The Impact of Aspirin Resistance on the Long-term Cardiovascular Mortality in Patients with Non-ST Segment Elevation Acute Coronary Syndromes

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ABSTRACT

Background: Aspirin resistance has been associated with an adverse long-term outcome in patients with atherosclerotic coronary artery disease, but more studies are needed.

Hypothesis: The aim of this study was to investigate the impact of aspirin resistance, assessed by the Platelet Function Analyzer-100 (PFA-100) (Dade Behring Inc., Deerfield, Ill., USA) on the long-term prognosis in patients with non-ST segment elevation acute coronary syndromes (NSTE-ACS).

Methods: A total of 496 consecutive patients were studied. The 1-y incidence of cardiovascular death was the prespecified study endpoint. The patients were divided, according to the values of PFA-100 collagen epinephrine closure time (CEPI-CT) upon presentation, into aspirin sensitives (those with a PFA-100 CEPI-CT >193 sec) and aspirin resistants (those with a PFA-100 CEPI-CT ≤ 193 sec).

Results: Aspirin resistants were younger (p-value $= 0.04$), and less frequently hypertensives (p-value $= 0.05$) or diabetics (p-value $= 0.04$) than aspirin sensitives. By 1 y, the incidence of cardiovascular deaths in the entire cohort was 12.9% (64/496), and aspirin resistants were at significantly higher risk of cardiovascular death (23.1% versus 9.6%; hazard ratio [HR] $= 2.6$; 95% confidence interval [CI] $= 1.6–4.3$; p-value <0.001), than aspirin sensitives. By multivariate Cox regression analysis, aspirin resistance (a PFA-100 CEPI-CT ≤ 193 sec) was among the most potent predictors of the 1-y incidence of cardiovascular death (HR $= 2.8$; 95% CI $= 1.7–4.6$; p-value <0.001).

Conclusion: According to the present data, aspirin resistance, assessed by the PFA-100, is an independent predictor of long-term cardiovascular mortality in patients with NSTE-ACS.

Key words: aspirin resistance, Platelet Function Analyzer-100, acute coronary syndromes, prognosis

Introduction

Platelet function has a key role in the induction and progression of fatal and nonfatal thrombotic complications in patients with non-ST segment elevation acute coronary syndromes (NSTE-ACS). Aspirin drug therapy substantially reduces the risk of these thrombotic complications and has been the standard of care in these patients. However, a substantial percentage of patients with stable or unstable coronary artery disease, ranging from 5%–45%, display an inadequate response to aspirin drug therapy. This phenomenon has been connected with an adverse outcome in these patients,[1–7] but more studies are needed.

The aim of this study was to prospectively investigate the impact of aspirin drug resistance on the incidence of 1-y cardiovascular death in patients with NSTE-ACS.

Patients and Methods

Study Patients

Consecutive, eligible patients with an NSTE-ACS who were admitted to the Department of Cardiology at Tzanio Hospital (Piraeus, Greece) from April 2003 through August 2004 were recruited. Patients were required to have had angina-like chest pain at rest in the previous 24 h, lasting 5 min or more, with associated ST-segment depression of ≥ 0.1 mV in 2 or more contiguous leads, or elevated levels of cardiac troponin I (cTnI) upon presentation. Patients were excluded for the following: (1) had angina of secondary etiology; (2) were treated with a glycoprotein IIb/IIIa inhibitor within the previous 1 mo; (3) were treated with nonsteroid anti-inflammatory or anticoagulant drugs within the previous 2 wk; (4) had a platelet count $<100{,}000$/mL or hematocrit count $<30\%$; (5) had a significant hepatic or renal dysfunction; or (6) had an active infection, chronic inflammatory diseases, and malignancy.

All patients received aspirin orally in a dose of 325 mg upon presentation, and it was continued as a daily dose indefinitely. Heparin was given in a bolus dose of 5,000 units upon admission to all patients, followed by intravenous infusion titrated to a therapeutic activated partial thromboplastin time. Heparin was continued in uncomplicated

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cases for 24 h followed by subcutaneous administration of enoxaparin. Clopidogrel was routinely administered in all patients following blood sampling for platelet function analysis. Further medical therapy was left to the discretion of the attending physician.

The study complies with the Declaration of Helsinki. The ethics committee of the attending hospital approved the research protocol and informed consent was obtained from all participants.

Platelet Function and Biochemical Assays

Venous blood samples were obtained, either upon presentation for baseline platelet function assay in those patients with prior aspirin use within the previous 7 d, or 6 h following oral aspirin administration in those without such prior therapy. Platelet function was assessed by the Platelet Function Analyzer-100 (PFA-100) (Dade Behring Inc., Deerfield, Ill., USA). After rejection of a few milliliters, blood for PFA-100 analysis was collected into citrate tubes and was analyzed within 1 h. The PFA-100 simulates an injured blood vessel by aspirating blood through test cartridges coated with either collagen and adenosine diphosphate (CADP), or collagen and epinephrine (CEPI). Blood (900 μL) flows under high shear rates (5000–6000 per sec) through a capillary and a microscopic aperture (147 μm) cut into the coated membrane. When blood comes into contact with the membrane, platelets adhere, aggregate, and form a plug that occludes the aperture with consecutive cessation of blood flow. The time required to occlude the aperture is automatically reported as the closure time (CT). Measurements are terminated after a maximum of 300 sec. Aspirin prolongs the CEPI-CT, but does not extend the CADP-CT and subsequently the CEPI cartridges were used. All samples were analyzed in duplicate and the mean value was used for statistical analysis. Values of CEPI-CT were not decoded before the end of the study.

Upon presentation, venous blood samples were obtained for cTnI and high sensitivity C-reactive protein (hs-CRP) measurements. Plasma cTnI levels were measured by an enzyme-based immunoassay (AxSYM cTnI, Abbot Diagnostics, Abbott Park, Ill., USA) with an analytical sensitivity at 0.02 ng/mL (at a 95% level of confidence), a coefficient of variation range at 0.16–0.27, and a threshold level for the diagnosis of myocardial infarction $\geq$0.4 ng/mL. Plasma hs-CRP levels were measured by a highly sensitive nephelometric method (BNII, Dade Behring Inc., Deerfield, Ill., USA) with a lower limit of detection at 0.1 mg/L, and intraassay and interassay coefficients variation of 3.3% and 3.2%, respectively.

Clinical Follow-up and Study Endpoint

In-hospital and post-discharge follow-up data were prospectively collected on predesigned case report forms. After discharge, patients were followed-up with at 1, 3, 6, and 12 mo on an outpatient basis or by telephone interview. The incidence of cardiovascular death during the first year was the prespecified study endpoint. Cardiovascular death was considered as sudden unexplained death, death due to fatal myocardial infarction, death after rehospitalization due to heart failure or possible acute myocardial ischemia, and death related to stroke or fatal complications (e.g., gangrene) of peripheral artery disease. The diagnosis of cardiovascular death was verified by review of death certificates, discharge medical reports, hospital records, or contact with the attending physicians.

Statistical Analysis

Values were expressed as mean±standard deviation (SD). Comparisons of continuous variables were made using *t* test or Mann-Whitney U test. Dichotomous variables were presented as percentages. Associations between dichotomous variables were tested by χ^2 or Fisher's exact test. On the basis of the recent literature, the study population was divided into aspirin sensitives and aspirin resistants, according to the values of the PFA-100 CEPI-CT and using a prespecified cutoff point of 193 sec. Aspirin sensitives were considered the patients with a CEPI-CT >193 sec, while aspirin resistants were those with a CEPI-CT $\leq$193 sec.[8,9]

The number of patients included in this study was tailored to achieve a statistical significance in the incidence of 1-y cardiovascular death between patients with or without aspirin resistance, with an expected incidence of 1-y cardiovascular death of 11% in the entire study population, and 17% in patients with aspirin resistance. On the basis of these assumptions, it was estimated that 450 patients were needed. Owing to an estimated 10% loss in follow-up, it was planned that a total of 495 patients would be recruited. The power of the study to detect a difference between the study groups was set at 80% with a 2-tailed α level of 0.05.

Correlations between continuous variables were evaluated by Spearman ρ. Event-free survival between the study groups was analyzed with the Kaplan-Meier method, and log-rank was used for comparisons among the curves. Univariate and multivariate Cox analyses were constructed for the evaluation of univariate and multivariate predictors of the primary endpoint. All variables presented in Tables 1 and 2 were evaluated as possible univariate predictors, and those with a p-value <0.1 were introduced in the multivariate model. Patients who died because of noncardiac causes were censored at the time of death. All tests were 2-tailed, and a p-value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 13.0 (SPSS Inc., Chicago, Ill., USA).

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TABLE 1: Baseline characteristics between aspirin sensitives and aspirin resistants

	Aspirin sensitives (n = 375)	Aspirin resistants (n = 121)	p-value
Age (y), mean±SD	69.8±8.8	67.9±9.2	0.04
Age ≥65 y (%)	72	63.6	0.08
Male gender (%)	69.1	70.2	0.81
Cardiovascular risk factors (%)			
Hypertension	62.9	52.9	0.05
Smoking	39.5	42.1	0.61
Diabetes mellitus	34.7	24.8	0.04
Hypercholesterolemia	64.3	62.8	0.77
Medical history (%)			
History of stable angina (>1 mo prior)	10.7	9.9	0.82
History of myocardial infarction	33.9	36.4	0.62
History of coronary angioplasty	14.1	17.4	0.39
History of coronary bypass grafting	10.1	8.3	0.55
Prior aspirin use (≥7 d prior) (%)	40.3	45.5	0.31
ST depression on admission ECG (%)	76.5	78.5	0.65
Red blood cell count, (M/μL), mean±SD	4.5±1.3	4.5±1.2	0.72
Platelet count ($\times 10^3/\mu L$), mean±SD	216.8±45.3	224.2±54.1	0.14
cTnI, (ng/mL), mean±SD	3.7±6.8	4.2±9.8	0.52
cTnI ≥0.4 ng/mL (%)	79.5	83.5	0.43
hs-CRP (mg/L), mean±SD	6.1±7.3	5.3±6.5	0.29
hs-CRP ≥3 mg/L (%)	63.3	57.1	0.23
TIMI risk score, mean±SD	4.3±1.4	4.4±1.3	0.69
Left ventricular ejection fraction (%), mean±SD	47.7±8.2	46.4±9.1	0.22

Abbreviations: cTnI = cardiac troponin I; ECG = electrocardiogram; hs-CRP = high-sensitivity C-reactive protein; SD = standard deviation; TIMI = thrombolysis in myocardial infarction.

Results

Baseline Characteristics

A total of 496 patients were included in the study. Values of the 2 measurements of PFA-100 CEPI-CT in each patient were significantly correlated (Spearman $\rho = 0.94$; p-value <0.001) (Figure 1). On the basis of the values of PFA-100 CEPI-CT, the patients were classified into aspirin sensitives (375/496; 75.6%) and aspirin resistants (121/496; 24.4%). Aspirin sensitives had significantly higher values of PFA-100 CEPI-CT than did aspirin resistants (median and interquartile range, 298 and 263–300 sec versus 142 and 116–65 sec; p-value <0.001). Baseline clinical characteristics of the study groups are presented in Table 1. Aspirin resistants were younger (p-value = 0.04) and less frequently hypertensives (p-value = 0.05), or diabetics (p-value = 0.04).

There was no difference in the values of PFA-100 CEPI-CT between patients with or without prior use of aspirin (median and interquartile range, 285 and 189–300 sec versus 275 and 199–300 sec; p-value = 0.51). Moreover, there was no difference in the values of PFA-100 CEPI-CT among patients treated with 100 mg (median and interquartile range, 281 and 208–300 sec), 160 mg (median and interquartile range,

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TABLE 2: In-hospital coronary revascularization strategies and drug therapy during the first year between aspirin sensitives and aspirin resistants

	Aspirin sensitives (n = 375)	Aspirin resistants (n = 121)	p-value
In-hospital coronary revascularization strategies (%)			
Coronary angioplasty	75.7	74.4	0.76
Coronary bypass surgery	9.1	12.4	0.29
Drug therapy during the first year (%)			
Aspirin	97.6	98.3	0.63
Clopidogrel	75.6	75.2	0.93
β-blockers	87.5	88.4	0.78
ACE inhibitors or ARBs	80.3	82.6	0.56
Statins	86.1	85.1	0.78

Abbreviations: ACE = angiotensin-converting enzyme; ARBs = angiotensin receptor blockers.

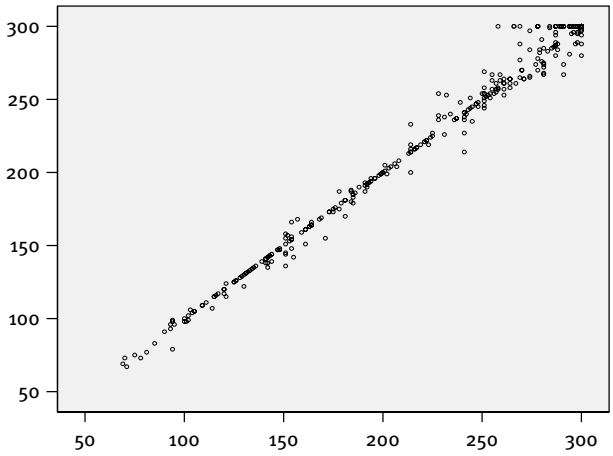

Figure 1: Scatter plot representing the correlation between the values of the 2 measurements of PFA-100 CEPI-CT in each patient.

280 and 156–300 sec), and 325 mg (median and interquartile range, 286 and 201–300 sec) of aspirin (p-value = 0.47).

Study Endpoint

No patients were lost during the follow-up. There were no significant differences with regard to the rates of in-hospital revascularization strategies and drug therapy between the study groups (Table 2). By the first year, 64/496 patients (12.9%) died due to cardiovascular reasons. In particular, 19/496 deaths (3.8%) occurred during the index event and 38/496 patients (7.7%) died suddenly after re-hospitalization for myocardial infarction or possible myocardial ischemia. Five deaths (5/496; 1%) were attributed to acute heart failure, 2/496 deaths (0.4 %) were attributed to stroke, and none of the deaths were attributed to peripheral artery disease. Aspirin resistants were at significantly higher risk of cardiovascular death (23.1% versus 9.6%; HR = 2.6, 95% CI = 1.6–4.3; p-value <0.001) than aspirin sensitives (Figure 2). Aspirin resistance (a PFA-100 CEPI-CT ≤193 sec) was among the most potent predictors of the 1-y incidence of cardiovascular death by both univariate and multivariate Cox regression analysis (Table 3).

Discussion

The study, in a relatively large cohort of patients with NSTE-ACS, has shown that resistance to aspirin drug therapy confers an increased risk for the incidence of 1-y cardiovascular death, and it was true by both univariate and multivariate Cox regression analysis.

The term aspirin resistance describes the inability of aspirin to achieve a predefined effect on an ex vivo or in vitro measure of platelet function, or to protect individuals from thrombotic complications.[10] Previous studies, using several methods for the assessment of aspirin resistance, evaluated the possible clinical relevance of this phenomenon in patients with atherothrombotic complications.[1–9] Grotemeyer et al., in a study of 180 patients suffering from ischemic stroke, have demonstrated that long-term fatal or nonfatal cardiovascular events were significantly more

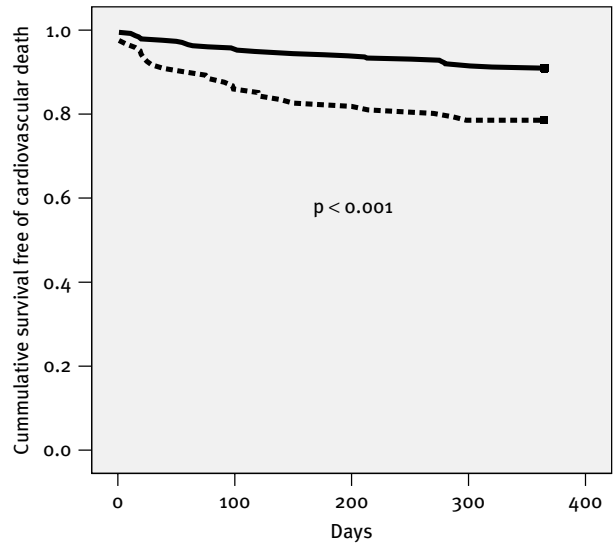

Figure 2: Kaplan-Meier curves between aspirin sensitives (solid line) and aspirin resistants (dashed line) for the cumulative incidence of 1-y cardiovascular death.

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TABLE 3: Univariate and multivariate predictors of 1-y cardiovascular death

	Univariate Cox regression		Multivariate Cox regression	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age ≥65 y	1.9 (1.1–3.6)	0.04	1.4 (0.6–2.9)	0.44
Male gender	0.7 (0.4–1.1)	0.1	0.9 (0.5–1.6)	0.84
Smoking	0.6 (0.3–0.9)	0.04	0.7 (0.5–1.4)	0.59
Diabetes mellitus	1.7 (1.1–2.8)	0.04	1.4 (0.8–2.3)	0.25
History of myocardial infarction	2.5 (1.5–4.1)	<0.001	1.5 (0.8–2.7)	0.23
History of coronary bypass grafting	1.6 (0.9–3.3)	0.1	1.1 (0.5–2.3)	0.85
Prior aspirin use (≥7 d prior)	3.6 (1.8–4.7)	<0.001	1.4 (0.6–3.3)	0.43
cTnI ≥0.4 ng/mL	1.8 (1–3.7)	0.05	1.7 (0.8–3.6)	0.16
hs-CRP ≥3 mg/L	1.9 (1.1–3.5)	0.01	1.6 (1.1–3.2)	0.02
TIMI risk score	1.7 (1.4–2.1)	<0.001	1.7 (1.4–2.1)	<0.001
Left ventricular ejection fraction <35 (%)	3.4 (1.8–5.1)	<0.001	3.5 (1.9–5.7)	<0.001
Aspirin resistance[a]	2.6 (1.6–4.3)	<0.001	2.8 (1.7–4.6)	<0.001

[a] A value of Platelet Function Analyzer 100≤193 sec. *Abbreviations*: CI = confidence interval; cTnI = cardiac troponin I; HR = hazard ratio; TIMI = thrombolysis in myocardial infarction.

frequent in aspirin resistants than in aspirin sensitives.[1] Eikelboom and co-workers, in a nested case-control substudy of the Heart Outcomes Prevention Evaluation (HOPE) study, reported that aspirin resistance estimated by urinary 11-dehydro thromboxane B2 levels was a significant predictor of future myocardial infarction or cardiovascular death.[2] Gum et al., in a study of 326 stable cardiovascular patients, also indicated a significant independent association of aspirin resistance and major atherothrombotic long-term events using standard aggregometry.[3] Lev et al.[4] and Chen et al.[5] have shown that resistance to aspirin, assessed by the rapid platelet function analysis, was associated with a significantly higher incidence of post-procedural myocardial necrosis following successful coronary stenting. Moreover, Gianetti and colleagues demonstrated an independent positive relation of aspirin resistance, estimated by the PFA-100, and 6-mo clinical recurrence of symptoms in 175 patients with stable or unstable coronary artery disease who underwent successful coronary stenting.[6] Another report by Fuchs et al.,[7] with a study population of 208 patients with unstable coronary artery disease, also yielded a significant independent association of aspirin resistance, assessed by PFA-100, and major atherothrombotic long-term events. Although all aforementioned reports support the present study, limited data exist regarding the relation between aspirin resistance and long-term cardiovascular mortality in patients with NSTE-ACS. Particularly, the larger study has shown that aspirin resistance, assessed by PFA-100, has an adverse impact on the 1-y incidence of the hard endpoint of cardiovascular death in a well defined population with NSTE-ACS.

Limitations of the Study

There are some possible limitations to this study that should be taken into account. First, of the several available methods for the assessment of aspirin resistance, the PFA-100 was used in the present study. Each method used for the evaluation of aspirin responsiveness, including PFA-100, has its own drawbacks and the diagnostic agreement among these methods has not been thoroughly evaluated. Standard platelet aggregometry and flow cytometry are the methods against which all others are compared; however, both methods are technologically intensive and expensive, require highly trained laboratory personnel, and are subsequently of low clinical usefulness. On the other

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hand, PFA-100 is a simple, rapid, and widely available point-of-care method, which has been designed to overcome many drawbacks of other available techniques, such as standard platelet aggregometry or flow cytometry. Second, it has been shown that the values of PFA-100 CEPI-CT are highly dependent on the von Willebrand factor,[10] and it is possible that differences in von Willebrand factor levels (not measured in the present study) could, at least partially, account for the outcome differences between aspirin sensitives and aspirin resistants. However, Fucsh et al., in a study of patients with ACS, have shown that inclusion of von Willebrand factor levels in a multivariate Cox regression model did not alter the predictive value of a low PFA-100 CEPI-CT for the incidence of long-term recurrent instability.[7] Third, patients with NSTE-ACS, and therefore probably with some degree of platelet hyperactivity, were included in this study. Subsequently, low values of PFA-100 in the study patients may reflect a transient phenomenon of the acute phase. However, the high predictive value of a low PFA-100 CEPI-CT that was observed in this study denotes an incremental prognostic usefulness of platelet function assessment in this setting. Fourth, because of the inclusion criteria of the study, it is possible that the values of platelet function assessment cannot be generalized in patients with other forms of coronary artery disease. Fifth, long-term antiplatelet therapy adherence was based on answers to questionnaires, and salicylate levels or pill counts were not performed.

Conclusions

According to the data, aspirin resistance assessed by the PFA-100 is an independent predictor of long-term cardiovascular mortality in patients with NSTE-ACS. Further studies to confirm these results and investigate more effective antiplatelet therapies for the patients with resistance to aspirin are warranted.

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