High On-Treatment Platelet Reactivity Predicts Cardiac Events in Patients with Drug-Eluting Stents

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Abstract

Background: The role of platelet reactivity (PR) tests in the prediction of long-term events in Latin-American patients treated with drug-eluting stents (DES) has not been established.

Objectives: To assess the role of PR tests in the prediction of events after DES implantation.

Methods: From May 2006 through January 2008, 209 Brazilian patients who underwent elective treatment with DES were included. PR was assessed 12 to 18h after the procedure by light transmittance aggregometry with 5μM of ADP. Patients were prospectively followed for up to 4.8 years. Seventeen (8%) individuals were lost to follow-up and the final cohort comprised 192 patients. Receiver operating curve (ROC) was used to determine the best 5μM of ADP cutoff to predict events. The primary endpoint was a combination of cardiovascular death, acute myocardial infarction, definite stent thrombosis, and target-artery revascularization. Cox proportional hazard models were used to determine the variables independently associated with the time to the first event.

Results: The best ADP 5μM cutoff was 33%. One hundred and seven (55.7%) patients had ADP 5μM ≥33%. Event-free survival rate at 1,800 days was 55% vs. 70% for individuals with ADP5 above and below such cutoff, respectively (p=0.001). Independent predictors of time to first event were current smoking (HR 3.49; 95% CI 1.76-6.9; p=0.0003), ADP 5μM ≥33% (HR 1.95; 95% CI 1.09-3.51; p=0.025) and age (HR 1.03; 95% CI 1.0-1.06; p=0.041).

Conclusions: In this study, 55.7% of the patients had high on-treatment platelet reactivity. ADP 5μM ≥33% was an independent predictor of long-term events (Arq Bras Cardiol. 2013;100(3):221-228).

Keywords: Platelet Aggregation; Angioplasty, Balloon, Coronary; Coronary Thrombosis; Drug-Eluting Stents.

Abbreviations

ADP = adenosine diphosphate
AUC = area under the curve
DES = drug-eluting stents
HPR = high on-treatment platelet reactivity
PCI = percutaneous coronary intervention
PRT = platelet reactivity tests
ROC = receiver operating curve

Introduction

In patients treated with drug-eluting stents (DES), dual antiplatelet therapy is mandatory and treatment with clopidogrel and aspirin has been the most frequent regimen used in clinical practice[1]. However, a great number of individuals are poor responders to clopidogrel as assessed by platelet reactivity tests (PRT)[2-4]. A number of studies have demonstrated an association between on-treatment platelet reactivity at a single time point during clopidogrel therapy and the risk of cardiac events in Caucasian patients who have undergone percutaneous coronary intervention (PCI)[5-10]. However, the role of such tests in Latin-American patients treated with DES is not clear. This is an important issue, as response to clopidogrel may be influenced by genetic factors such as P450 polymorphisms[11-15] and the distribution of these genetic polymorphisms may differ among ethnic groups. For instance, the loss-of-function CYP2C19*2 is common in diverse populations and occurs in 24% of Caucasian individuals. Nevertheless, the frequency of this allele is somewhat lower in Mexican-Americans (18%), higher in African-Americans (33%), and markedly higher in Asian populations (51%)[16-18]. Furthermore, the strength of effect of CYP2C19*2 on clopidogrel response may depend on other factors, such as genetic background or environmental exposures, which may differ among ethnicities. Therefore, PRT may have different performances according to the ethnic background.

In this study, we sought to assess the role of on-treatment platelet reactivity measured by optical aggregometry in the prediction of long-term cardiovascular events in Brazilian patients treated with DES.

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Manuscript received June 20, 2012, revised manuscript August 26, 2012, accepted October 209, 2012.

DOI: 10.5935/abc.20130044

Methods

Patient population and study design

This study was approved by the Medical Research Ethics Committee of our hospital and conformed to the Declaration of Helsinki. All patients provided written informed consent. The study was funded by our institution.

This was an observational, prospective cohort. From May 2006 through January 2008, 209 consecutive Brazilian patients who underwent elective PCI with DES implantation in a cardiology hospital were prospectively included. Mean age was 67 ± 10.4 years and 142 (74%) were males. All patients had stable coronary artery disease. The indication of PCI was left to the primary physician's discretion. Baseline clinical data were collected immediately before the intervention. Before PCI, patients were treated with clopidogrel according to our institution protocol. Clopidogrel-naive patients were treated with a loading dose of 600 mg 2 h before the procedure. Patients who were already on clopidogrel for at least five days did not receive the loading dose. The type of stent was Cypher in 117 (56%) patients, Taxus in 86 (41%), Endeavour in 10 (4.7%), and others in 6 (2.8%) individuals.

Platelet reactivity was assessed at a single-time point in all patients, 12 to 18 h after PCI by light transmittance aggregometry with 5μM of ADP. At discharge, all patients were on aspirin 100 mg and clopidogrel 75 mg per day and were maintained on this regimen for at least one year. All decisions regarding therapeutic issues were left at the discretion of the primary physician.

Follow-up was achieved by telephone interviews and was complemented with information from primary physicians and hospital records. Data on clinical status and on drug regimen, including the use of clopidogrel, were collected. Patients were followed for up to 4.8 years (mean 2.2 ± 1.2 years). Seventeen (8%) patients were lost to follow-up and the final cohort comprised 192 patients.

Platelet function analysis

Blood samples (5 tubes with 4.5 mL) were collected from all patients 12 to 18 h after PCI. Platelet function was assessed by light transmittance aggregometry. Platelet-rich plasma was prepared by centrifuging blood samples at 250 x g for 10 min. Aggregation studies were performed with a Chrono-log optical aggregometer (Chrono-log Corporation, USA) and the AGGRO/LINK® 810-CA software for Windows. ADP 5μM and arachidonic acid (1 μM) were used as agonists.

Endpoints determination and statistical analysis

The primary endpoint was a combination of cardiovascular death, nonfatal myocardial infarction, definite stent thrombosis, and percutaneous target-artery revascularization. Definite thrombosis was considered as acute coronary syndrome with an angiogram showing stent thrombosis. According to time from PCI, it was classified into acute (≤ 24 h), subacute (1 to 30 days), late (1 to 12 months), and very late (> 12 months).

Categorical variables are expressed as frequencies and percentages. Continuous variables were analyzed for a normal distribution with the Kolmogorov-Smirnoff test. Normally distributed variables are presented as mean ± standard deviation. Receiver operating curve (ROC) was used to determine the optimal ADP 5μM cutoff to predict events. Kaplan-Meier event-free survival curves were constructed and log-rank test was used to assess differences between curves. Cox proportional hazard models were used to determine the variables independently associated with time to first event. Covariates included in this analysis were age, gender, history of hypertension, history of diabetes mellitus, history of dyslipidemia, current smoking status, prior myocardial infarction, prior coronary artery bypass graft, prior PCI, aspirin, beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, statins, periprocedural clopidogrel regimen, use of clopidogrel for more than 1 year after PCI, stent implanted in the left anterior descending (LAD) coronary artery, multivessel PCI, number of stents implanted, minimal stent diameter, total stent length, arachidonic acid, and ADP 5μM. Variables were selected using the stepwise forward method. A p value < 0.05 was considered statistically significant. The analysis was performed using the statistical software SAS® System, version 6.11 (SAS Institute, Inc., Cary, North Carolina).

Results

ADP 5μM optimal cutoff as determined by ROC curve analysis was 33%, with the area under the curve (AUC) of 0.58 (95% confidence interval, 0.49-0.67). Sensibility, specificity, positive predictive value and negative predictive value were, respectively, 66.7%, 48.2%, 31.8%, and 80%. Using this cutoff, high on-treatment platelet reactivity (HPR) was present in 107 (55.7%) out of 192 patients included in the analysis. Baseline characteristics of patients with HPR as compared with patients with low platelet reactivity are shown in Table 1. Patients with HPR were more likely to have prior myocardial infarction and were more frequently on beta-blocker therapy at the moment of PCI. The majority of the patients used clopidogrel for more than 1 year after PCI.

During follow-up, 51 (26.5%) patients reached the primary endpoint. Mean ADP 5μM was higher in these patients as compared with those without endpoints (37 ± 13.5 vs. 32.8 ± 14.1, p = 0.06). The cardiovascular events observed were 34 (17.7%) target-artery percutaneous revascularizations, 23 (12%) acute myocardial infarctions, 10 (5.2%) cardiovascular deaths, and 5 (2.6%) definite stent thrombosis. Median time to first event was 371 days (interquartile range 180-732 days). Of note, 51% of the events occurred after one year of follow-up. Acute stent thrombosis was observed in 2 patients, subacute in 1, and very late in 2 patients (2 and 3.6 years post-stent implantation). Among these 5 cases, 3 (60%) resulted in death. All patients who experienced stent thrombosis were on dual antiplatelet therapy at the moment of the event. In all but one patient ADP 5μM was above the optimal cutoff, indicating HPR. The only patient with ADP 5μM below the cutoff had acute stent thrombosis.

Event-free survival rates were lower for current smokers and patients with HPR (Figures 1 and 2). Covariates independently associated with the primary endpoint in the final Cox multivariate regression model were current smoking, ADP 5 μM ≥ 33%, and age (Table 2).

Table 1 - Baseline characteristics of patients with and without high on-treatment platelet reactivity (ADP 5 µM ≥33%)

Variables	ADP 5 µM ≥33% n=107	ADP 5 µM <33% n=85	p value
Age (years)	67.5 ± 11.2	66.9 ± 9.8	0.69
Male gender	80 (74.7%)	62 (72.9%)	0.77
Hypertension	81 (75.7%)	58 (68.2%)	0.25
Diabetes mellitus	34 (32.4%)	21 (24.7%)	0.24
Dyslipidemia	78 (72.9%)	60 (70.6%)	0.72
Current smoking	16 (15%)	8 (9.4%)	0.24
Prior myocardial infarction	30 (28%)	13 (15.3%)	0.035
Prior CABG	29 (27.1%)	17 (20%)	0.25
Prior PCI	40 (37.4%)	30 (35.3%)	0.76
Aspirin	97 (90.7%)	70 (84.3%)	0.18
Beta-blocker	68 (63.5%)	38 (44.7%)	0.027
ACE inhibitors/ARB	63 (58.8%)	51 (60%)	0.82
Statins	85 (79.4%)	64 (75.2%)	0.47
Loading dose clopidogrel	59 (55.1%)	46 (54.1%)	0.88
Use of clopidogrel >1 year	101 (94.4%)	81 (95.3%)	0.96
Stent implanted in LAD	58 (54.2%)	40 (47%)	0.32
Multivessel PCI	44 (41%)	41 (48.2%)	0.51
Number of stents implanted	2.08 ± 0.99	2.16 ± 0.15	0.86
Minimal stent diameter (mm)	2.72 ± 0.44	2.61 ± 0.43	0.10
Total stent length (mm)	25.5 ± 6.7	25.7 ± 7.4	0.83
Arachidonic acid (%)	5.63 ± 4.5	5.66 ± 7.98	0.11
ADP 5µM (%)	43.6 ± 9	21.6 ± 8.6	-

ACE: angiotensin-converting enzyme; ADP: adenosine diphosphate; ARB: angiotensin-receptor blocker; CABG: coronary artery bypass graft surgery; LDA: left anterior descending coronary artery; PCI: percutaneous coronary intervention.

Table 2 - Influence of covariates on primary endpoint in the final Cox multivariate regression model

Variable	Adjusted Hazard ratio	95% CI	p value
Current smoking	3.49	1.76 - 6.9	0.0003
ADP 5µM ≥33%	1.95	1.09 - 3.51	0.025
Age	1.03*	1.00 a 1.06	0.041

ADP: adenosine diphosphate; CI: confidence interval.
** For each 1-year increment.*

Discussion

The primary finding of the present study was that HPR as measured by light transmittance aggregometry was independently associated with long-term cardiovascular events in Brazilian patients with stable coronary artery disease who underwent PCI with DES implantation. The optimal cutoff level for ADP 5μM in this analysis was 33%. Our study is the first one to include only Latin-American patients from Brazil and confirmed that PRT are useful in predicting long-term outcomes in this scenario as demonstrated with Caucasian patients.

Platelet function can be assessed by different methods. Light transmittance aggregometry has been used by other authors to address the value of such test in the prediction of cardiovascular events after coronary stent implantation[5-7]. These studies demonstrated that platelet function assessment was able to identify high-risk patients. However, they only provided information after 30-day, 6-month, and 1-year follow-up. In this regard, our study adds information to the previous studies using light transmittance aggregometry, as it provides data on long-term follow-up, demonstrating that PRT can identify patients at

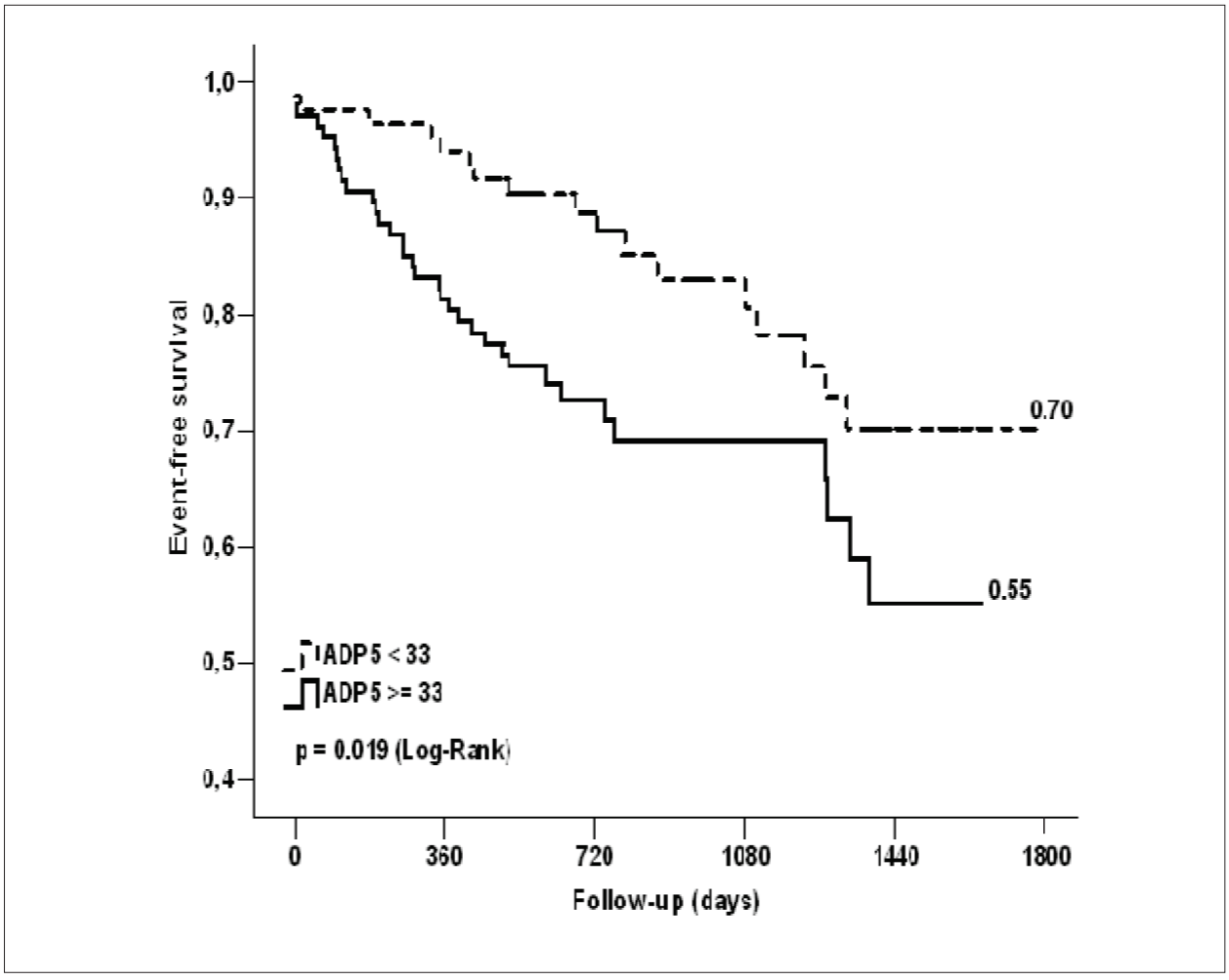

Figure 1 - *Kaplan-Meier event-free survival curve in patients with and without high on-treatment platelet reactivity (ADP 5 μM ≥33%).*

risk beyond one year after DES implantation. More recently, a new method used to assess platelet function has been introduced. The Multiplate Analyser is a point of care test that has been shown to be superior to the conventional light transmittance aggregometry[19].

Head-to-head comparison of platelet function tests has been reported in only one study. The POPULAR study[6] compared the ability of six different PRTs in predicting atherothrombotic events in clopidogrel-pretreated patients undergoing PCI with stent implantation. Only light transmittance aggregometry, VerifyNow, and Plateletworks were significantly associated with the primary endpoint. The IMPACT-R, Dade PFA collagen/ADP, and Inovance PFA P2Y were unable to discriminate between patients with and without events at 1-year follow-up. Of note, although the 3 former tests were able to identify patients at risk, the area under the curve was modest (0.63, 0.62, and 0.61, respectively). In the present study this was also true. The area under the curve for ADP 5μM >33% was only 0.58. Likewise, we observed a good negative predictive value, but low positive predictive value as was also observed in the POPULAR study. A possible explanation for this finding is that in all these studies, including ours, platelet function was assessed in a single time point. As a matter of fact, it has been demonstrated that in patients on clopidogrel, platelet function varies over time and, therefore, serial measurements improve the accuracy of these tests[20]. Using the VerifyNow P2Y12 assay, Campo et al. demonstrated that platelet reactivity decreased from baseline to one month in patients on clopidogrel, improving the area under the curve from 0.69 at baseline to 0.87 at 30 days. On-clopidogrel platelet reactivity at 1 month was the strongest predictor of adverse outcomes[20].

Another concern regarding platelet function measurements in this setting refers to the optimal cutoff level for event prediction. Some studies have used prespecified cutoffs, while others have used ROC curves to determine the optimal cutoff. Two studies using ADP 5μM have suggested cutoffs of 42.9% and 46% based on ROC curves[6,21]. We found a lower cutoff of 33%. This may be explained by a longer follow-up in our study and differences in disease severity. Furthermore, recent studies have found lower cutoffs than those suggested by previous studies. For example, in the GRAVITAS study, the VerifyNow P2Y12 test was used to identify patients with HPR[22]. A prespecified cutoff of 240 PRU

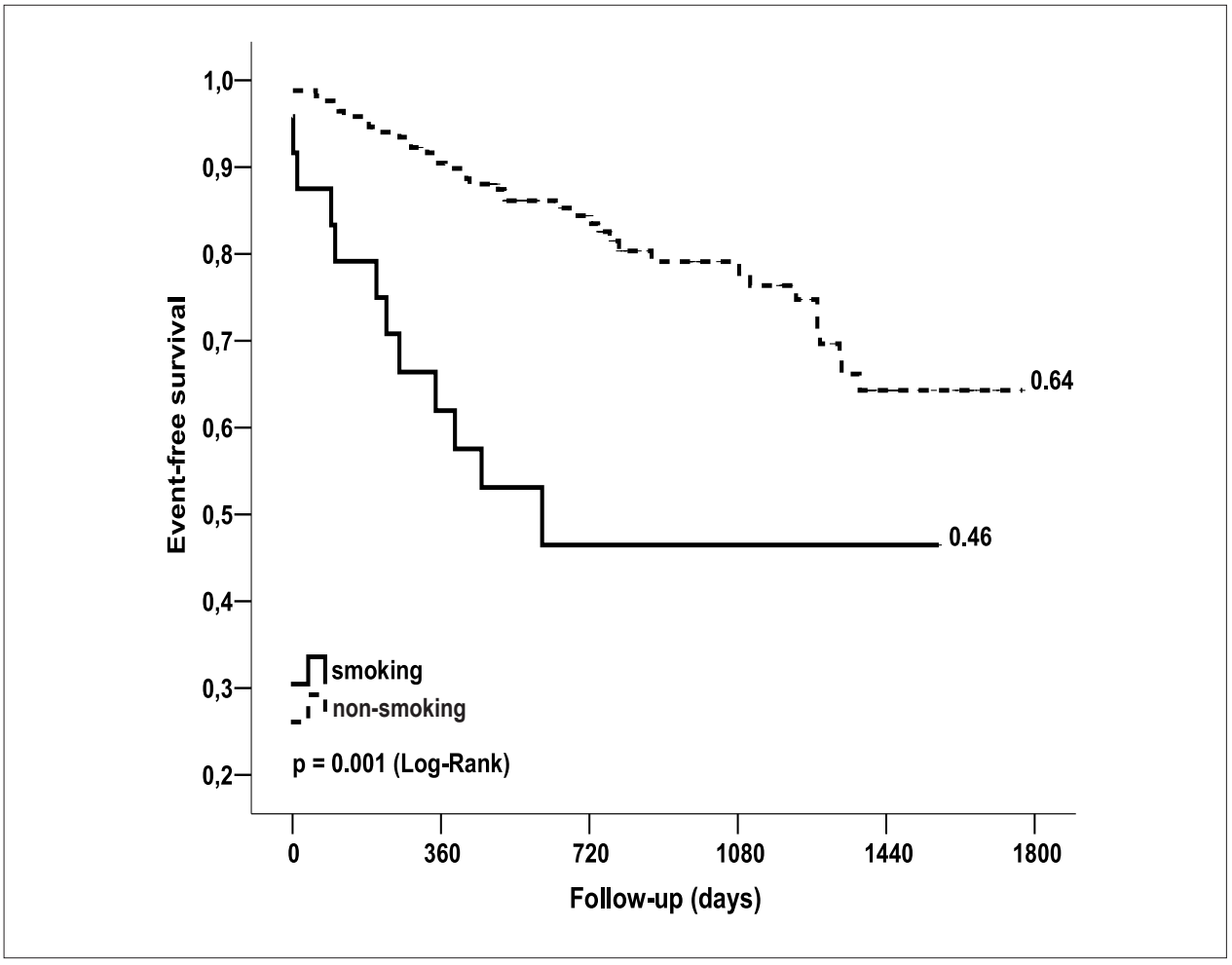

Figure 2 - *Kaplan-Meier event-free survival curve in current versus non-smokers.*

was used based on previous data[23]. Patients with HPR were randomized to either a fixed regimen of high-dose clopidogrel (600 mg followed by 150 mg daily for 6 months) or the standard-dose clopidogrel (75 mg daily). No differences in outcomes were observed between the two regimens. However, in a subanalysis of this study, the prespecified cutoff of 240 PRU was not associated with ischemic events in the follow-up. On the contrary, a post-hoc cutoff of 208 PRU assessed at 12 to 24 h after PCI or at 30 days was associated with the primary endpoint[24]. Of note, using this lower cutoff, the proportion of patients with HPR was very similar to the one observed in our study (49.6% and 55.7%, respectively).

Although most of the studies have shown that platelet reactivity tests are useful in predicting events in patients treated with stent implantation, a recent study using the VerifyNow P2Y12 assay failed to show any benefit[25]. A possible explanation for this finding is that event rates were lower than expected. Two-year event rates in patients with and without HPR were 2.8% and 2.4%, much lower than that observed in our study. This could also be related to racial or ethnic differences, since this study was carried out in an Asian population, in contrast with a predominantly western population in other studies.

Half of the endpoints in our study occurred after 1 year of follow-up. This is an important finding, as dual antiplatelet therapy in patients with DES has been recommended for up to 1 year. We demonstrated that many events still occur after that time point and patients at risk, despite the use of aspirin and clopidogrel, can be identified by PRT. Among 5 definite stent thromboses, 2 occurred at 2 and 3.6 years post-PCI, in patients on dual antiplatelet therapy. On the other hand, a transitory 'rebound' increase in platelet reactivity within 3 months after clopidogrel discontinuation has been observed[26] and may explain the increase in ischemic events that occur early after clopidogrel discontinuation[27,28], although some believe that these events are rather a result of late stent epithelization[29]. The ideal time on dual antiplatelet therapy post-stent implantation is a matter of debate and deserves further studies. Nevertheless, we had the unique opportunity to study patients who were on clopidogrel for more than 1 year after the intervention, at the discretion of their primary physicians. Our data suggest that patients with HPR have increased risk of events even if they persist on clopidogrel beyond 1 year post-PCI.

The strongest predictor of cardiovascular events in this study was current smoking. Although smoking has been related to late stent thrombosis[29,30], few studies have addressed the association between smoking, platelet function analysis, and prognosis in this scenario. In some studies, a greater proportion of smokers was observed in patients with low platelet reactivity[5,22]. In others, the opposite[7] or no difference was observed[6]. However, the so-called smoking paradox has been described. According to this hypothesis, smoking stimulates the CYP1A2 at the cytochrome P450, resulting in higher production of the active clopidogrel metabolite and less stent thrombosis[31]. Our findings do not support this theory. Most importantly, in our study ADP 5μM>33% predicted cardiac events regardless of current smoking.

The best strategy for the management of patients with high on-treatment platelet reactivity is not clear. The strategy of fixed regimen of high-dose clopidogrel, tested in the GRAVITAS STUDY, was not superior to the standard-dose regimen[22]. Alternatively, one may choose one of the new antiplatelet drugs, prasugrel or ticagrelor, which have been found to be superior to clopidogrel in clinical trials, in the context of acute coronary syndromes[32,33].

Although most authors agree that currently available evidence supports the concept of a threshold for on-treatment platelet reactivity that may be used to stratify patient risk to ischemic events following PCI[34], guidelines have not recommended platelet function tests on a routine basis[1,35-37]. This may be due to some drawbacks related to these tests. There have been no large-scale clinical trials to date demonstrating that the adjustment of antiplatelet therapy based on any of these cutoffs improves clinical outcomes. Additional issues are the lack of consensus on the optimal method to evaluate HPR and the cutoff value associated with clinical risk. However, we believe such tests are of particular importance in patients who have suffered an acute coronary syndrome. The guidelines recommend that these patients be kept on dual antiplatelet therapy ideally for one year. In fact, the incidence of very late thrombosis in the HORIZONS-AMI[30] study was elevated, regardless the type of stent, justifying monitoring these patients with PRT overtime.

Study limitations

This study has some limitations. First, this is a relatively small single-center study and one should generalize our findings with caution. In spite of that, the study had enough power to detect differences between groups due to the very long follow-up. We did not collect data on bleeding events, which are important endpoints related to mortality. We did not perform serial measurements of platelet function, which has been shown to improve the test accuracy. And finally, our data refers mostly to first-generation stents and may not apply to new generation stents.

Conclusions

In this study, a cutoff value of 33% for ADP 5μM was associated with long-term events in Brazilian patients with stable coronary artery disease treated with DES. Using this cutoff, 55.7% of patients had high on-treatment platelet reactivity. Current smoking was the strongest predictor of cardiovascular events, followed by HPR and age. HPR predicted long-term events independently of clopidogrel maintenance beyond 1 year.

Acknowledgement

This work was supported by our institutions and no external financial source was provided.

Author contributions

Conception and design of the research: Villacorta AS, Villacorta Junior H, Helmuth B; Acquisition of data: Villacorta AS, Batista MJS, Gomes RV, Macedo LA; Analysis and interpretation of the data: Villacorta AS, Villacorta Junior H, Mesquita ET; Statistical analysis: Villacorta Junior H; Writing of the manuscript: Villacorta AS; Critical revision of the manuscript for intellectual content: Villacorta Junior H, Batista MJS, Gomes RV, Mesquita ET, Helmuth B.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Aline Sterque Villacorta, from Universidade Federal Fluminense.

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