The influence of aspirin resistance on non-fatal coronary events following percutaneous coronary interventions

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Abstract

Introduction: Aspirin resistance is associated with unfavourable prognosis, including a higher incidence of myocardial infarction, stroke, and cardiovascular death among stable cardiovascular patients, a higher incidence of re-occlusion after peripheral angioplasty, and myonecrosis following elective percutaneous coronary interventions (PCI). The objective of this study was to evaluate the relationship between aspirin resistance and non-fatal clinical endpoints during the long term follow-up following successful PCI.

Material and methods: A total of 100 subjects with angiographically diagnosed coronary artery disease and treated with elective, non-urgent intracoronary stent implantation between October 2001 and June 2002 were enrolled in the study. All patients were under regular aspirin (300 mg) treatment. PFA-100 analyzer was used to assess the platelet functions. Aspirin resistance was defined as a collagen/epinephrine closure time (CTCEPI) < 186 s. The study end-point was the composite of non-fatal coronary events which included non-fatal MI, coronary artery bypass graft surgery (CABG) or repeat PCI, during the 2-year follow-up period after the index PCI.

Results: The incidence of aspirin resistance was found to be significantly higher \((p = 0.021)\) in patients with non-fatal coronary events \((22.4\%)\) compared to those who did not have \((5.9\%)\). Aspirin resistance was found to be an independent risk factor for non-fatal coronary events after adjusted for other potential risk factors \((p = 0.019)\).

Conclusions: Despite regular treatment with aspirin, the incidence of aspirin resistance was significantly higher in patients who developed non-fatal coronary events on long term follow-up following elective PCI. Thus, these findings suggest that aspirin resistance might be an important risk factor that could affect the outcome following PCIs.

Key words: aspirin resistance, platelet, percutaneous coronary intervention.

Introduction

Clinical trials have shown the efficacy of aspirin in both the primary and secondary prevention of myocardial infarction (MI), stroke, and cardiovascular death [1, 2]. Despite its well known beneficial effects, coronary artery disease patients treated with aspirin have a recurrent vascular event during long-term follow-up [3]. It appears that aspirin’s antiplatelet effect is not uniform in all patients, and a substantial proportion of patient taking aspirin have normal, ex-vivo determined, platelet functions [4-7]. Although none of these
tests have been validated, there are several studies demonstrating unfavourable prognosis, including higher incidences of MI, stroke, and cardiovascular death among stable cardio-vascular patients, a higher incidence of re-occlusion following peripheral angioplasty, and myonecrosis after elective percutaneous coronary interventions (PCI) in patients who had aspirin resistance detected in laboratory tests [8-11]. PFA (platelet function analyzer)-100 is a point-of-care test to determine platelet functions *ex-vivo* [12]. This method evaluates platelet adhesion/aggregation, and is being frequently used in the clinical practice to detect hemostatic alterations.

The objective of this study is to assess the incidence of aspirin resistance by using PFA-100, in patients with non-fatal coronary events following elective non-urgent PCIs compared to event-free PCI patients.

**Material and methods**

**Study population**

A total of 100 consecutive subjects with angiographically diagnosed coronary artery disease and treated with elective, non-urgent PCI between October 2001 and June 2002 were enrolled in the study. In 49 of these patients, a non-fatal coronary event (non-fatal MI or repeat revascularization) was observed during the 2-year follow-up period after the index PCI, whereas 51 patients were event-free. Exclusion criteria included age below 21 years, use of drug eluting stents, severe co-morbidity with a life expectancy less than 12 months, thrombocytopenia (< 100,000/mm³) or thrombocytosis (> 400,000/mm³), anemia (hemoglobin < 10 g/dl), polycythemia (hematocrit > 50%), neutropenia (leukocyte < 4000/mm³), end-stage renal disease, acute or chronic liver disease, hematologic diseases, malignancies, aspirin intolerance or contraindication for the use of aspirin, use of glycoprotein IIb/IIIa inhibitor within the last 3 days, use of oral anticoagulants or antithrombotic agents other than aspirin more than 30 days after the procedure and use of regular non-steroidal anti-inflammatory drugs within the last 3 months. Five patients were excluded who met at least one of these criteria, during the enrolment period. The remaining 100 patients were considered as the study population. As a policy aspirin tablets are provided by the hospital and refilled every 90 days for PCI patients. Compliance with aspirin therapy was ascertained by a personal interview and confirmed by pill count. All patients gave a written informed consent, and the study was approved by the institutional ethics committee.

**Percutaneous coronary intervention**

Percutaneous coronary intervention was performed as described before [13]. Aspirin treatment at a dose of 300 mg/day was started 3 days prior to the procedure and continued indefinitely. A loading dose of clopidogrel (300 mg) was also administered before the procedure and followed by 75 mg/day for 4 weeks. Glycoprotein IIb/IIIa inhibitor was administered according to the discretion of the cardiologist who performed the intervention. A successful procedure was defined as a residual stenosis of less than 20% of the vessel diameter when stent was implanted and less than 30% of the vessel diameter when only balloon angioplasty was performed. Coronary angiograms were obtained following the intracoronary injection of 100-300 μg of nitrate. Quantitative analyses of all angiographic data before, during, and after the procedure were performed by experienced interventional cardiologists using edge-detection techniques. The luminal diameter of the coronary artery and the degree of stenosis were measured before and after the procedure.

**Platelet function tests and biochemical analysis**

Venous blood samples were obtained 2 to 4 h after aspirin intake, for the measurement of platelet functions and blood count analysis. For this purpose, blood samples were collected in tubes containing 3.8% sodium citrate for platelet function tests (buffered pH 5.5, Vacutainer, Becton Dickinson, Plymouth, UK) and ethylenediaminetetraacetic acid (EDTA) for blood count.

PFA-100 analyzer was used to assess the platelet functions. The PFA-100 uses a disposable test cartridge that simulates an injured blood vessel for primary hemostasis by forcing the whole blood through an aperture (147 mm diameter) cut into a collagen-coated membrane. The analyzer aspirates whole blood at high shear rate through the capillary where it comes in contact with the membrane; the platelets adhere to the membrane surface and aggregate. Platelet plug formation occurs following the occlusion of the aperture and cessation of blood flow. The device has two types of cartridges: collagen/epinephrine and collagen/ADP. Administration of aspirin usually affects collagen/epinephrine measurement. The normal reference ranges for closure time measured with PFA-100 in our laboratory were 98-185 s and 81-113 s for Col/Epi (CT_CEP) and Col/ADP (CT_CADP) cartridges, respectively [14]. Patients under aspirin therapy are expected to exhibit a prolonged CT_CEP and a normal CT_CADP. Aspirin resistance was defined as having a CT_CEP < 186 s, despite regular aspirin therapy. Platelet function test was performed within 1 h of blood sampling and repeated 6 months after the initial assessment. Two patients treated with thienopiridines in addition to aspirin were re-evaluated with PFA-100 one month after discontinuation of their thienopiridines.
Glomerular filtration rate (GFR), calculated using the simplified modification of Diet in Renal Disease Prediction Equation \[186 \times \text{serum creatinine}^{-1.154} (\text{mg/dl}) \times \text{age}^{-0.203} (\text{years}) \times 0.742, \text{if female} \times 1.212, \text{if black}, \] was used to determine the renal function [15]. GFR level below 60 ml/min was defined as having chronic renal failure [16].

### Endpoint of the study

The endpoint of this study was the composite of non-fatal coronary events which included non-fatal MI, coronary artery bypass graft surgery (CABG) or repeat PCI, during the 2-year follow-up period following the index PCI. Myocardial infarction was defined as the presence of new pathological Q waves by electrocardiogram plus an increase in creatine kinase (CK) more than twice the upper limit of the normal range with a concomitant increase in its MB isoenzyme or an increase in cardiac troponins above the upper limit of the normal range with typical symptoms. To meet this endpoint, patients who had initially presented with Q-wave MI had to have symptomatic recurrent ST-segment elevations with elevated CK and/or cardiac troponins and/or angiographically verified target vessel occlusion. Creatinin kinase and cardiac troponin levels were determined 24 h after the intervention. The target-lesion revascularization (TLR) was defined as any repeated PCI or CABG due to restenosis (stenosis ≥ 50%) within the stent or in 5 mm distal or proximal segments along with symptoms or signs of ischemia. Target vessel revascularization (TVR) was characterized by repeated PCIs or surgical interventions of the treated vessel. Repeat revascularization implied to revascularization procedures (except for the staged procedure) applied to any of the coronary segments after the index procedure. Bleeding complications requiring blood transfusion were also followed. The endpoints were adjudicated by an independent clinical-events committee. Collection of the clinical, angiographic and other data was completed before blinded determination of aspirin resistance.

### Statistical analysis

Discrete variables were presented in percentages and continuous variables in mean ± SD. To test the differences between study groups for discrete variables, a \(\chi^2\) test or Fisher’s exact test were used. Continuous variables were analyzed by using \(t\) test for unpaired samples. The logistic regression analysis was used for the analysis of the influence of covariables on the relation between aspirin resistance and non-fatal cardiac events. The following variables were evaluated in the stepwise regression process: age, gender, hypertension, diabetes mellitus, LDL cholesterol, body mass index (BMI), smoking, GFR, prior MI and prior PCI or CABG.

Cumulative hazard rate between patients with or without aspirin resistance was compared by Kaplan-Meier and log rank tests. A value of \(p < 0.05\) in the 2-tailed test was regarded as statistically significant. Analyses were done using SPSS statistical software package version 11.5 (Chicago, Illinois).

### Results

#### Baseline characteristics

A total of 100 patients were enrolled in the study and all patients completed the 2-year follow-up. None of the patients died. Forty-nine patients had a non-fatal coronary event whereas 51 patients were event-free following the index PCI, whereas 51 patients were event-free following the index PCI. The mean age was 60.5 ± 9.3 years (range: 37-84 years) and 77% of the patients were males. The percentages of the patients who had previous MI, CABG or PCI history were 25, 12 and 26%, respectively. As demonstrated in Table I, the baseline clinical characteristics of the patients were similar in both groups. In the group with non-fatal events, hypertension tended to be significantly higher compared to event-free patients (77.6 and 58.8% respectively, \(p = 0.055\)). The compliance rate of aspirin use was 100%.

The quantitative coronary angiogram (QCA)-determined lesion characteristics and calculated parameters at baseline and immediately after PCI were similar in both groups (Table II). The target vessel was the left anterior descending coronary artery in 44 patients (44%), the right coronary artery in 40 patients (40%), and the left circumflex artery in 36 patients (36%). Stenting was performed in 89 patients (89%). None of the patients received platelet glycoprotein Ib/IIa inhibitors. Procedural success was 100% in both groups.

#### Incidence of aspirin resistance and non-fatal coronary events

The incidence of aspirin resistance in patients with non-fatal coronary events (22.4%, \(n = 11\)) was significantly higher (\(p = 0.021\)) compared to event-free patients (5.9%, \(n = 3\)) (Figure 1). Significance continued after adjustment for age, gender, hypertension, diabetes mellitus, LDL cholesterol, BMI, smoking, GFR, prior MI and prior PCI or CABG. Aspirin resistance was found to be an independent risk factor for the occurrence of non-fatal events (odds ratio: 6.83, 95% confidence interval: 1.37-34.12, \(p = 0.019\)) (Table III). Among the 49 non-fatal coronary events, 15 (30.6%) were non-fatal MIs and 34 (69.4%) were repeat revascularizations (CABG or repeat PCI) at the end of the 2 year follow-up. All of the repeat revascularizations were TVRs (25 of 34 TVRs were TLR).

Platelet aggregation tests, repeated 6 months after the initial assessment, revealed that
14 aspirin-resistant patients were still resistant and 86 aspirin-responsive patients continued to be responsive. Two patients treated with thieno-piridines were evaluated one-month after their discontinuation of thieno-piridines. Both were detected to be aspirin responsive in their second evaluation.

**Association between aspirin resistance and cardiovascular risk factors**

The evaluation of the association of aspirin resistance with cardiovascular risk factors (i.e. age, gender, hypertension, diabetes mellitus, low density lipoprotein (LDL) cholesterol, BMI, family history and...
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Smoking) by univariate analysis followed by a logistic regression showed that the aspirin-resistant and -responsive groups were similar in this regard (Tables I and III).

### Aspirin resistance and event-free survival

The cumulative hazard ratio determined by Kaplan-Meier analysis revealed a significant difference in favour of aspirin responsive patients regarding event-free survival ($p = 0.011$) (Figure 2).

### Bleeding complications

None of the patients had any bleeding complication requiring blood transfusion.

### Discussion

Our study demonstrated that despite regular treatment with aspirin, the incidence of aspirin resistance is significantly higher in patients with non-fatal coronary events during the long term.
follow-up after elective PCI, compared to event-free patients. Aspirin reduces the risk of cardiovascular events by approximately 25% in a broad category of patients with atherothrombosis [1]. Aspirin has also been shown to reduce acute thrombotic complications of balloon angioplasty [17, 18]. However, its effectiveness is limited because 10 to 20% of the patients with arterial thrombosis treated with aspirin have a recurrent vascular event during long-term follow-up [3].

Aspirin resistance describes the observation of the inability of aspirin to prevent thrombotic complications in its clinical sense or refers to inadequate platelet response, detected by biochemical means in patients on aspirin therapy. However, aspirin resistance currently is not clearly-defined. Among the diagnostic tools, light transmittance aggregometry is the gold standard for determination of platelet functions. However, this technique can only be performed in the laboratory settings. Thus, it is not easy to interpret the data due to inter-laboratory variations [19]. The VerifyNow Rapid Platelet Function Assay (RPFA)-ASA (Accumetrics, San Diego, CA) and PFA-100 have been designed as point-of-care tests to allow ease of use and widespread distribution. PFA-100 is a semiautomatic analyzer of platelet function that mimics the \textit{in vivo} conditions. It is a thromboxane-dependent platelet function test. According to previous studies it is a sensitive and reproducible method for detecting aspirin intake and its effect on platelet function compared to several other tests. In addition PFA-100 test is a widely used standardized method. However, the PFA-100 may not be specific to assess the thromboxane-dependent platelet activation for some reasons. During the test, platelets are activated by high concentrations of epinephrine and collagen as well as by the shear stress on platelets [8]. In addition, platelets hyperactivity to ADP and collagen had been described in patients treated by aspirin [19, 20]. Therefore, aspirin inhibition of TxA2 pathway could not be evaluated specifically.

Secondary, the PFA-100 remains to be sensitive to many variables other than TxA2 production including plasma Von Willebrand Factor [8, 17, 21]. In the present study, we preferred the PFA-100 system because it has been found to be comparable with optical platelet aggregation technique for detection of aspirin resistance [20, 22].

Platelet activity is an important process that could lead to thrombo-embolic events following

### Table III. The influence of covariables on non-fatal coronary events

<table>
<thead>
<tr>
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<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.94-1.04</td>
<td>0.663</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.65</td>
<td>0.20-2.12</td>
<td>0.475</td>
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<td>Hypertension</td>
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<td>LDL cholesterol</td>
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<td>0.99-1.02</td>
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</tr>
<tr>
<td>Body mass index</td>
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<td>0.80-1.08</td>
<td>0.340</td>
</tr>
<tr>
<td>Active smoker</td>
<td>0.93</td>
<td>0.29-3.05</td>
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<tr>
<td>GFR</td>
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<td>0.96-1.03</td>
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<tr>
<td>Previous MI</td>
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<td>0.15-1.28</td>
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<tr>
<td>Previous CABG</td>
<td>0.51</td>
<td>0.12-2.25</td>
<td>0.373</td>
</tr>
<tr>
<td>Previous PCI</td>
<td>0.84</td>
<td>0.30-2.41</td>
<td>0.751</td>
</tr>
<tr>
<td>Aspirin resistance</td>
<td>6.83</td>
<td>1.37-34.12</td>
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</table>


*Figure 2. Kaplan-Meier analysis of the event-free survival rate of aspirin-resistant and aspirin-responsive patients.*

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PCIs, particularly in patients with acute coronary syndromes [21]. The incidence of aspirin resistance was evaluated in four previous PCI studies, using the same PFA-100 definitions [23-26]. The incidence ranged between 16-42%. The incidence of aspirin resistance was 14% in our study population which was slightly lower than the previous studies. We observed a relatively high incidence of non-fatal coronary events in our study, which could be due to complex lesion intervention and use of bare metal stents only. Prior studies demonstrated an association between adverse coronary events and aspirin resistance [8-11]. Gum et al. reported a nearly three-fold increased risk of death, MI, or cerebrovascular events in aspirin resistant patients [20]. Eikelboom et al. have reported an increased risk of death and MI in high risk cardiovascular disease patients with aspirin resistance [8]. Aspirin resistance was also found to be significantly higher in patients with evolving MI [27]. Chen et al. investigated the effect of aspirin resistance on myocardectomy after non-urgent PCI among 151 patients and found that 19.2% of the aspirin-resistant patients had an increased risk of myocardectomy following PCI, despite pre-treatment with aspirin [28]. The incidence of aspirin resistance was detected to be higher in patients who had a coronary event following a successful PCI for the treatment of acute MI [29]. In our study we found a significantly increased incidence of aspirin resistance in patients with non-fatal coronary events after non-urgent PCI suggesting that aspirin resistance could be an important predictor of non-fatal events following PCIs.

Although both 81 and 325 mg/day of aspirin are known to be equally effective in prevention of stroke, MI, or death, there is conflicting data on the dose-dependent response to aspirin treatment [9, 19, 29, 30]. Several controlled studies demonstrated a remarkable intra-individual variability regarding aspirin resistance at low doses (i.e. 80 mg or below) [19]. In our study, the standard dose of aspirin was 300 mg which was the highest recommended dose for coronary artery disease patients available in our country, therefore we could not analyze the dose response of aspirin on aspirin resistance. In addition, to further test the variability of platelet aggregation over time we repeated the analysis 6 months after the initial measurement and observed that platelet aggregation did not differ at these two time points. This finding differs from the study by Poulsen et al., who demonstrated variability in aspirin resistance over time, in patients with cardiovascular disease [31].

The slightly lower incidence of aspirin resistance in our study population, compared to previous studies, might be the result of the higher dose of aspirin used in our study, whereas in the previously reported studies the dose of aspirin was ranging between 100-325 mg. In addition we did not observe any bleeding complications in our study. Although the study is not designed with enough power to postulate the use of higher doses of aspirin safely and effectively, the results of our study encourages the use of 300 mg of aspirin post-PCI.

Aspirin resistance was associated with decreased survival rates in acute MI patients treated with PCI [29], whereas, in a previous study, it was not related with survival benefits in patients with known cardiovascular disease [31]. Our study demonstrated a significant event-free survival benefit in aspirin responsive patient.

There are several potential limitations of this study. First, the initial assessment of aspirin resistance was 2 years following the index PCI procedure. Therefore we lack baseline aspirin resistance information. These findings should be investigated prospectively in a patient population undergoing PCI, with baseline and repetitive follow-up measurements of platelet aggregation. Secondly, we did not perform an angiographic follow-up, which could detect clinically insignificant restenosis. Third limitation is that, although the study size is adequate to show a difference in the incidences of aspirin resistance among groups, it is not enough to analyze event-free survival rates. Therefore, our observations need to be confirmed by other larger prospective studies.

As a conclusion, despite regular treatment with aspirin, the incidence of aspirin resistance determined by a point-of-care assay was significantly higher in patients who developed non-fatal coronary events on long term follow-up after elective PCIs compared to those without these events. These findings raised the possibility that aspirin resistance might be an important risk factor influencing the outcome of PCIs; thus, screening might be recommended before or after the procedure.

References
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