Thrombosis Research (2007) **121**, 407–412

ELSEVIER

THROMBOSIS RESEARCH

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REGULAR ARTICLE

Identification of platelet hyper-reactivity measured with a portable device immediately after percutaneous coronary intervention predicts in stent thrombosis

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Received 25 January 2007; received in revised form 18 April 2007; accepted 22 April 2007
Available online 6 June 2007

KEYWORDS
PFA-100;
Platelet activation;
PCI;
Stent thrombosis

Abstract

Introduction: Platelet hyper-reactivity, despite a standard anti-thrombotic therapy, is a recognized risk factor for recurrent myocardial ischemia and in-stent thrombosis following PCI. We have investigated whether this detrimental condition, measured by collagen–epinephrine closure times (CEPI-CT) with the Platelet Function Analyzer (PFA-100) device could predict IST defined as the composite of cardiovascular death or myocardial infarction.
Materials and methods: CEPI-CT was measured in 256 consecutive patients with stable angina ($n=103$) or ACS ($n=153$) 30 ± 8 h after PCI (T_0) and 1 month later (T_1). All patients were followed up for a mean period of 9 months. Platelet hyperactivity was defined as a CEPI-CT <190 s.
Results: Baseline CEPI-CT <190 s was associated with a higher rate of death or MI (LogRank $\chi^2=4.23$, $p=0.039$) as compared with CEPI-CT >190 s (4.6% vs. 0.7%). Multivariable analysis after adjustment for other risk factors confirmed that baseline CEPI-CT <190 s was an independent correlate for death or MI (Hazard ratio 6.981, $p=0.008$). At T_1 there was a significant prolongation of CEPI-CT ($p=0.03$) from 208 ± 64 s to 240 ± 59 s but T_1 did not predict any event.

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0049-3848/$ - see front matter
doi:10.1016/j.thromres.2007.04.009

Conclusions: A CEPI-CT < 190 s measured within the first 24 h following PCI predicts IST defined as the occurrence of death or MI.

Introduction

In-stent thrombosis (IST) is a dramatic and mostly unpredictable complication affecting, in the real world, around 3% of otherwise successful PCI [1]. New generation drug eluting stents (DES) are related to a potentially higher risk of IST, due to the late process of endothelialization [2]. Platelet hyperreactivity, which is a powerful risk factor for recurrent myocardial ischemia in patients undergoing PCI, is strongly implicated in the pathophysiology of IST [3]. Point-of-care tests have been developed in an attempt to more easily measure platelet function and to monitor the effects of antiplatelet therapy. Platelet Function Analyzer-100 (PFA-100) is a rapid and reproducible test allowing a quantitative measurement of platelet function. It has been shown to be sensitive to aspirin response [4,5] but also to predict subsequent coronary events following PCI [6]. In the present study, we hypothesized that platelet function assessment with the PFA-100 POC assay immediately after PCI was an independent predictor of IST. For that purpose, we performed a prospective study of patients who underwent PCI and measured platelet function using the POCT PFA-100 immediately and 1 month after PCI. All patients were followed up for 9 months.

Methods

Study design

The study protocol was approved by the local Ethical Committee and informed consent was obtained from all subjects ($n = 256$) who entered the study after successful PCI. A total of 153 patients underwent PCI in the context of an evolving STEMI ($n = 47$) or within 48 h of admission for NSTEMI ($n = 106$). The remaining patients underwent PCI for stable angina (Table 1). All the patients underwent platelet function evaluation within an average of 30 ± 8 h after PCI and at least more than 24 h after the interruption of IIb/IIIa receptor antagonists (Tirofiban) (T_0) and 1 month later (T_1): at T_0 we identified early poor responders to antiplatelet therapy, whereas at T_1 we verified the compliance to therapy. A cut-off value of 190 s was chosen to define platelet hyperactivity because it has been shown to identify aspirin non-responders at risk for subsequent acute coronary events [7–9]. A p value less than 0.05 is indicated as significant. All the patients received a dual oral antiplatelet regimen (aspirin and thienopyridine) for a planned 6-month period. Clopidogrel was given in the majority of patients (92%), as a loading dose of 300 mg followed by a maintenance dose of 75 mg/day. Ticlopidine was given in the remaining 8% of the patients, at the dose of 500 mg/day. IIb/IIIa receptor antagonists were used in 84 patients with NSTEMI with positive Troponin I before PCI. Assuming from previous data [6] an event rate (death or MI) of 5% in the CEPI-CT < 190 s and 1% in the CEPI-CT > 190 s, the number of patients planned to provide the study with 80% power with a type I error rate equal to 5% was calculated to be 250.

Blood collection

Blood samples for analysis of platelet function were collected into evacuated tubes (Vacutainer, Becton Dickinson) containing 3.8% citrate. Platelet function was evaluated using the Platelet Function Analyzer-100 (PFA-100; Dade Behring) which provides a quantitative measure of primary platelet-related hemostasis at high shear stress [10]. It is a rapid, simple, and reproducible test. A total of 0.8 ml of citrated whole blood is transferred into the reservoir of a disposable test cartridge within 4 h of blood sampling. The anticoagulated blood is warmed to 37 °C and drawn under vacuum through a 200-μm-diameter stainless steel capillary (that mimics a small or stenotic blood vessel) and a 150-μm-diameter aperture in a nitrocellulose membrane coated with collagen and epinephrine (CEPI). In response to the high shear rates of 5000–6000 s^{-1} and the agonists, a platelet aggregate forms that blocks blood flow through the aperture; the time taken to occlude the aperture is reported as the closure time and is measured to a maximum of 300 s [11]. All measurements were done from 1 to 4 h after blood sampling. The reference range in normal subjects was 76–184″. Coefficients of variation for duplicate analysis averaged 15% with a day-to-day variability that was around 10% for both cartridges, as elsewhere reported [12]. It has also been used for monitoring GpIIb/IIIa antagonists in patients undergoing percutaneous transluminal coronary angioplasty [13] and for identifying of aspirin resistance [8].

Table 1 Demographics and clinical characteristics of patients recruited

	Total	CEPI-CT<190 s	CEPI-CT>190 s	*P* value
Number	256	109	147	
Male sex (%)	60	62	58	0.62
Medical history, laboratory and instrumental data				
Diabetes mellitus (%)	26	28	23	0.34
Hypertension (%)	32	29	33	0.46
Smoking habitus (%)	38	42	35	0.42
Hypercholesterolemia (%)	41	45	38	0.51
Diagnosis at admission				
Acute coronary syndromes (%)	60	62	58	0.79
Medications during follow-up (intention-to-treat)				
Aspirin, low dose <160 mg/die (%)	79	75	83	0.73
Aspirin, high dose >160 mg/die (%)	21	26	17	0.66
Thienopyridines (%)	100	100	100	0.96
Warfarin (%)	2	2	2	0.96
Clinical events during 9-month follow-up				
Intrastent thrombosis = composite of death/MI/TIMI 0–1 (%)			0.7	0.04
Re-hospitalization for coronary restenosis (%)	3.9	6.4	2	0.06
Total coronary events	10.1	17.3	4.8	0.01
Major bleedings (%)	1.6	1.8	1.4	0.75
Hemodynamic findings and data				
Coronary artery disease (mean ± SD)	1.68 ± 0.7	1.58 ± 0.7	1.65 ± 0.6	0.66
Number of treated vessels (mean ± SD)	1.36 ± 0.5	1.26 ± 0.5	1.37 ± 0.6	0.54
Cumulative length (mm) of stents implanted (mean ± SD)	38 ± 20	40 ± 20	37 ± 16	0.73
Percentage of medicated stents	47	48	46	0.79
In-hospital adjuvant therapy				
GpIIb/IIIa antagonists (%)	30	32	28	0.85
Fibrinolytic therapy (%)	4	4	4	0.96

Clinical follow-up

Patients' follow-up was obtained by phone interview for a mean period of 9 ± 0.3 months. Non-invasive identification of residual myocardial ischemia was performed by treadmill at 3 and 6 months after PCI. A coronary angiography was repeated in patients with symptomatic ischemia or residual ischemia.

Objectives

The primary end-point was defined as the composite of death or MI or by a thrombolysis in myocardial infarction (TIMI) flow 0 or 1 by angiography at the site of stent implantation. Residual ischemia and target lesion revascularizations were considered as secondary end-points.

Statistics

Comparisons among continuous variables were performed by unpaired Student's *t* test, whereas nominal variables were compared by the Chi-squared test (Statview 5.0.1, SAS Institute). Regressions among continuous variables were calculated by means of simple or multiple (step-wise) regression analysis. Spearman's correlation coefficient was used to test correlation among non-continuous variables. Cumulative survival free of events during follow-up was calculated by means of a Kaplan–Meier analysis (Statview 5.0.1, SAS Institute). The excess of risk related to parametric variables was calculated by a Cox regression. All odds ratio are given with their 95% confidence interval (CI).

Results

Patients characteristics

Demographic and clinical data at the time of recruitment are presented in Table 1. Our population included 256 unselected coronary patients: 40.5% stable angina, 18.3% STEMI, 41.2% NSTEMI at low to intermediate risk. Cardiogenic shock and

Killip class III and IV were excluded. 109 Patients (43%) showed a CEPI-CT<190 s. There were no significant differences between the two groups of patients (CEPI-CT < or >190 s) regarding both clinical characteristics and procedural data.

Clinical end-points

A total of 18 events were identified during FU including 6 IST (primary end-point). Five patients had a subacute IST (during the first 4 weeks following PCI) and 1 had a late IST. The IST-free survival for the patients with CEPI-CT<190 s was 95.4% compared with 99.3% for the patients with CEPI-CT>190 s. The cumulative survival free of IST based on Kaplan–Meier analysis is reported in Fig. 1 (LogRank $\chi^2=4.23$, $p=0.039$). Three of the 5 subacute IST occurred during the first week, whereas the late IST occurred within 4 months of the procedure. Of note, the case of late IST was associated with a spontaneous interruption of the clopidogrel assumption, despite the standard indication given by our center to continue the dual antiplatelet therapy for the first 6 months following PCI.

At 9-month follow-up the overall event-free survival for the patients with CEPI-CT<190 s was 83.6% compared with 96.4% for the patients with CEPI-CT>190 s. The cumulative survival free of total coronary events based on Kaplan–Meier analysis is reported in Fig. 2 (LogRank $\chi^2=6.981$, $p=0.008$).

In Fig. 3 is reported the cumulative survival free of stable angina/ischemia with an angiographic documentation of coronary restenosis: 7 of 10 patients had a baseline CEPI-CT<190 s (LogRank $\chi^2=3.401$, $p=0.065$).

CEPI-CT distribution at T_0 and T_1 is reported in Fig. 4 (panels A and B): we observed a significant prolongation of CEPI-CT (from 208±64 s to 240±59 s, respectively, $p=0.03$), with a percentage of CEPI-CT<190 s reduced from 43 to 25%. In panel C the T_0-T_1 gradient is illustrated, with a mean value of +35±57 s.

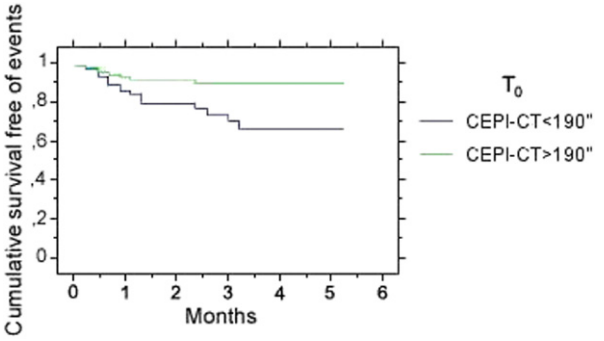

Figure 2 Kaplan–Meier cumulative survival free of MACE, LogRank $\chi^2=6.981$, $p=0.008$.

Independent predictors of IST

Univariate and multivariate analysis by Cox model of regression showed that variables associated with a significant hazard risk for IST were bifurcation lesions and CEPI-CT<190 s at T_0 (Table 2).

Discussion

Our study provides the first evidence that identification of post-PCI platelet hyper-reactivity using the PFA-100 POCT can predict IST.

Identification of high ADP-induced platelet activity using standard optical aggregometry and the VASP test has been shown to correlate with the risk of IST [3]. In the present study, we demonstrate that a POCT test for platelet function, with the advantage of easy reproducibility, is also relevant and reliable. Moreover, although the CEPI-CT by PFA-100 is poorly sensitive to the effect of clopidogrel alone, it is quite sensitive to the effect of dual antiplatelet therapy, aspirin plus a thienopyridine, with an all-or-none modality of response that can be convenient to assess the global effect of combined antiplatelet regimen [14].

Our findings further emphasize the pathophysiologic relationship between the platelet activation

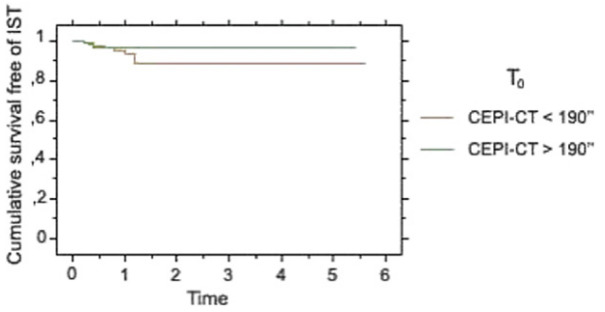

Figure 1 Kaplan–Meier cumulative survival free of IST, LogRank $\chi^2=4.236$, $p=0.039$.

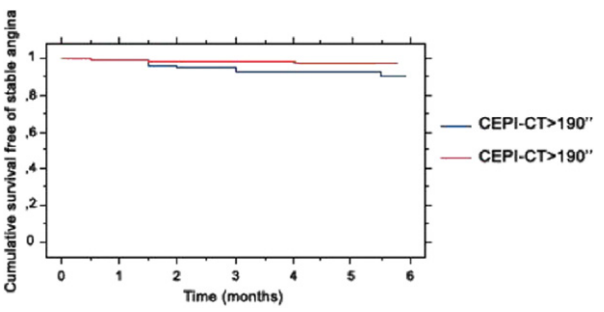

Figure 3 Kaplan–Meier cumulative survival free of stable ischemia/angina, LogRank $\chi^2=3.401$, $p=0.06$.

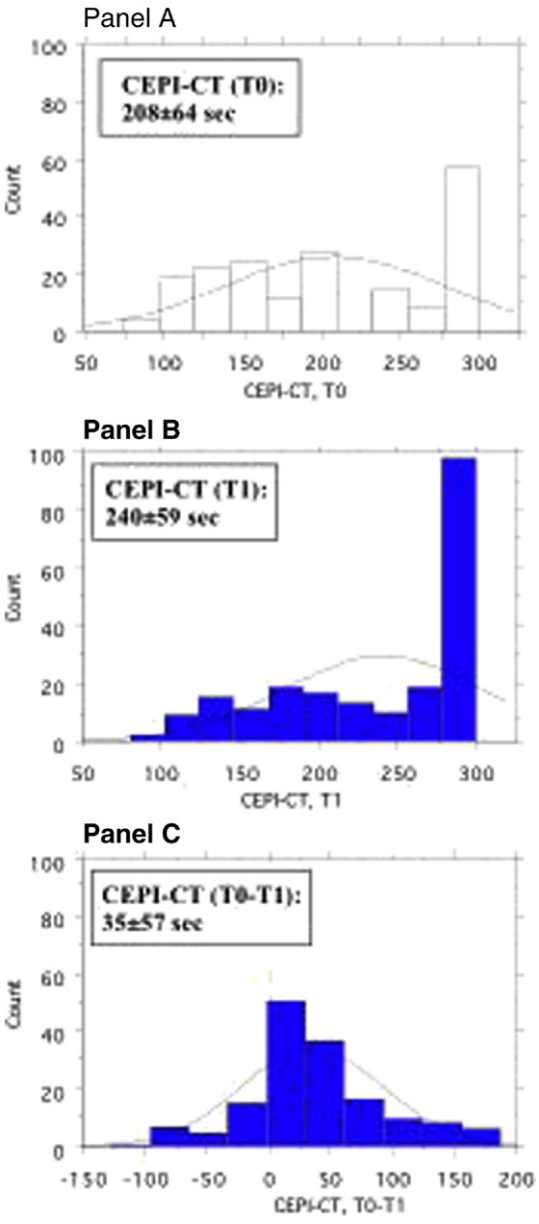

Figure 4 Distribution of the patients for CEPI-CT values, at T_0 (panel A) and T_1 (panel B). In panel C the T_0-T_1 gradient is illustrated.

state and IST, an often devastating complication of otherwise successful PCI, with an hypothetical higher incidence in the era of DES [15,16].

The availability of POC tests in the evaluation of platelet function, such as PFA-100, makes it possible for the risk stratification of patients undergoing an elective or urgent PCI with subsequent recurrent acute coronary event or IST and eventually to adapt the standard anti-thrombotic therapy on the basis of the individual risk profile.

The threshold of CEPI-CT (< or >190 s) we have selected to define platelet hyperactivity is the one accepted to discriminate the state of aspirin resistance [10,11].

We have already noted that patients with a CEPI-CT<190 s after PCI are exposed to higher risk of clinical recurrent events [6]; therefore, this value maybe considered a plausible threshold to establish whether a more aggressive anti-thrombotic strategy is indicated. In our study the rate of baseline CEPI-CT<190 s in patients with recurrent stable ischemia due to coronary restenosis was higher but still not statistically significant ($p=0.06$, Fig. 3) and this result can be justified by the weaker relationship between platelet activation and the process of restenosis. Since most of the total coronary events reported in Fig. 2 were secondary to stable ischemia/angina, occurring after several weeks from PCI, this makes reasonable the weaker relationship with CEPI-CT at T_1.

We also observed that the platelet activation state is significantly time related: the longer the distance from the acute phase post-PCI, the higher the probability to observe a reduced platelet activation. In fact, the rate of aspirin non-responder patients decreased in about 30 days (44.2% vs. 24.8%). The evaluation of the platelet activation state at T_1 loses most of its prognostic value, but it may depend on the very small number of late IST in our population (Table 2). Moreover, the role of PFA-100 in assessing the risk of late IST remains open, since the only late event in

Table 2 Results of time-to-event analysis by Cox model of regression

	Univariate HR (95% CI)	*P* value
Age	1.03 (0.98–1.05)	0.84
Diabetes	1.97 (0.41–5.93)	0.12
Smoking habitus	1.27 (0.34–4.73)	0.68
Hypertension	0.84 (0.4–1.76)	0.64
Cholesterol-LDL	0.81 (0.4–1.68)	0.57
CEPI-CT<190 s at T_0	4.95 (1.63–15.03)	0.01
CEPI-CT<190 s at T_1	1.71 (0.63–4.59)	0.28
CEPI-CT (T_0-T_1)	0.91 (0.42–1.78)	0.76
Biforcation lesions	12.23 (2.76–53.97)	0.001
Renal insufficiency	1.31 (0.603–2.89)	0.61
Ejection fraction	0.73 (0.168–3.178)	0.67

Results of multivariate time-to-event analysis by Cox model of regression

	Multivariate HR (95% CI)	*P* value
Biforcation lesions	57.42 (7.98–412.82)	0.0001
CEPI-CT<190 s at T_0	9.74 (2.22–42.86)	0.002

Legend: CI=confidence interval; HR=hazard ratio; CEPI-CT=collagen epinephrine closure time.

our population was due to the interruption of clopidogrel. The significant prolongation of CEPI-CT after 1 month can be justified by the relationship between the peri-procedural inflammatory response and the pro-thrombotic phenotype [17]: a higher thrombin level may be the pathophysiological link with the state of platelet activation. T_1 measurements were also performed to check the treatment compliance, that was almost complete (98% at 30 days) in our population.

We recruited both acute and stable coronary syndromes, similarly to large cohort studies focusing on the incidence of IST, since the rate of IST after coronary stent deposition is independent on the clinical syndrome leading to PCI [16,17]. The relatively higher rate of IST in our population (2.3%) as compared with other larger registry [18] can be explained by the relatively higher percentage of bifurcation lesion. In fact, we confirmed that the bifurcation lesion is the most powerful predictor of IST, without abolishing the significance of CEPI-CT $<$ 190 s at T_0 in the multivariate model of regression (Table 2). Recent observations report the role of dual antiplatelet therapy interruption as a major risk factor for IST [18,19]: we also observed a case of late IST in a patient who prematurely interrupted clopidogrel assumption; however, we also report a higher risk of IST in patients on standard dual antiplatelet therapy, in case of persistent platelet hyperactivity, as detected by PFA-100.

Conclusions

Even if these data are limited to a relatively small number of cases and require a confirmation by larger multicentric studies, the observation of the significant predictive value of CEPI-CT for the risk of IST suggests the opportunity to stratify the thrombotic risk of patients undergoing PCI with a rapid evaluation of platelet function by PFA-100. If confirmed that PFA-100 may help to target poor responders to dual antiplatelet therapy who might need higher loading dose of clopidogrel, as recently suggested by the ALBION study [20].

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