). For studies with LPS-cultured WB, blood was collected in heparin, and aspirin (30  $\mu$ mol/L, Flectadol, Sanofi Synthelabo Spa, Milan, Italy) was added to prevent COX-1 activity. Serum was prepared from WB, after spontaneous clotting at 37°C for 30 min. Morning urine samples were divided into aliquots and stored at  $-80^\circ\text{C}$ .

### Platelet aggregation and thromboxane synthesis

PRP was prepared by blood centrifugation (160 x g for 15 min) and platelet aggregation was performed by a ChronoLog Optical Aggregometer (model 490, Mascia Brunelli, Milan, Italy) without adjusting platelet count (16, 17). Pilot experiments were performed in order to select the more appropriate agonists. Although arachidonic acid is considered the most COX-1-selective among platelet agonists, we found a high degree of variability in the aggregatory response to this agonist. Therefore, collagen type I (4  $\mu$ g/ml, Mascia Brunelli) and ADP (4  $\mu$ M, Sigma Chemical Co., Milan, Italy) were used. Aggregation was expressed as change in transmittance (%) detected 5 min after the addition of the stimulus. In vitro studies were carried out in PRP samples incubated with aspirin (100  $\mu$ M Flectadol) for 30 min before the addition of collagen.

Following aggregation with collagen, PRP samples were centrifuged (700 x g for 15 min) and TXB<sub>2</sub> levels were determined as a measure of COX activity (exclusively platelet-derived) by enzyme immunoassay (EIA kit, Cayman Chemical, Ann Arbor, MI, USA). Serum TXB<sub>2</sub> levels were also determined as a measure of the overall COX activity of blood cells (largely platelet-derived).

The inter-assay coefficients of variation were  $<10\%$  for both platelet aggregation and TXB<sub>2</sub> immunoassay.

### Urinary 11-dehydrothromboxane B<sub>2</sub> (11-dh TXB<sub>2</sub>) measurement

11-dh TXB<sub>2</sub> was measured using enzyme immunoassay (EIA kit, Cayman Chemical) after extraction and purification on SPE (C18)

columns (Waters Associates, Milford, MA, USA). Data were normalised for urinary creatinine concentration.

### Cyclooxygenase-2-dependent thromboxane biosynthesis in whole blood

Aliquots of WB were cultured at 37°C with or without 10 µg/ml LPS (*Escherichia coli* 0111:B4, Sigma) for 24 h (18). WB was then centrifuged (700 x g for 15 min) and TXB<sub>2</sub> was measured in plasma.

### Platelet function analyser (PFA-100®)

The PFA-100® point-of-care assay (Dade-Behring) assesses platelet activation in WB under high shear. Standard collagen/epinephrine (CEPI) or collagen/ADP (CADP) cartridges were used. Aspirin treatment usually prolongs CEPI closure time (CT) but not CADP-CT. Patients were defined as non-responder to aspirin when CEPI-CT was in the normal range (<193 sec) despite the treatment (9). The inter-assay coefficient of variation was <15%.

**Table 1: Characteristics of the study patients.**

	Aspirin 100 mg (Group A)	Aspirin 325 mg (Group B)	P-value
Patients	28 (57%)	21 (43%)	-
Males	22 (79%)	18 (86%)	0.71
Age (years)	65 ± 8.3	63 ± 7.2	0.38
Cardiovascular risk factors			
Hypertension	15 (54%)	13 (62%)	0.56
Diabetes	10 (36%)	4 (19%)	0.34
Smoking (current)	1 (4%)	1 (5%)	0.99
Dyslipidaemia	19 (68%)	16 (76%)	0.52
Preoperative medications			
Aspirin	23 (82%)	19 (90%)	0.68
β-blockers	9 (32%)	9 (43%)	0.44
Lipid-lowering agents	7 (25%)	6 (29%)	0.78
Glucose-lowering agents	4 (14%)	0 (0%)	0.13
Diuretics	7 (25%)	9 (43%)	0.19
Calcium channel blockers	5 (18%)	1 (5%)	0.22
Nitrates	12 (43%)	13 (62%)	0.19
Surgery			
Distal anastomoses	3.1 ± 0.92	2.6 ± 0.73	0.07
Transfused patients *	9 (32%)	4 (19%)	0.35

Data are presented as mean ± SD or number (%) of patients. \* Red blood cells.

### Statistics

A sample size of 30 subjects per group was planned in order to detect as significant (alpha = 0.05, power = 90%) a difference of 35% in the frequency of patients with TXB<sub>2</sub> levels in serum <15 ng/ml, assuming that this value would be reached in 95% of patients on 325 mg dose and in 60% of those on 100 mg. With the actual sample size reached (21 and 28 in the two groups, respectively) the power was reduced to approximately 80%. Continuous variables with skewed distributions are presented as medians and interquartile ranges and were compared by Wilcoxon signed-rank test (within subjects) and by the Kruskal-Wallis and Wilcoxon rank-sum test (between groups). Variables with nearly normal distributions are presented as means ± SD and were compared by paired *t*-test (within subjects) and by ANOVA and two sample *t*-test (between groups). Categorical variables are presented as frequencies and percentages and were compared by chi-square or Fisher exact test, when appropriate. Associations between variables were assessed by Spearman correlation. A value of *P*<0.05 was considered significant. All analyses were two-sided and were performed with SAS statistical package 9.13 (SAS Institute Inc., Cary, NC, USA).

### Results

#### Treatment

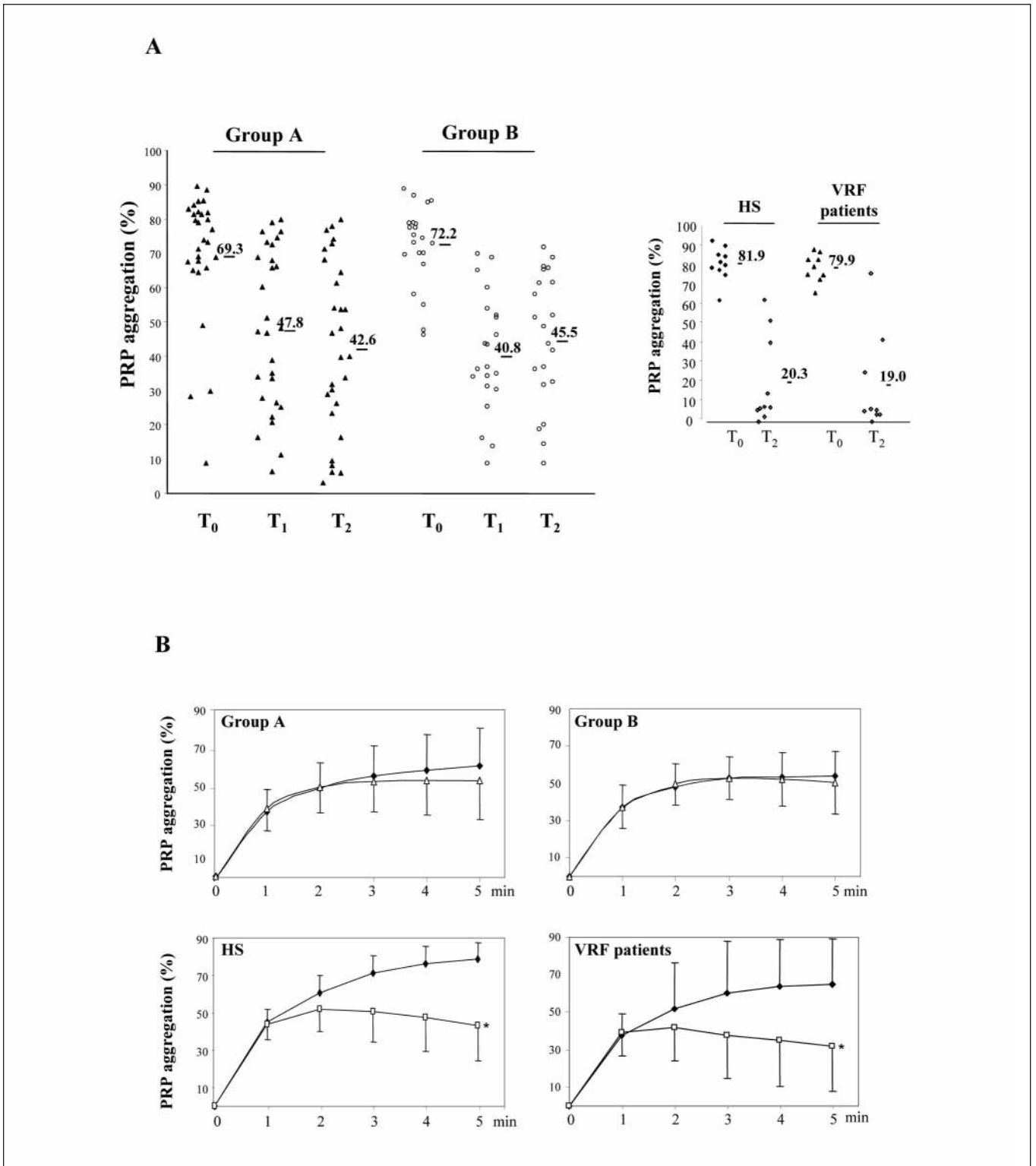
Sixty patients were enrolled in the study. Four declined to participate after the enrolment. There were two discontinuations because of inadequate venous access, three for excessive bleeding and two due to technical failures (unusable samples). A total of 49 patients underwent all the analytical procedures. Twenty-eight received 100 mg aspirin (Group A) and 21 received 325 mg aspirin (Group B). 11-dh TXB<sub>2</sub> could be measured in 20 and 17 patients assigned to Group A and B, respectively.

► Table 1 shows the clinical characteristics and medications of the study population.

Before surgery, mean platelet counts were within the normal range and were similar in the two groups (220 ± 60 and 210 ± 51 x 10<sup>3</sup>/µl for Groups A and B, respectively). At T<sub>1</sub> (3 days after surgery), because of haemodilution during extracorporeal circulation, platelet counts were 190 ± 53 and 165 ± 33 x 10<sup>3</sup>/µl, respectively. At T<sub>2</sub>, platelet counts were higher than at T<sub>0</sub> (324 ± 83 and 252 ± 77 x 10<sup>3</sup>/µl, *P* = 0.005 vs T<sub>0</sub>). At T<sub>1</sub> and T<sub>2</sub> mean platelet counts were not significantly different between the two groups (*P* = 0.07).

#### Effect on platelet aggregation

Platelet aggregation in response to 4 µg/ml collagen was similarly reduced by 100 or 325 mg aspirin (38.5 and 36.9 %, respectively, at T<sub>2</sub>) remaining, however, >40% in about half of the patients in each group, both at T<sub>1</sub> and T<sub>2</sub> (► Fig. 1A). Aggregation values were in-



**Figure 1: Effect of two aspirin doses (100 and 325 mg, Group A and Group B, respectively) on platelet aggregation.** A) Platelet-rich plasma (PRP) aggregation induced by collagen (4 µg/ml) was measured by light transmission aggregometry before (T<sub>0</sub>) and 3 and 5 days after surgery (T<sub>1</sub> and T<sub>2</sub>, respectively). Inset: PRP aggregation induced by collagen (4 µg/ml) in healthy subjects (HS) and in patients with vascular risk factor (VRF patients) before (T<sub>0</sub>) and after (T<sub>2</sub>) 100 mg aspirin for five days. Individual aggregations

and mean values are shown. Platelet aggregation was similarly reduced by the two doses (-38.5%, Group A, and -36.9%, Group B, at T<sub>2</sub>) in patients after CABG. In HS and VRF patients, platelet aggregation was reduced by more than 75%. B) PRP aggregation induced by adenosine diphosphate, ADP (4 µM), in patients after CABG and in HS and VRF patients before (T<sub>0</sub>, filled symbol) and after (T<sub>2</sub>, open symbol) 5 day aspirin treatment. Data are % aggregation (mean ± SD) recorded every minute for 5 minutes. \*P = 0.05 vs T<sub>0</sub>.

deed more than double of those measured in HS or VRF patients who received 100 mg aspirin for 5 days (Fig. 1A, inset;  $P = 0.003$  vs Group A patients for both control groups). In these latter groups platelet aggregation was reduced by more than 75%.

Five day aspirin treatment, either 100 or 325 mg, did not affect ADP-induced platelet aggregation in patients after surgery (Fig. 1B); by contrast, 100 mg aspirin reduced ADP-induced aggregation in HS or VRF patients, as evidenced by the reversal of the second-phase of aggregation (Fig. 1B).

Taken together, data indicate that increased residual platelet activity, as reflected by collagen- or ADP-induced PRP aggregation, was observed in patients after CABG and that the phenomenon was not prevented by the higher aspirin dose.

### Effect on platelet thromboxane biosynthesis

TX biosynthesis by collagen-stimulated PRP was, instead, differently reduced by the two aspirin doses: TXB<sub>2</sub> levels (that did not differ between Group A and Group B at T<sub>0</sub>) remained significantly higher in Group A than in Group B, both at T<sub>1</sub> and at T<sub>2</sub> (► Fig. 2), and than in HS or VRF patients ( $P = 0.003$  and  $P = 0.001$  vs Group A patients, respectively) (Fig. 2, inset). At T<sub>2</sub>, aspirin reduced TXB<sub>2</sub> biosynthesis by 94.7 and 98.6 % in Group A and B, respectively.

TX biosynthesis was measured also in serum to assess the residual capacity of blood cells to produce this metabolite. The obtained

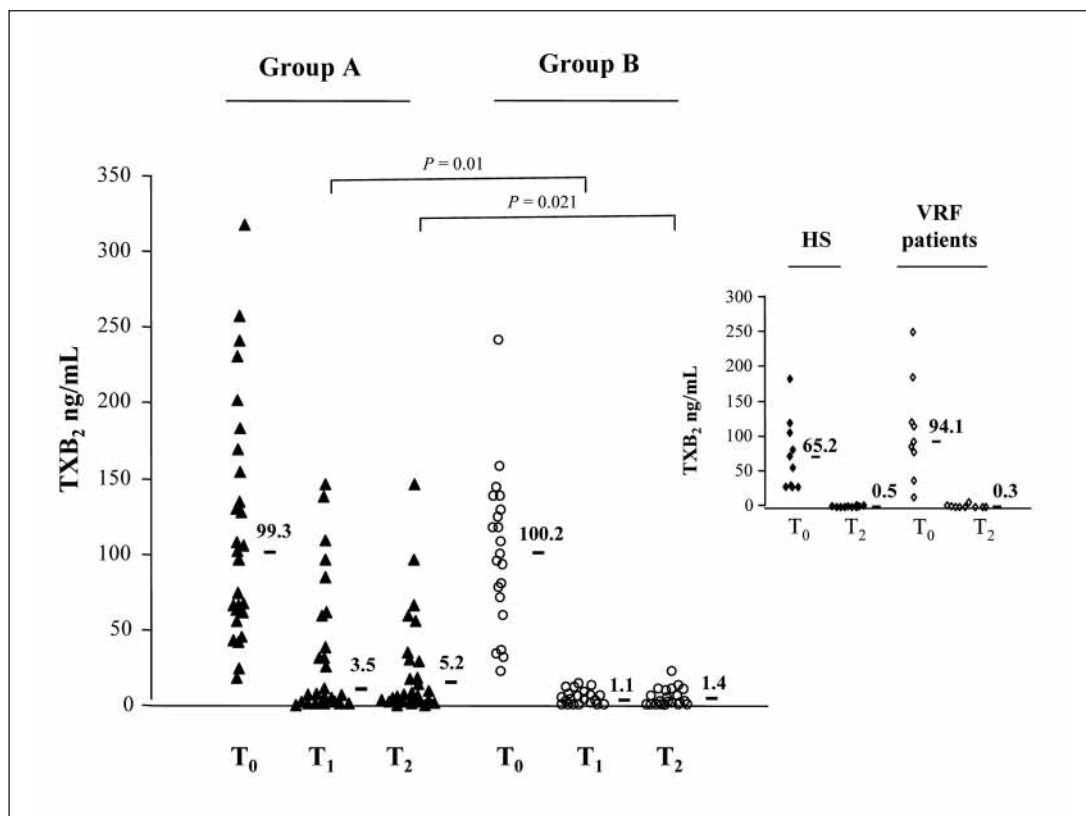
results closely paralleled those in collagen-stimulated PRP. Serum TXB<sub>2</sub> levels were significantly higher in Group A than in Group B either at T<sub>1</sub> or at T<sub>2</sub> (at T<sub>2</sub>: 13.42 [8.9, 35.6] and 8.33 [4.7, 14.1] ng/ml in Group A and B respectively,  $P = 0.04$ ). TXB<sub>2</sub> reduction was indeed <90% in nearly half (46%) patients from Group A and only in 14% patients from Group B ( $P = 0.017$  vs Group A).

Noteworthy, TXB<sub>2</sub> levels measured either in serum or in collagen-stimulated PRP were highly correlated in both groups of patients ( $r = 0.72$  and  $r = 0.79$  for Group A and B, respectively,  $P < 0.0001$  for both).

When dividing Group A patients into good (A1, n=13) and poor (A2, n=15) responders, based on a cut-off for serum TxB<sub>2</sub> of 15 ng/ml at T<sub>2</sub> (19), collagen-induced platelet aggregation was significantly different in the two subgroups accounting for 56.8 ± 23.4% and 30.3 ± 18.7% in subgroup A1 and A2, respectively ( $P = 0.002$ ). Collagen-induced TXB<sub>2</sub> biosynthesis was reduced by 52.0 ± 53.0% (residual concentrations: 29.8 [11.7, 59] ng/ml) and by 94.0 ± 10.0% (residual concentrations: 2.68 [0.27, 6.07] ng/ml) in subgroup A1 and A2, respectively ( $P = 0.0013$ ).

However, when aspirin (100 μM) was added *in vitro*, platelet aggregation in patients from subgroup A1 was further reduced, reaching values that were fully comparable to those measured in subgroup A2 (► Table 2). TX biosynthesis was suppressed by >90% in both subgroups (residual levels: 0.62 [0.52, 0.92] and 0.15 [0.05, 0.22] ng/ml in subgroup A1 and A2, respectively).

Interestingly, collagen-induced platelet aggregation measured at T<sub>2</sub> in patients from the two subgroups correlated with serum



**Figure 2: Effect of two aspirin doses (100 and 325 mg, Group A and Group B, respectively) on platelet thromboxane B<sub>2</sub> (TXB<sub>2</sub>) biosynthesis.** TXB<sub>2</sub> biosynthesis induced by collagen (4 μg/ml) was measured in PRP before (T<sub>0</sub>) and 3 (T<sub>1</sub>) and 5 days (T<sub>2</sub>) after surgery. Inset: TXB<sub>2</sub> levels measured in collagen-stimulated PRP of HS and VRF patients, before (T<sub>0</sub>) and after 100 mg aspirin for 5 days (T<sub>2</sub>). Individual TXB<sub>2</sub> levels and medians are reported. Platelet TXB<sub>2</sub> biosynthesis was reduced in patients after CABG by 94.7% and 98.6% (Group A and B, respectively, at T<sub>2</sub>), whereas in HS and VRF patients it was reduced by more than 99%.

TXB<sub>2</sub> ( $r = 0.49$ ,  $P = 0.009$ ). This finding suggests that residual levels of serum TXB<sub>2</sub> >15 ng/ml after 100 mg aspirin for 5 days may predict residual platelet activity, as detected by aggregation.

### Effect on 11-dehydro-thromboxane B<sub>2</sub> excretion

Aspirin significantly reduced 11-dh TXB<sub>2</sub> levels in both groups of patients (-55.3%, Group A and -55.2%, Group B) (► Fig. 3), without difference between doses ( $P = 0.14$  at T<sub>2</sub>). Levels measured in patients after CABG at T<sub>2</sub> were much higher than those measured in HS and VRF patients: in these latter groups, 11-dh TXB<sub>2</sub> levels decreased from 31.2 [25, 68.2] to 8.3 [4.5, 9.8] and from 75.5 [73.5, 77.8] to 17.1 [14.1, 22.5] ng/mmol creatinine, respectively ( $P < 0.001$  vs Group A patients for both control groups).

### Effect on thromboxane biosynthesis by LPS-cultured whole blood

Similarly to what was observed for 11-dh TXB<sub>2</sub>, aspirin reduced LPS-induced TX biosynthesis both at T<sub>1</sub> and at T<sub>2</sub>, with no difference between doses (20.6% and 22.2% at T<sub>2</sub> for Group A and B, respectively) (► Fig. 4).

### Effect on platelet function detected by PFA-100®

CEPI-CT detected at T<sub>2</sub> was <193 sec in 23 (82%) out of 28 and in 11 (52%) out of 21 patients from Group A and Group B, respectively ( $P = 0.02$ , ► Fig. 5), whereas it was >300 sec for all HS and

**Table 2: *In vitro*-effect of aspirin (100 μM) on collagen-induced platelet aggregation of Group A (100 mg aspirin) patients after CABG stratified according to serum thromboxane B<sub>2</sub> levels.**

Aspirin	Subgroup A1* (n=13)		Subgroup A2† (n=15)	
	-	+	-	+
T <sub>0</sub>	66.8 ± 24.1%	36.3 ± 23.3%	71.4 ± 14.0%	32.2 ± 16.2%
T <sub>2</sub>	56.8 ± 23.4%	31.8 ± 17.3%	30.3 ± 18.7%‡	27.0 ± 15.1%

Data are presented as % of aggregation (mean ± SD). \*Subgroup A1 patients: serum TXB<sub>2</sub> > 15 ng/ml. †Subgroup A2 patients: serum TXB<sub>2</sub> < 15 ng/ml. ‡P = 0.002 vs Subgroup A1.

VRF patients. Interestingly, CEPI-CT and TXB<sub>2</sub> levels in collagen-stimulated PRP were inversely correlated ( $r = -0.36$ ,  $P = 0.004$ ). No changes of CT were detected when the CADP cartridge was used (data not shown).

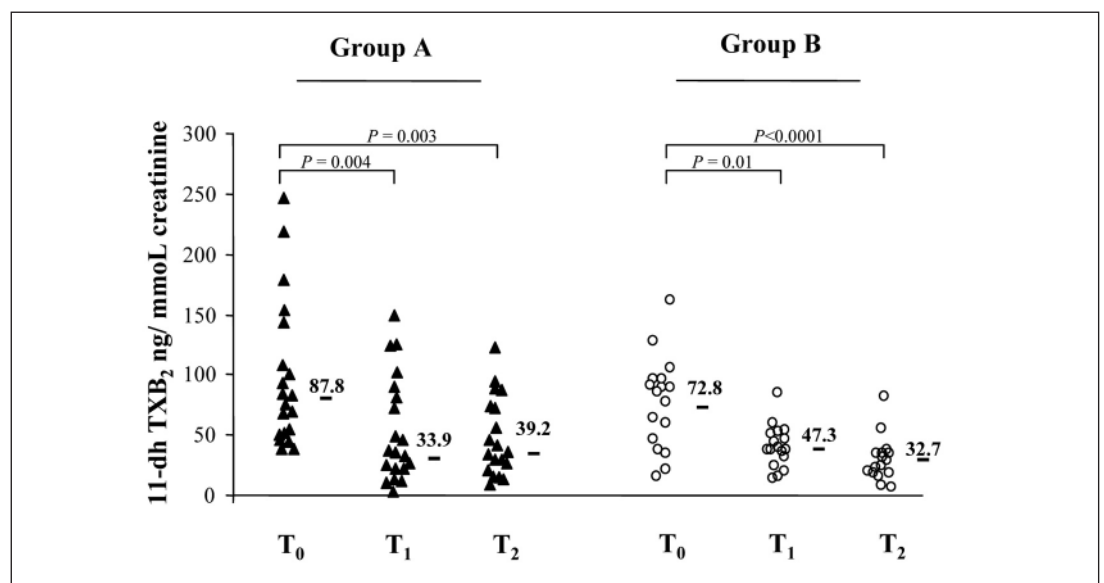
### Discussion

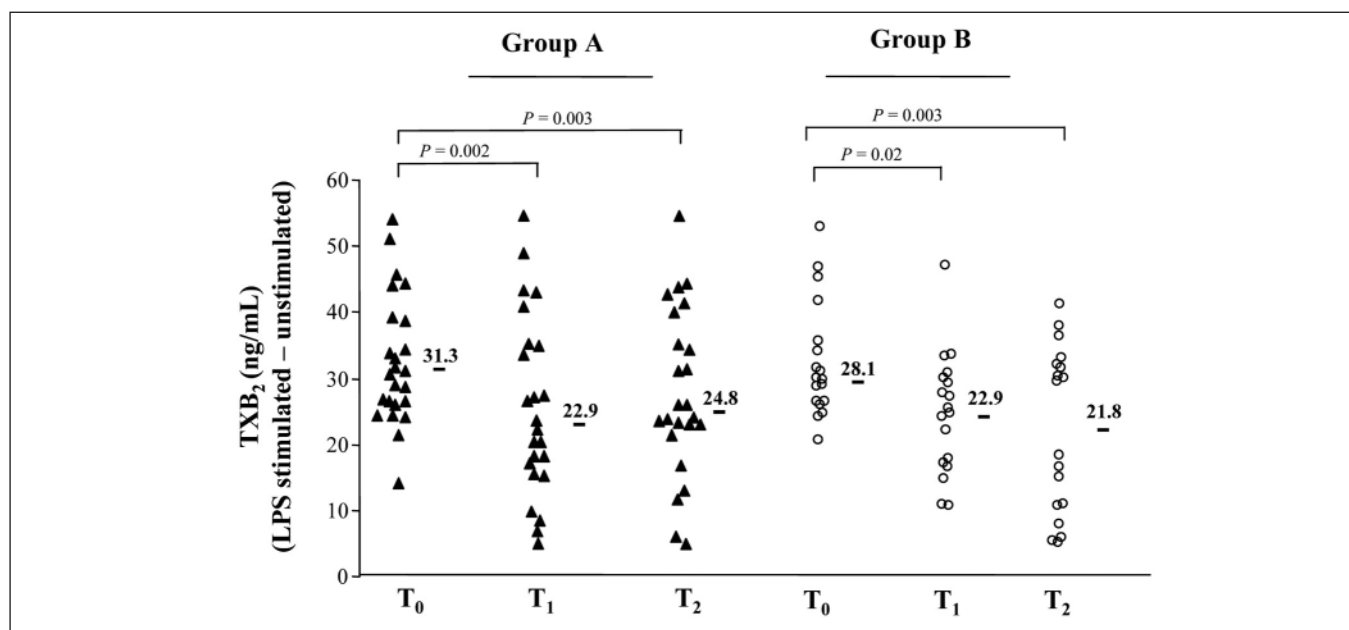
Results show that the incidence of residual platelet activity detected in patients who received 100 mg aspirin after CABG surgery can be reduced by increasing the dose.

The insufficient TX inhibition was highlighted by measuring either platelet- or serum-derived TX levels or by PFA-100®, but not by the other methods used. This indicates that different methods yield different results in monitoring platelet function during aspirin treatment, rendering them not interchangeable, as already reported in patients with stable coronary artery disease (9) and, more recently, in normal subjects (11, 12).

Our data indicate that 100 mg aspirin treatment for 5 days did not adequately inhibit TX biosynthesis in 46% of patients after

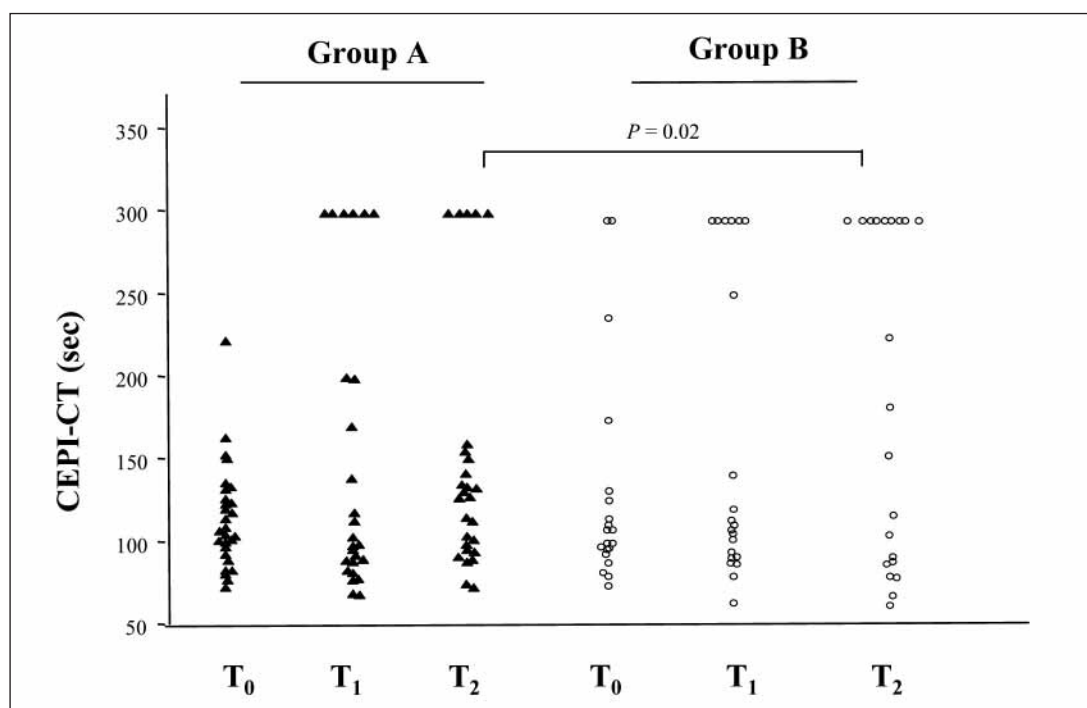
**Figure 3: Distribution of urinary 11-dehydro-thromboxane B<sub>2</sub> (11-dh TXB<sub>2</sub>) levels in patients after CABG.** 11-dh TXB<sub>2</sub> levels were measured before (T<sub>0</sub>) and 3 (T<sub>1</sub>) and 5 days (T<sub>2</sub>) after aspirin treatment (100 mg, Group A and 325 mg, Group B). Individual urinary 11-dh TXB<sub>2</sub> levels and medians are reported. 11-dh TXB<sub>2</sub> levels were similarly reduced in patients after CABG by the two doses (-55.3%, Group A, and -55.2%, Group B, at T<sub>2</sub>).





**Figure 4:** Distribution of cyclooxygenase-2 (COX-2)-dependent TXB<sub>2</sub> levels measured in lipopolysaccharide (LPS)-cultured whole blood of patients after CABG. COX 2-dependent TXB<sub>2</sub> levels were measured in LPS-cultured whole blood of patients before (T<sub>0</sub>) and 3 (T<sub>1</sub>) and 5 days (T<sub>2</sub>) after aspirin treatment (100 mg, Group A, n = 24, and 325

mg, Group B, n = 18). Individual TXB<sub>2</sub> levels (difference between LPS-stimulated and unstimulated samples) and medians are reported. TXB<sub>2</sub> levels were similarly reduced by the two doses in patients after CABG (-20.6%, Group A, and -22.2%, Group B, at T<sub>2</sub>).



**Figure 5:** Distribution of closure times (CT) measured by PFA-100<sup>®</sup>. CT was assessed by Collagen/Epinephrine (CEPI)-cartridge before (T<sub>0</sub>) and 3 (T<sub>1</sub>) and 5 days (T<sub>2</sub>) after aspirin treatment (100 mg, Group A, and 325 mg, Group B). Individual values are reported.

CABG surgery. This finding is in agreement with a previous report showing that, in the same clinical setting, platelet inhibition by the same aspirin dose and formulation (enteric coated) is compromised within several days after the intervention (13). Although it is

known that the bioavailability of enteric coated aspirin is lower and more variable than that of immediate-release aspirin (20, 21), other mechanisms may account for the impaired platelet inhibition observed after CABG. First, the surgical intervention itself

may play a critical role; indeed, it has been previously shown that CABG causes platelet activation (22–24) and increases platelet turnover (14). Secondly, it has been reported that platelet activation results in de novo-COX-1 synthesis and TXB<sub>2</sub> production despite the block of preformed COX-1 by aspirin (25). This mechanism, together with the entry of new platelets containing active COX-1 into circulation, may counteract the complete and persistent suppression of TX biosynthesis by aspirin. Finally, an impaired interaction between aspirin and platelet COX-1 has been proposed to occur in patients after CABG (13). *In vitro* addition of aspirin to PRP samples, however, further reduced either platelet TX biosynthesis or aggregation indicating that COX-1/aspirin interaction was not altered in these patients.

Contrary to what was observed with platelet- or serum-derived TXB<sub>2</sub>, platelet aggregation was not further reduced by increasing the dose, fully in agreement with data by Cornelissen et al. obtained in the same clinical setting (26). This discrepancy supports the hypothesis that signalling pathways, independent of COX-1, may contribute to the residual platelet aggregation observed in these patients (26). In the present study, both aspirin doses reduced aggregation only partially (~50%) and to a lower extent than that detected in VRF patients not undergoing surgery (~80%). This finding suggests that aspects other than the phenotypic variance related to cardiovascular risk factors (27) may account for the aspirin failure to fully prevent collagen-induced platelet aggregation in patients after CABG, and strengthens the role of surgical intervention.

A poor correlation between aspirin non-responsiveness defined by serum or platelet-derived TXB<sub>2</sub> and platelet aggregation was observed in patients after CABG, as already reported in patients with stable coronary artery disease (9) and in normal subjects (11, 12). However we found a significant correlation between TXB<sub>2</sub> and the degree of reduction of platelet aggregation in patients who received 100 mg aspirin for 5 days. In this respect, even if indirectly, data indicate that serum TXB<sub>2</sub> levels may be predictive for residual platelet activity, as reflected by aggregometry.

Although our results support the scanty relationship among different assays used to monitor platelet function during aspirin treatment, both the measurement of TXB<sub>2</sub> levels (either platelet- or serum-derived, that we show to be highly correlated) and platelet function assessed by PFA-100® documented the insufficient inhibition of platelet reactivity in patients receiving 100 mg aspirin. Of note, in our study, PFA-100® yielded the higher frequencies of persistent platelet reactivity despite treatment, confirming data recently reviewed (28). This finding, observed also in other clinical settings (10), suggests that PFA-100® measures non-thromboxane-related residual platelet activity which, in patients after CABG, is most likely increased by the surgical stress. Although this assay is not COX-1 specific, it may carry prognostic information in aspirin-treated cardiovascular patients (29–31).

The effect of aspirin on TX biosynthesis was also assessed through the measurement of the urinary excretion of 11-dh TXB<sub>2</sub> that is considered an index of global TX synthesis. Both aspirin doses similarly reduced 11-dh TXB<sub>2</sub> excretion in patients after CABG, with the extent of reduction being lower (55% in both groups) than that measured in HS or VRF patients (>70%). Our

finding indicates that levels of TX derived from sources other than platelets were scarcely affected by aspirin in patients after CABG, as in other clinical settings (32, 33). Moreover, we show that the measurement of platelet TXB<sub>2</sub> and urinary 11-dh TXB<sub>2</sub> were not equally effective in assessing the anti-platelet effect of aspirin, supporting previously reported data (34).

The inflammatory response to surgery and healing may activate COX-2. The possible contribution of this pathway to the global TX levels was therefore assessed by ex vivo-experiments carried out in LPS-cultured whole blood. Aspirin treatment reduced COX-2-dependent TX synthesis. As already observed for urinary 11-dh TXB<sub>2</sub>, however, TXB<sub>2</sub> levels measured after aspirin treatment were neither negligible nor reduced even by the higher dose. This finding indicates that aspirin-insensitive TX biosynthesis from extra-platelet source may occur in patients after CABG and supports the critical role of COX-2 induced in response to a local inflammatory milieu, as in other clinical settings (33).

In conclusion, although limited by the small number of patients and the short clinical follow-up, our results indicate that 100 mg enteric coated aspirin caused insufficient platelet TX inhibition and that this effect could be overcome by increasing the dose.

The clinical significance of the present functional data deserves confirmation from properly powered trials with an adequate follow up.

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