Platelet reactivity monitoring of clopidogrel loading dose in Unprotected Left Main Coronary Artery Stenting.

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Summary

Aims : Recent studies have suggested that optimized platelet reactivity (PR) inhibition could translate into decreased post-procedural thrombotic events in patients undergoing percutaneous coronary interventions (PCI). We aimed to investigate if PR monitoring of the response to clopidogrel in patients undergoing left main coronary artery (LMCA) stenting could decrease thrombotic events post-PCI.

Methods and result: A cohort of 136 consecutive, unselected patients with unprotected LMCA stenosis who underwent elective stenting by sirolimus eluting stents (SES), with a clopidogrel loading dose(LD) adjusted to PR monitoring using the VASP index (Group1) was compared to a historical cohort of 228 consecutive unselected patients treated with elective bare metal stents(BMS) receiving a single 450mg LD of clopidogrel (Group2). The primary end-point was the one month death rate. One patient (0.7%) in Group 1 died within 30 days compared to 13 patients (5.7%) in Group 2(p=0.02). No difference was observed in the rate of in-hospital minor and major bleeding (2.2 vs 2.2%; p=1). No additional gain of mortality or "catch-up" phenomenon was observed between 30 days and one year between Group 1 and Group 2.

Conclusions: The present study suggests VASP-adjusted clopidogrel LD is associated with decreased mortality at one month in patients undergoing LMT stenting.

Keywords
Vasodilator stimulated phosphoprotein, percutaneous coronary intervention, Stent, Sirolimus, High on-treatment platelet reactivity, thrombosis.

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INTRODUCTION

Improvement in the anti-platelet therapy with the addition of thienopyridines to aspirin has significantly reduced the risk of recurrent thrombotic event including stent thrombosis after percutaneous coronary intervention (PCI) with stent implantation. However, although reduced, post-PCI thrombotic events have not been abolished with the advent of dual anti-platelet therapy (1,2). Following reports of a large inter-individual variability in response to clopidogrel, numerous trials have suggested a link between high on-treatment PR (HTPR) and thrombotic events post-PCI including stent thrombosis (4-8). Studies have demonstrated that higher loading dose (LD) of clopidogrel could decrease both the mean platelet reactivity (PR) and the number of patients considered to have HTPR (9,10). Further reports have observed a decreased in post-PCI thrombotic events with higher (600 mg) clopidogrel LD (11-12). However despite the use of high clopidogrel LD, some patients are still considered to have HTPR which is associated with stent thrombosis and major adverse cardiovascular events (MACE). These findings together with the inability of clinical characteristics to predict clopidogrel responsiveness support the need for PR monitoring. Interestingly, there are growing evidences of a stepwise relationship between PR and thrombotic events post-PCI (5,8,13). Accordingly, we have observed that a threshold of PR predicted thrombotic events. In this study a PR below 50% using the VASP index had a very high negative predictive value for thrombotic events confirming previous findings (5,14).

The concept of a potential benefit of adjusted clopidogrel LD according to PR monitoring is supported by the results of VASP studies (ref).

In these trials we have demonstrated that adjusted clopidogrel LD according to PR monitoring could be used to optimise PR inhibition in patients with HTPR which translates into a reduction in post-PCI MACE and early stent thrombosis rates (16,17). Left main coronary artery stenting (LMCA) is gaining acceptance as a therapeutic alternative to surgery (18).

However because of higher rates of MACE and in particular of death following PCI despite great improvements in techniques, devices and pharmacology, this procedures is mainly performed for patients considered at high surgical risk. In fact, LMCA stenting is considered a high risk procedure since acute or sub-acute stent thrombosis in such location has a dramatic outcome. We aimed to investigate if adjusted clopidogrel LD according to PR monitoring using the VASP index could decrease the 30 days death rate in patients undergoing LMCA PCI.

MATERIAL AND METHODS

Patient population: Using our institution registry we were able to identify 136 unselected consecutive patients with significant unprotected LMCA stenosis treated by PCI with SES between January 2003 and May 2005 (group 1). In these patients clopidogrel LD was individually tailored according to VASP measurement to reach an optimal PR inhibition before PCI. This group of patients was compared to a historical cohort of 228 unselected consecutives patients treated with BMS between January 2001 and December 2002 (group 2). In this group patients received a single LD of 450 mg of clopidogrel before PCI.

Unprotected LMCA was defined as > 50% stenosis on angiography without any patent graft to the left coronary arteries. The indications of PCI in both groups were a surgical contra-indication or preference by patient and physician for a non-surgical approach. The protocol is in accordance with the declaration of Helsinki and was approved by the local ethic committee. Before the procedure, informed consent was obtained from all patients.

VASP analysis: VASP phosphorylation was determined with Platelet VASP kits according to the manufacturer's instructions (Diagnostica Stago, Asnières, France). Briefly, blood samples, punctionned on sodium citrate, were incubated *in vitro* with ADP and/or PGE1 before fixation. Indirect immunolabelling on each sample was performed with a first incubation with 16C2 Mab, followed by the staining with a goat anti-mousse FITC polyclonal reagent (BioCytex, Marseille, France). Flow cytometric analysis was performed on a Coulter, EpicsXL cytometer. Platelet population was identified on its forward and side scatter distribution and 3.000 platelet events were gated and analyzed for Mean Fluorescence Intensity (MFI) using EpicsXL software. MFI corresponding to each experimental condition (ADP, ADP+PGE1) was determined to establish a ratio directly correlated with VASP phosphorylation state. The ratio, $100*(1-MFI^{ADP+PGE1}/MFI^{PGE1})$, is expressed in this study as a VASP index corresponding to a ratio of the VASP phosphorylation of activated platelets versus resting platelets (5,14,19). The VASP index was measured at least 6 hours after each clopidogrel loading dose.

Adjunctive anti-platelet therapy: Group 1: In this group, PR was measured using the VASP index. According to previous studies, the aim of PR monitoring was to ensure a VASP index < 50%, which was considered an optimal PR inhibition, before PCI (5,14,17). The LD of clopidogrel was individually adjusted before PCI.

Patients under chronic clopidogrel therapy received a loading dose of 300 mg, for those not under chronic clopidogrel therapy the loading dose was 600 mg. Patients with a VASP index <50% were considered good responders and could undergo PCI without further LD. On

the contrary if after the first LD, PR was ≥ 50%, patients were considered to have HTPR and received additional 300 mg LD in order to obtain a VASP index <50% before PCI.

Group 2: In group 2, a single clopidogrel loading dose of 450 mg was given at least 24 hours for all patients before the procedure. No platelet function analysis was performed.

Angioplasty procedure: Stent delivery was performed based on angiography without intravascular ultrasound (IVUS) guidance. For bifurcation lesions located in the distal LMCA final kissing balloon was performed at the end of the procedure in all cases. The stenting technique was based on the LMCA anatomy and included T-stenting, Y-stenting or V-stenting. Prophylactic intra-aortic balloon pump support was not used.

All patients were pre-treated with aspirin 160 mg at east 12 hours before the procedure. Maintenance dose of anti-platelet therapy were identical in both group and included clopidogrel 2 x 75mg/day for one month followed by 75mg for at least one year in association with aspirin (160 mg/day). Glycoprotein IIb/IIIa inhibitors were used according to the guidelines.

End-point: The primary end-point was the death rate at one month. MACE included: death, myocardial infarction (MI) and TLR (either by PCI or coronary artery bypass surgery) were also recorded.

Death was defined as all causes of mortality. Cardiac death included all death of cardiovascular origin. Myocardial infarctions (MI) were defined as a troponin elevation above the 99th percentiles of normal associated with ischemic symptoms or EKG changes suggesting of ischemia. TLR and TVR were characterized by repeat percutaneous or surgical intervention of treated lesion or vessel, respectively.

There is no program of angiographic follow-up in our center, therefore all coronary angiography performed during the follow-up were clinically driven.

Follow-up: Pre-specified clinical and laboratory data during hospitalization periods were obtained from hospital charts reviewed by independent research personnel who were unaware of the objectives of the study. The data were entered prospectively into the database. Clinical follow-up at 1 month, 6 months, and 1 year were conducted by telephone contact, chart review or office visits. The occurrence of major late clinical events was recorded, including death (all-cause), MI, TLR, and TVR. All clinical events were adjudicated by source documentation by independent physicians who were not involved in the procedures.

Statistical analysis: Data were analysed with the Statview 5 package (SAS Institute Inc). The clinical and angiographic data were collected prospectively and stored in a computerized database. Data are reported as mean +/- SD. Variables were compared using either the chi-square test or Fischer's exact test. Statistical significance was defined as p <0.05. The confidence intervals for our rates are 95 %. To determine the occurrence of death and MACE at one month and one year, the univariate analysis consisted of the chi-square for categorical variables; the variables that were found to be significant (p <0.05) by univariate analysis were entered into multivariate analysis using step-wise logistic regression analysis. Patients had a one-month and one-year actuarial survival follow-up evaluated according to Kaplan-Meier analysis. Comparison of survival was performed using the Log-Rank test.

RESULTS

Baseline characteristics are displayed in table 1. The two groups were well matched in terms of demographic and clinical characteristics. In particular there was no difference in the incidence of cardiovascular risk factors including diabetes (23.5 vs 18.9 %; p=0.3) or in the clinical presentation (unstable angina 42.6 vs 39.5%; p= 0.6). Group 1 more frequently had distal left main disease (61.8 vs 48.2%; p= 0.01).

Table 1 : Baseline characteristics

	Group 1 (n=136)	Group 2 (n=228)	p Value 0.46
Age (years)	71.5 ± 10.9	72.4 ± 11.4	0.71
Males (%)	78.7	80.3	0.78
Hypertension (%)	58.3	56.6	0.28
Diabetes mellitus (%)	23.5	18.9	0.52
Smoking (%)	22.0	25.0	0.22
Hypercholesterolemia (%)	58.8	52.1	0.55
Unstable Angina (%)	42.6	39.5	0.2
Prior MI (%)	13.2	18.4	0.83
Euroscore	7.4 ± 3.7	7.3 ± 4.9	0.41
LV EF	61.4 ± 16.3	59.9 ± 17.0	

MI: myocardial infarction
LVEF: left ventricular ejection fraction

A high number of patients had three vessels disease in both groups (57.4 vs 64.5 %; p= 0.2). Finally patients in group 2 more frequently received glycoprotein IIb/IIIa inhibitors at the time of PCI (36.4 vs 18.4%; p< 0.001). The mean EUROSCORE was identical (7.4 ±3.7 vs 7.3 ± 4.9; p= 0.8).

Interventional procedure: A high number of patients had three vessels disease in both groups and they had similar number of treated vessels (Group 1 vs Group 2: 58.4 vs 64.5 %; p=0.2 and 2.36 ±1.5 vs 2.43 ±1.5; p= 0.7 respectively). The reference and post procedure minimal lumen diameter were higher in group 2 compared to group 1 (3.52 ±0.41 vs 3.68 ±0.56; p=0.004 and 3.47 ±0.39 vs 3.7 ± 0.58; p< 0.001 respectively). Intra aortic balloon counter pulsation was rarely used in both groups (0.7 vs 1.3 %; p=1) (table 2).

Table 2 : Angiographic and procedural characteristics.

	Group 1 (n=136)	Group 2 (n=228)	p Value
Distal Location (%)	61.8	48.2	0.01
RCA occlusion (%)	25.0	27.6	0.58
RCA Stenosis (%)	33.1	36.0	0.58
Three vessel disease (%)	58.4	64.5	0.22
Lesions treated	2.36 ± 1.5	2.43 ± 1.5	0.67
Ref diameter (mm)	3.52 ± 0.41	3.68 ± 0.56	0.004
MLD pre (mm)	1.27 ± 0.90	1.31 ± 0.62	0.62
MLD post (mm)	3.47 ± 0.39	3.70 ± 0.58	<0.001
Max. balloon pressure (bars)	16.2 ± 4.34	17.6 ± 4.3	0.003
Rotablator (%)	0.7	3.1	0.27
IABP (%)	0.7	1.3	0.99
Anti-GP IIb/IIIa (%)	18.4	36.4	< 0.001

Data are mean+/- SD; MI : myocardial infarction;
RCA: right coronary artery; IABP: intra-aortic balloon pump
MLD: minimal lumen diameter (QCA)
IABP: intra-artic balloon pump

Platelet monitoring: In group 1, which includes patients with VASP-guided clopidogrel LD, 53 (39%) patients were considered to have HTPR (VASP ≥50%) after the first LD of clopidogrel and therefore received additional bolus of 300 mg of clopidogrel. This high frequency of patients with HTPR was observed despite the fact that 42% of patients were already under chronic clopidogrel therapy (75mg per day).

A mean additional 848 ± 414mg of clopidogrel was necessary to obtain a VASP index < 50% before PCI. As illustrated by the high standard deviation in the mean additional LD, the wide inter-individual variability persists with very high dose of clopidogrel. In 2 (1.5%) patients, despite up-to a total of 2 400 mg of clopidogrel, we were unable to decrease the VASP index below 50%, these patients were referred to surgery. The mean VASP index after dose adjustment in group 1 was 36.7 ± 15.8 % (5.2-49.9 %).

One-month outcome is summarized in table 3: Patients who had a tailored LD of clopidogrel according to PR monitoring (group 1) had a significantly better one month outcome than those in group 2.

Table 3 : One month outcome

	Group 1 (n=136)	Group 2 (n=228)	p Value
Death	1 (0.7%)	13 (5.7%)	0.02
QWMI	0	2 (0.9%)	0.53
TLR	0	3 (1.3%)	0.3
-Re PCI	0	1 (0.4%)	1
-CABG	0	2 (0.9%)	0.53
Other Revasc.	1 (0.7%)	0	0.37
-Re PCI	1 (0.7%)	0	0.37
-CABG	0	0	-
MACE	2 (1.5%)	18 (7.9%)	0.02

QWMI: Q-wave myocardial infarction
TLR: target lesion revascularization
Re-PCI : re-percuatnous coronary intervention
CABG : coronary artery bypass surgery
MACE: major adverse cardiovascular events

In particular, the one month death rate was significantly lower in group 1 compared to group 2 (0.7 vs 5.7%; p= 0.02). In the SES group, only 1 patient (0.7%) died 5 days after a complex LMCA stenting, his EUROSCORE was 18. This death was related to end-stage congestive heart failure. Kaplan-Meier curve of freedom form death demonstrates a significantly lower rate of death at one month in group 1 (log rank p= 0.02) (Fig. 1).

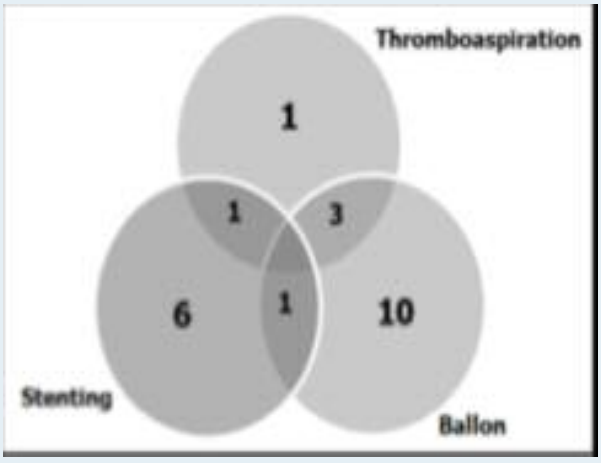

Figure n°1 : Kaplan-Meier Survival for death at one month for Group I (SES) in yellow, Group II (BMS + clopidogrel) in green.

In multivariate analysis the only iondependent predictor of death at one month was group 1 (VASP-adjusted clopidogrel LD) with an odd ratio of 15.7 (95%CI: 1.57 to 45.3; p <0.02). The rate of MACE at one month was also significantly lower in group 1 compared to group 2 (1.5 vs 7.9 %; p=0.02). No intra-cerebral bleeding was recorded. No patients had major bleedings. Three patients (2.2 %) patients had minor bleedings in group 1 and 5 (2.2%) in group 2 (p= 1).

One-year follow-up data: No patient was lost of follow-up at one year (Table 4). The incidence of death was significantly lower in group 1 compared to group 2 (5.9 vs 12.7 %, p < 0.05). Furthermore, the incidence of cumulative mortality (Fig. 2) indicated a lower mortality in group 1 compared to group 2 (log rank= 0.03). This improved survival rate at one year is mainly driven by a significantly lower cardiac death rate in group 1 (3.7 vs 10.9%; p=0.02). Left ventricular dysfunction (LVEF <50%) was the only independent predictor of death at one year in multivariate analysis (OR= 2.62; 95% CI: 1.6-4.3; p<0.001).

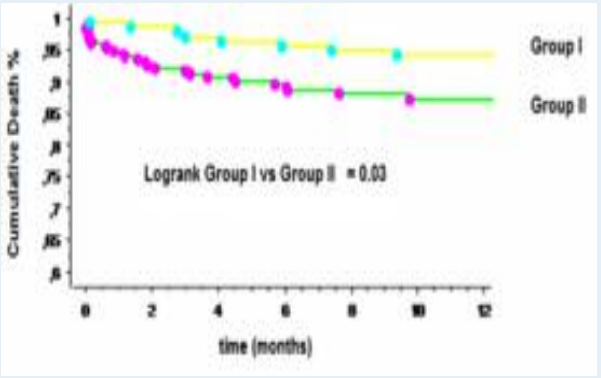

Figure n°2 : Kaplan-Meier Survival for death at one year for Group I (SES) in yellow, Group II (BMS + clopidogrel).

Table 4 : One year outcome

	Group 1 (n=136)	Group 2 (n=228)	p Value
Death	8 (5.9%)	29 (12.7%)	0.04
Cardiac Death	5 (3.7%)	25 (10.9%)	0.01
QWMI	0 (0%)	3 (1.3%)	0.3
TLR	8 (5.9%)	31 (13.6%)	0.02
-Re-PCI	8 (5.9%)	20 (8.8%)	0.3
-CABG	0	11 (4.8%)	0.02
Other Revasc.	17 (12.5%)	23 (10.1%)	0.47
-Re-PCI	17 (12.5%)	20 (8.8%)	0.25
-CABG	0	3 (1.3%)	0.3
MACE	33 (24.3%)	83 (36.4%)	0.02

QWMI: Q-wave myocardial infarction
TLR: target lesion revascularization
Re-PCI : re-percuatnous coronary intervention
CABG : coronary artery bypass surgery
MACE: major adverse cardiovascular events

The incidence of MACE was significantly lower in group 1 compared to group 2 (24.3 vs 36.4%; p=0.02). In particular group 1 had lower rates of TLR (5.9 vs 13.6%; p= 0.02). A lower incidence of cumulative TLR was also noted between group 1 versus group 2 (log rank =0.02) (Fig.3). Further as illustrated by the Kaplan Meier curve there was no catch-up phenomenon in terms of MACE in group 1 after the first month. In fact, at one month post-PCI the death and myocardial infarction rates were similar between the two groups.

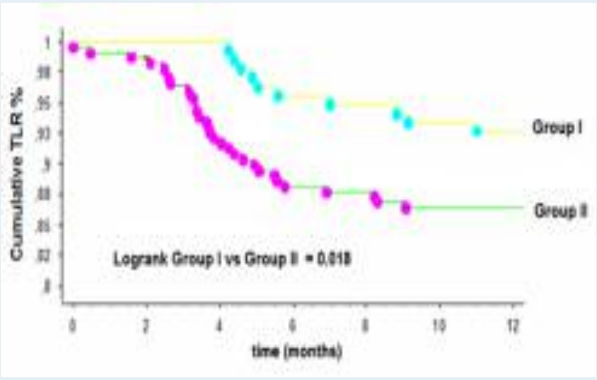

Figure n°3 : Kaplan-Meier Survival for TLR at one year for Group I (SES) in yellow, Group II (BMS + clopidogrel).

DISCUSSION

The results of the present study suggest that adjusted clopidogrel loading dose according to platelet reactivity monitoring using the VASP index reduces the rate of death at 30 days in patients undergoing left main coronary artery stenting. Of interest this reduction in thrombotic events post-PCI was not associated with any increase in bleedings. This is the first study suggesting a survival benefit of improved PR inhibition in patients undergoing PCI. The present results therefore represent a step towards the validation of a personalized medicine approach to guide the intensity of oral antiplatelet therapy based on *ex-vivo* measurements of platelet function to improve the clinical outcome of PCI.

Clopidogrel is a second generation $P2Y_{12}$-ADP receptor antagonist which acts by a selective and irreversible blockade of the receptor inhibiting platelet aggregation (20). Following reports of a great inter-individual variability in the response to clopidogrel, studies have linked biological clopidogrel resistance to thrombotic

events post-PCI (4-8). This biological resistance may be related to various factors from clinical factors such as diabetes, body mass index to genetic polymorphisms of the $P2Y_{12}$-ADP receptor or of cytochrome P450. Of interest, some of these mechanisms of resistance could be overcome by dose adjustment (21). Because of the large inter-individual variability in response to clopidogrel and the numerous mechanisms of the variability, the use of platelet reactivity assays is critical to determine the effect of the drug in each individual and to tailor therapy to patient's susceptibility. Several platelet assays have been proposed to measure clopidogrel responsiveness. The VASP index is a flow cytometric assay based on the quantification of the VASP phosphorylation state which is a marker for platelet inhibition because of its good correlation with fibrinogen receptor inhibition (19,22-23). Because it is a standardized, reproducible and highly specific assay, the VASP index has recently gained interest. Further, studies focusing on the relation between thrombotic events and PR have observed a high negative predictive value of a threshold of 50 % of PR inhibition, as used in the present study, to predict thrombotic events (5,14,16,24).

Prasugrel, is a new thienopyridines which achieves higher PR inhibition compared to standard clopidogrel therapy (25). This new thienopyridine was associated with a significant reduction of post-PCI MACE, including stent thrombosis, compared to clopidogrel standard therapy in the TRITON TIMI 38 trial (15,26). However in this study prasugrel did not reduce the overall mortality. Further, prasugrel use is associated with a significant increase in bleeding complications including fatal haemorrhage. Consistently with the findings of a reduction in thrombotic events post-PCI with enhanced PR inhibition, we have recently demonstrated that adjusted LD of clopidogrel according to PR monitoring, in patients with clopidogrel low response, using the VASP index, decrease the rate of MACE without increased bleeding. We later confirmed that the reduction in MACE was mainly related to decrease early stent thrombosis rates. These findings support a potential therapeutic window for $P2Y_{12}$-ADP receptor (17,18). Consistently, in the present trial, LD adjustment according to PR monitoring, resulted in an improved survival at one month. This is consistent with the fact that PR is critical in stent thrombosis which has a dramatical outcome in LMCA stenting.

Percutaneous coronary intervention for lesions located in the LMCA remains debated and to date guidelines advocate surgery despite encouraging results with the use of DES (19,28-38). Although PCI has demonstrated favourable long-term outcome for ostial and shaft left main lesions, stenting of the distal left main, which include the bifurcation, is still controversial (28). A recent study has observed similar rates of death and myocardial infarction at three year follow-up for DES stenting and coronary artery bypass surgery for LMCA stenosis. However a trend toward increase death rates with the use of DES was noted during the follow-up (19). Further in the DELEFT registry the one month cardiac death rate was 3.3% and the rate of definite stent thrombosis 1.1% (33). This figure illustrates the relatively high rate of thrombotic events within the first month after PCI in this high risk location and its dramatical outcome. Of importance a recent study by Migliorini et al. has observed that post-treatment PR in patients undergoing left main stenting was the only independent predictor of death and stent thrombosis (36). Accordingly implementation of our strategy of LD adjustment could translate into an improved survival.

Finally in the present study, the use of DES did not affect the rate of death and myocardial infarction as demonstrated by the similar rates of these events in the 2 groups between one month and 1 year. This result is re-assuring in view of recent concerns of late stent thrombosis with DES use in LMT stenting (35). However the potential survival benefit of DES in PCI remains controversial (38-41). In the present study we did not observe any evidence of increased in late stent thrombosis with DES. However, consistently with previous studies, we observed a significant reduction in the rate of restenosis with SES compared to BMS (28-33). Our data therefore confirmed the positive impact of SES on restenosis with lower rate of TLR compared to BMS despite the large diameter of LMCA.

CONCLUSIONS

The present study suggests that optimized anti-platelet therapy using tailored clopidogrel loading dose according to platelet reactivity monitoring in patients undergoing left main coronary artery stenting is associated with an improved survival at one month without increased bleeding.

No conflict of interest for any of the authors.

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