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ORIGINAL ARTICLE

Long-term serial changes in platelet activation indices following sirolimus elution and bare metal stent implantation in patients with stable coronary artery disease

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Abstract *Background:* Platelet activation is crucial in the development of stent thrombosis following percutaneous coronary intervention (PCI). We carried out a long-term assessment of multiple factors implicated in the thrombotic process and monitored markers of platelet activation after the implantation of sirolimus-eluting stents (SES) in patients with stable coronary artery disease (CAD). Additionally, we compared these findings with those after bare-metal stent (BMS) implantation.
Methods: A cohort of 47 consecutive patients, aged <70 years, with severe stenosis (>70% narrowing of the lumen) of one major epicardial coronary artery and stable CAD underwent successful elective PCI. Patients were randomly allocated to SES (n = 25) or BMS (n = 22). Venous blood was obtained 24 hours before and 24 hours, 48 hours, 1 month, and 6 months after PCI for measurements of plasma levels of sP-selectin, von Willebrand Factor (vWF), fibrinogen, d-dimer, sCD40, factor VIII, b-thromboglobulin (b-TG) and platelet factor 4 (PF-4).
Results: There were no significant differences between the two groups in levels of fibrinogen or d-dimers in peripheral blood. However, we observed a significant kinetic effect ($p < 0.001$) and stent-effect ($p < 0.015$) on vWF levels and a significant kinetic effect ($p = 0.012$) on factor

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VIII, sP-selectin ($p = 0.04$), b-TG ($p < 0.001$), and PF4 ($p = 0.016$). A trend towards a significant stent effect on sCD40 was also detected ($p = 0.06$).
Conclusions: SES and BMS did not show significant differences in relationship to markers of platelet activation and coagulation in patients with stable CAD. Although some markers showed an increase after stent implantation, they returned to the initial levels 6 months later.

Although drug-eluting stents (DES) are associated with significantly lower rates of in-stent stenosis compared with bare-metal stents (BMS), early and late stent thrombosis remains an issue.[1,2] Stent thrombosis is a rare but potentially fatal complication of percutaneous treatment of coronary artery disease (CAD). In patients undergoing coronary stenting, antithrombotic drugs are needed to prevent intraluminal thrombus formation. Although these patients routinely receive dual antiplatelet treatment to reduce the risk of developing stent thrombosis, this issue remains a dramatic complication following stent implantation; it is estimated that the rate of acute and subacute thrombosis after percutaneous coronary intervention (PCI) with stent implantation may reach 16% in high-risk patients.[3]

One of the most important and most frequently used drugs in DES is sirolimus. Notably, it has been reported that sirolimus significantly potentiates agonist-induced platelet aggregation in a time- and dose-dependent manner.[4] Additionally, DES are associated with delayed endothelization, a condition that predisposes individuals to thrombosis, and for this reason, a longer period of antiplatelet therapy is mandated compared to BMS. However, the underlying mechanisms are not yet entirely clear, and the influence of these two types of coronary stent on platelet activation and the blood/hemostatic system has not yet been fully elucidated.

Platelet activation is crucial in the development of stent thrombosis following PCI. Herein, we assessed the behavior of platelet activation markers and other factors implicated in the thrombotic process after DES implantation in patients with stable CAD. We compared those results with data from patients following BMS implantation. The DES used were sirolimus-eluting stents (SES), while the levels of sCD40, fibrinogen, factor VIII, von Willebrand factor (vWF), d-dimer, sP-selectin, platelet factor 4 (PF-4), and beta-thromboglobulin (b-TG) were used as indices of thrombogenesis in peripheral blood.

1. Methods

We enrolled 47 consecutive patients aged <70 years with severe stenosis (>70% narrowing of the lumen) of one major epicardial coronary artery and stable CAD who had undergone successful elective PCI. Patients were randomly allocated to SES ($n = 25$) or BMS ($n = 22$). Exclusion criteria were as follows: in-stent restenosis, disease of the left main coronary artery, chronic total occlusion (>3 months), bifurcation stenting, adjacent stented segments >3 cm, periprocedural complications, prior PCI or bypass surgery; ejection fraction <55%; allergy to aspirin, heparin, or clopidogrel; significant valvular disease; pregnancy, myocarditis, a history or signs of neoplastic or hematological disease; heart, renal or hepatic failure; history of any inflammatory disease during the last 6 months; and being a heavy smoker. Other exclusion criteria included a personal or family history of bleeding disorders or thrombotic events, hematocrit levels <35% or >50%, and platelet counts <150,000/μL or >500,000/μL.

Cardiovascular medications were not discontinued during the study and treatment remained unchanged throughout the study period. All patients were on dual antiplatelet therapy with 100 mg of aspirin and 75 mg of clopidogrel. Clopidogrel loading (300 mg) was administered one day before coronary intervention, while patients were on aspirin for at least 7 days before the procedure.

Blood samples were obtained 24 hours before PCI, at 24 and 48 hours after, and then on the first visits 1 and 6 months after coronary intervention. Study subjects were asked to refrain from eating food and drinking alcohol or coffee for 12 hours before each blood sampling. All studies were performed between 9:00 and 11:00 a.m., and all participants rested for >30 min in a supine position. A medical history was obtained and a full clinical examination was performed at each visit.

All participants provided written informed consent for participation in the study. The ethics committee of our hospital approved the study protocol.

2. Biochemical assays

Venous blood was obtained atraumatically 24 hours before, and 24 hours, 48 hours, 1 month, and 6 months after PCI. Blood was used to measure the levels of plasma sP-selectin, vWF, fibrinogen, d-dimer, sCD40, factor VIII, b-TG and PF- 4. Blood samples were immediately centrifuged at 3000 rpm for 20 minutes at 4°C, and serum and plasma were separated and stored at −80°C until later analysis.

The levels of sP-selectin, sCD40, b-TG and PF- 4 were measured by enzyme-linked immunosorbent assay (ELISA, R&D Systems, Abingdon, United Kingdom) using commercial reagents, and the results were reported in nanograms per milliliter. The quantitative assay of vWF was based on the immunoturbidimetric determination of levels of vWF antigen (vWF Ag, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Fibrinogen was measured using the Clauss method with some modifications (Multifibren U, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The results were reported in grams per liter.

D-dimer levels were evaluated using a fully automated quantitative D-dimer assay—the particle-enhanced immunoturbidimetric assay Innovance D-DIMER (Siemens Medical Solutions, Marburg, Germany).

3. Statistical analysis

Summary descriptive data are presented as the means ± SD or frequency (%), as appropriate. Repeated-measures ANOVA was used to assess the kinetics (the within factor with 5 levels) of various parameters that were implicated in the thrombotic process and to compare the drug-eluting and bare-stent groups (the between factor with 2 levels). Post hoc Bonferroni-adjusted tests were employed in cases of overall significant findings to pinpoint differences. All statistical tests were performed at the two-sided 5% level of significance with the IBM-SPSS 21 statistical software package.

4. Results

Demographic and clinical data, angiographic findings, and procedural variables were similar between the two groups (Table 1). Fig. 1 shows serial changes of the measured parameters in the two groups, SES and BMS, between the study time points both before and after stenting. All baseline measurements were comparable between the two groups.

Table 1 Demographic and clinical data, angiographic findings and procedural variables in the BMS and SES groups.

	BMS (n = 22)	SES (n = 25)	p
Age (years)	67 ± 10	68 ± 11	NS
Sex (male/female)	14/11	12/10	NS
Smoking	11	14	NS
Diabetes mellitus	12	13	NS
Hypertension	15	10	NS
Hyperlipidemia	18	20	NS
Medication			
Beta-blockers	15	18	NS
ACE-i	11	10	NS
Statins	17	18	NS
Target vessel			
Left-anterior descending artery	15	16	NS
Right-coronary artery	5	5	NS
Circumflex	2	4	NS
Angiographic parameters			
Vessel size (mm)	2.8 ± 0.4	2.9 ± 0.5	NS
Minimum luminal diameter before stenting (mm)	0.9 ± 0.4	1.0 ± 0.3	NS
Stent length (mm)	29 ± 18	27 ± 20	NS
Minimum luminal diameter after stenting (mm)	2.9 ± 0.3	2.8 ± 0.4	NS

BMS: bare metal stents; SES: sirolimus-eluting stents; ACE-i: angiotensin-converting enzyme inhibitors.

Our analysis did not reveal any significant differences between the two stent groups in the levels of fibrinogen and d-dimers in peripheral blood. However, repeated-measures ANOVA revealed a significant kinetic effect ($p < 0.001$) and stent-effect ($p < 0.015$) on vWF levels and a significant kinetic effect ($p = 0.012$) on levels of factor VIII, sP-selectin ($p = 0.04$), b-TG ($p < 0.001$), and PF-4 ($p = 0.016$). A trend towards a statistically significant stent effect on sCD40 was also detected ($p = 0.06$; Fig. 1). Our analysis did not have sufficient statistical power to detect kinetic stent interactions between groups.

The results of the post hoc tests showed that vWF levels in the SES group were higher at 24 and 48 h compared to baseline ($p = 0.06$ and 0.02, respectively). At one and 6 months, there was a significant decline from the 48 h levels to levels slightly, but not significantly, above baseline. In contrast, no significant increase was observed in the BMS group. Additionally, patients with SES showed a trend towards a significant increase in factor VIII levels at 1 month and sP-selectin levels at 6 months ($p = 0.08$). Moreover, PF-4 showed a significant increase at one month ($p = 0.01$) only in the BMS group, and those levels returned to baseline at 6 months. Finally, b-TG also showed a significant increase at 48 h in the BMS group alone, and this difference remained significant at one month ($p < 0.01$ for both) but returned to baseline at 6 months.

Clinical parameters such as smoking, diabetes mellitus, hypertension, hyperlipidemia, and stent length did not significantly affect the time course of the parameters measured for either stent type.

5. Discussion

In this study, we investigated for the first time the changes over a long period of time in multiple markers of coagulation and platelet activation in the peripheral blood of patients with stable CAD who were undergoing elective PCI. We compared the effects of SES or BMS implantation. We found that the majority of factors measured did not show any substantial differences in behavior between the two stent groups. Our analysis did not reveal any differences in levels of fibrinogen and d-dimers in peripheral blood between the two stent groups. However, there was a trend towards increased levels of sCD40, vWF, factor VIII and sP-selectin after SES implantation, whereas PF-4 and b-TG levels intermittently showed a higher elevation during the study in the BMS group alone.

Increased activation of platelets has been a consistent finding after coronary stent implantation[5] and previous studies have reported changes in the expression of coagulation factors and platelet activation.[6,7] These trends could be modified by the selection of antithrombotic regimens. Similarly, we hypothesized that the drug eluted by SES could influence platelet activation and thrombogenic factors after implantation, and this possibility had not been previously assessed. Our study was the first to simultaneously examine many factors and over a long follow up after SES implantation and to compare our results with those from BMS in patients with stable CAD.

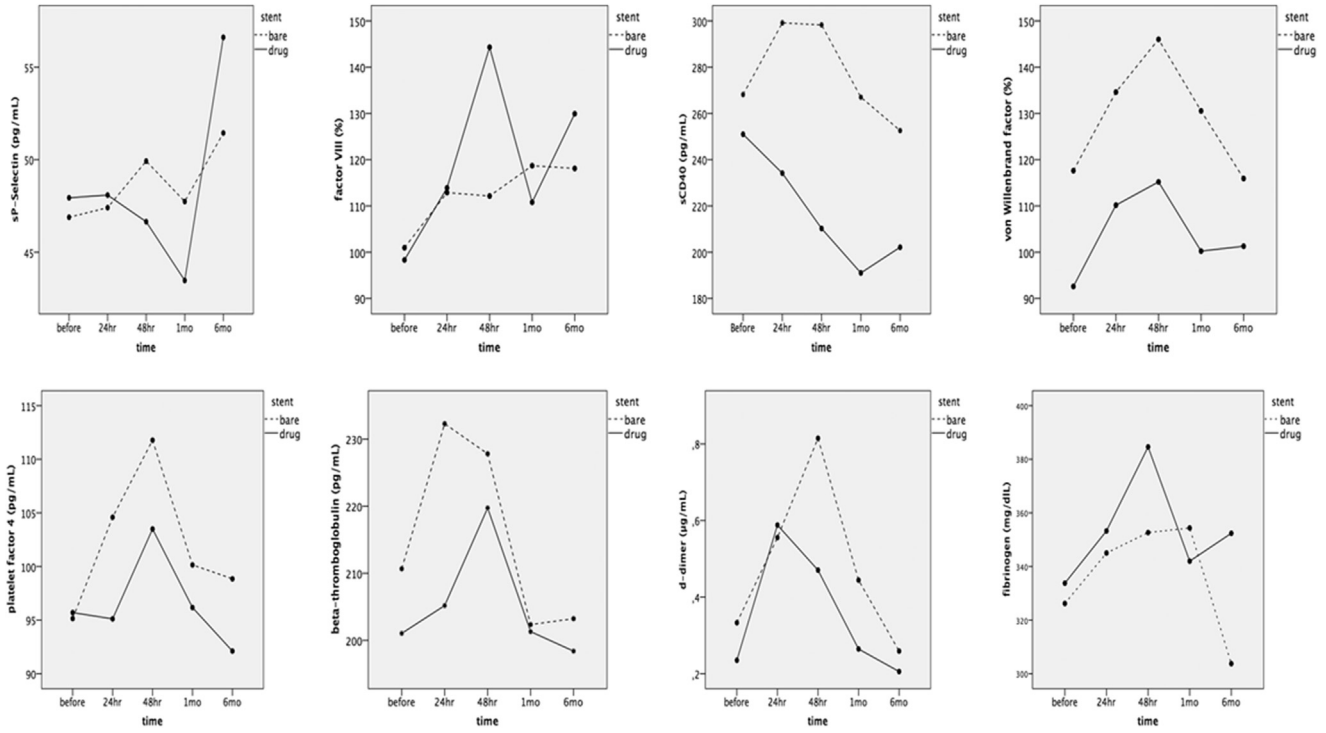

Figure 1 Serial time changes in coagulation and platelet activation indices in peripheral blood from patients.

Coronary thrombosis has long been recognized as a rare, but catastrophic complication after stent implantation. Stent thrombosis may lead to acute myocardial infarction and death, and it has been reported that 90% of these patients require an emergency reperfusion procedure.[3] In patients undergoing coronary stenting, antithrombotic drugs are needed to prevent intraluminal thrombus formation. Although these patients routinely receive dual antiplatelet treatment to reduce the risk of stent thrombosis, it continues to be, albeit in a small number of patients, a major complication following stent implantation. The creation of in-stent thrombosis initially begins with platelet adhesion, while at the same time the fibrinolytic and anticoagulant capability of the body is deficient as a result of factors that regulate the vascular endothelium. Following its formation, the thrombus undergoes endothelization and becomes perfused by leukocytes and monocytes, while smooth muscle cells also migrate to the same site.[8] Consequently, the onset, development, and formation of the thrombus within the stent, which can also cause restenosis, are a complication in which various factors are involved, influencing platelet adhesion, endothelial function, coagulation, and fibrinolysis. Various conditions, such as incorrect stent deployment or a poor choice of size, a small stent, small vessel, bifurcation of the vessel, implantation of multiple stents, eccentric lesions, acute coronary syndrome, low ejection fraction, or subtherapeutic antiplatelet medication,[9–12] are considered to be risk factors for thrombosis. However, there are some patients who show none of the above factors.

An understanding of the mechanisms and pathophysiology of stent thrombosis is critical to improve its treatment and prevention. The extent to which SES, which has been proved to reduce in-stent restenosis, tends to increase the incidence of thrombosis has been the subject of debate. Since thrombosis may not appear until late after stent implantation, we decided to conduct this long-term study.

The thrombogenicity of DES is the subject of debate and seems to result from a series of complex interactions that involve the presence of a thrombogenic surface and platelet activation. In a large cohort of unselected patients undergoing coronary stenting, there was a significant excess risk of stent thrombosis at 3 years for first-generation DES compared with BMS, which was driven by an increased risk of stent thrombosis events beyond 1 year. Second-generation DES was associated with a similar risk of stent thrombosis compared with BMS.[13] Another pooled analysis that included over 5000 patients in trials with drug-eluting stents showed similar rates of stent thrombosis in patients receiving BMS or DES.[14] However, little is known about the pathophysiology or mechanisms underlying this response, and changes in blood coagulation and the fibrinolytic system following stent implantation are of great interest. Platelet and thrombin activation are important factors in the development of stent thrombosis.[15] Stent thrombosis is a complex phenomenon that has not yet been fully elucidated. Herein, we investigated whether sirolimus may enhance thrombogenicity by activating platelets and coagulation proteins.

A previous in vitro study showed that there was no substantial difference in the thrombogenicity of BMS and SES.[16] However, that study compared the earliest effects of these two types of stent, so conclusions about their long-term effects cannot be drawn. We studied the long-term

thrombogenic effects of BMS and SES after implantation. Although we found small differences in some factors, they were not sufficient to have a major impact on the clinical outcome with respect to thrombotic events.

We demonstrated that patients with stable CAD undergoing SES implantation had significantly higher levels of vWF in peripheral blood immediately after the procedure compared with patients receiving BMS. The role of vascular endothelium in the pathogenesis of thrombus formation has been recognized for a long time.[17] Plasma vWF is a critical factor in the endothelium, and its increased levels suggest endothelial dysfunction.[18] Additionally, vWF can be released rapidly and locally at the arterial injury site and can mediate platelet adhesion to the exposed subendothelium.[19] Additionally, we found a trend towards increased levels, and in the later phases, the levels of factors that are related to platelet activation, such as sP-selectin, sCD40, and factor VIII, also increased. Previous studies found no such differences, perhaps because they were limited to the early phases following implantation.[16,20]

Additionally, we used substances degranulated from platelets as markers for platelet disruption and activation, and detected a kinetic effect of b-TG and PF-4 levels, especially in patients who had undergone BMS implantation. Although the reason for this elevation is unclear, it might be attributed to observations from experimental studies that suggested that sirolimus attenuate vascular wall inflammation and the release of substances such as b-TG following angioplasty.[21]

We did not detect any significant differences in d-dimers or fibrinogen levels between the two groups. D-dimers are markers of active fibrinolysis, and any differences in the levels of these markers would be accompanied by profound differences in clinical outcomes, which we did not observe.

6. Limitations

The sample size in this study was relatively small; however, we believe that our findings are representative of what occurs in these patient populations. We cannot exclude the possibility that the combination of dual antiplatelet agents that we administered to our patients had some effect on our findings and that the use of newer antiplatelet medications might have led to different results. However, this is the most commonly used combination in daily clinical practice, which makes our data relevant.

We did not obtain blood samples from the coronary sinus. Obviously, this would have revealed a better local picture of platelet activation at the site of the lesion. However, our initial aim and design was to investigate and compare long-term changes in platelet activation between SES and BMS. For ethical reasons, it was not possible to obtain serial blood samples from the coronary sinus. Additionally, a carefully designed study from Jaumdally et al[22] compared platelet activation indices from blood samples that were obtained from the aortic root, coronary sinus, and femoral vein. Although there were some small differences, that study concluded that the magnitude of platelet activation following PCI can be measured peripherally.

Finally, the present study was not designed to investigate the link between platelet activity and long-term outcomes in patients with stable CAD after coronary stenting, so we did not provide any additional information in this regard.

7. Conclusions

This was the first study to perform a long-term examination of many markers of coagulation and platelet activation in the peripheral blood of patients with stable CAD undergoing elective PCI and SES, compared to patients with BMS implantation. In the long term, these two categories of stent do not appear to show significant differences, at least in this category of patients. Although some indices showed an increase after stent implantation, these profiles at 6 months returned to approximately the same levels as before stent implantation. Likely, our results would be translated into similar behavior regarding the phenomenon of acute thrombosis and may thus confirm the recommendation that 6 months of dual antiplatelet therapy is sufficient.

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