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A Prospective, Blinded Determination of the Natural History of Aspirin Resistance Among Stable Patients With Cardiovascular Disease

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OBJECTIVES This study was designed to determine if aspirin resistance is associated with clinical events.

BACKGROUND Aspirin resistance, defined by platelet function testing and presumed clinical unresponsiveness to aspirin, has been previously reported by our group and others. However, little information exists linking the laboratory documentation of aspirin resistance and long-term clinical events.

METHODS We prospectively enrolled 326 stable cardiovascular patients from 1997 to 1999 on aspirin (325 mg/day for ≥7 days) and no other antiplatelet agents. We tested for aspirin sensitivity by optical platelet aggregation using adenosine diphosphate (ADP) and arachidonic acid (AA). The primary outcome was the composite of death, myocardial infarction (MI), or cerebrovascular accident (CVA). Mean follow-up was 679 ± 185 days. Aspirin resistance was defined as a mean aggregation of ≥70% with 10 μM ADP and ≥20% with 0.5 mg/ml AA.

RESULTS Of the patients studied, 17 (5.2%) were aspirin resistant and 309 (94.8%) were not aspirin resistant. During follow-up, aspirin resistance was associated with an increased risk of death, MI, or CVA compared with patients who were aspirin sensitive (24% vs. 10%, hazard ratio [HR] 3.12, 95% confidence interval [CI] 1.10 to 8.90, p = 0.03). Stratified multivariate analyses identified platelet count, age, heart failure, and aspirin resistance to be independently associated with major adverse long-term outcomes (HR for aspirin resistance 4.14, 95% CI 1.42 to 12.06, p = 0.009).

CONCLUSIONS This study demonstrates the natural history of aspirin resistance in a stable population, documenting a greater than threefold increase in the risk of major adverse events associated with aspirin resistance. (J Am Coll Cardiol 2003;41:961–5) © 2003 by the American College of Cardiology Foundation

Aspirin is the cornerstone of antiplatelet therapy in cardiovascular medicine today. It exerts its antiplatelet effect by acetylation of the platelet cyclooxygenase, resulting in an irreversible inhibition of platelet-dependent thromboxane formation (1). In their most recent meta-analysis of more than 200,000 patients from 287 randomized trials, the Antithrombotic Trialists' Collaboration has documented the powerful effect of aspirin in reducing ischemic vascular events by 22% compared with control in a wide array of

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atherothrombotic conditions (2). However, aspirin has been shown to have variable antiplatelet activity in individual patients. Previous studies have estimated that 5% to 45% of the population do not achieve an adequate antiplatelet effect from aspirin (3–7). Little prospective data are available for extended long-term follow-up of stable patients concerning the clinical consequences of aspirin resistance.

In a cohort of 326 stable cardiovascular patients, we previously demonstrated approximately 1 in 20 has laboratory evidence of aspirin resistance as measured by optical platelet aggregation (3). We used this prospectively collected population of patients to determine the long-term clinical significance of aspirin resistance.

METHODS

Study group. We prospectively enrolled 326 stable cardiovascular patients between January 1997 and September 1999. The patients were recruited from consecutive patients presenting to the outpatient clinic or for elective cardiac catheterization. All patients had prior history of cardiovascular disease as defined by previous documented coronary stenosis on cardiac catheterization of ≥60%, previous history of myocardial infarction (MI) or stroke, or previous invasive cardiovascular revascularization procedure. The study was reviewed and approved by the Institutional Review Board. All participants gave informed consent before enrollment. We evaluated these patients for laboratory evidence of aspirin resistance. Patients who were ≥21 years old and taking 325 mg of aspirin for ≥7 days immediately before were eligible for enrollment. Compliance on aspirin was determined by patient interview both at study enrollment and follow-up. Exclusion criteria included:

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Abbreviations and Acronyms

AA = arachidonic acid
ADP = adenosine diphosphate
CI = confidence interval
CVA = cerebrovascular accident
HR = hazard ratio
MI = myocardial infarction

ingestion of ticlopidine, dipyridamole, or other nonsteroidal anti-inflammatory drugs; use of other drugs containing aspirin; administration of heparin or low-molecular-weight heparin within 24 h before enrollment; major surgical procedure within one week before enrollment; malignant paraproteinemias; family or personal history of bleeding disorders; platelet count $<150 \times 10^3/\mu l$ or $>450 \times 10^3/\mu l$; hemoglobin <8g/dl, history of myeloproliferative disorders; or history of heparin-induced thrombocytopenia.

Blood samples. Four samples of whole blood were collected in 3.8% sodium citrate for platelet aggregation testing. One tube of blood anticoagulated with ethylenediaminetetraacetic acid was collected for baseline hemoglobin and platelet count. The last dose of aspirin was administered within 1 to 24 h before blood sampling. Room-temperature blood samples were processed within 1 h of blood collection. Whole-blood specimens were centrifuged for 10 min at 200 g to obtain platelet-rich plasma. Platelet-poor plasma was obtained on the remaining specimen by re-centrifugation at 2,000 g for 15 min. A platelet count was measured on the platelet-rich plasma and was adjusted to between $200 \times 10^3/\mu l$ and $300 \times 10^3/\mu l$ with platelet-poor plasma. The baseline optical density was set with platelet-poor plasma. Aggregation was performed using adenosine 5′-diphosphate (ADP) (BioData, Horsham, Pennsylvania) at 10 μM and arachidonic acid (AA) at 0.5 mg/ml with a BioData PAPS-4 platelet aggregometer (BioData).

For optical platelet aggregation, optical density changes were detected photoelectrically as platelets began to aggregate. Adenosine diphosphate promotes the release of endogenous ADP and thromboxane A_2 when added to platelet-rich plasma, causing irreversible aggregation. The test is abnormal in patients using aspirin or having aspirin-like release defects, storage pool disease, afibrinogenemia, or Glanzmann's thrombasthenia. Arachidonic acid is used to evaluate the degree of inhibition of platelet aggregation by aspirin. The addition of AA to platelet-rich plasma enhances platelet aggregation by producing thromboxane A_2. The test is abnormal in patients using aspirin, having aspirin-like release defects, and Glanzmann's thrombasthenia. Aspirin resistance was defined as a mean aggregation of $\geq$70% with 10 μM ADP and a mean aggregation of $\geq$20% with 0.5 mg/ml AA. Laboratory norms were established by screening 40 in-house normal samples.

Study end points. The primary end point was the composite of death, MI, or cerebrovascular accident (CVA). Secondary end points were the individual events of death, MI, and CVA. Death was defined as all-cause mortality due to MI, ischemic CVA, and other vascular and nonvascular causes. Myocardial infarction was defined as the presence of at least two of these criteria: prolonged angina >30 min; total creatinine kinase elevation >2 times the upper limit of normal as confirmed by creatine kinase-MB fraction isoenzyme elevation; electrocardiogram evidence of infarction, defined as ST-segment elevation of at least 0.1 mV (measured 0.2 s after the J-point) in two contiguous leads; or new significant Q-wave of >0.04 s duration or having a depth greater than one-fourth of the corresponding R-wave amplitude, or both. Cerebrovascular accident was defined as an acute neurologic vascular event with focal signs for more than 24 h.

Follow-up. Follow- up was performed by telephone interview and query of the Social Security Death Index on all patients enrolled regardless of aspirin resistance status between November 2000 and June 2001. Persons performing follow-up interviews were blind to aspirin sensitivity status. For those patients having reached at least one of the primary end points, a medical chart review was initiated to determine whether the event met the definitions described. Follow-up was complete on 96.9% of all patients enrolled. Of those determined to be aspirin resistant, follow-up was complete on 94.1%, and of those determined to not be aspirin resistant, follow-up was complete on 97.1%. Follow-up via telephone and chart review was complete on 293 patients and data concerning death were retrieved solely via the Social Security Death Index on 23 patients.

Statistical analysis. Categorical variables are presented as frequencies and percentages. For the categorical variables, patient demographics between groups were compared using chi-square tests or, if expected cell frequencies were small, exact tests. Continuous variables are presented as means $\pm$ SD. The p values associated with continuous variables were generated by Wilcoxon two-sample tests (because the factors were not normally distributed). Kaplan-Meier product limits were computed for freedom from death, MI, CVA, and the primary end point of composite death, MI, or CVA with respect to aspirin resistance status. Log-rank tests were used for screening univariable group results with respect to outcomes. Cox proportional modeling techniques were used to describe the risks for the composite end point of death, MI, or CVA. Variables entered into the model include: age, gender, race, history of tobacco use, diabetes, hypertension, hyperlipidemia, revascularization, MI, hemoglobin, platelet count, creatinine, and aspirin sensitivity.

RESULTS

Patient characteristics. Of the 326 patients enrolled, follow-up was available on 315 (97%). Mean follow-up was 679 days. Baseline characteristics comparing the two groups, aspirin resistant versus not aspirin resistant, are presented in Table 1. Of the 326 patients studied, 17 (5.2%) were aspirin resistant by optical aggregation. Patients who

Table 1. Baseline Characteristics of Patients

	Aspirin Resistant (n = 17)	Not Aspirin Resistant (n = 309)	p Value
Clinical factors			
Age, mean ± SD (yrs)	59 ± 15	62 ± 11	0.4
Female (%)	8 (47)	65 (21)	0.03
Tobacco use (%)	0 (0)	19 (6)	0.6
Diabetes (%)	3 (18)	77 (25)	0.8
Prior CABG (%)	7 (41)	111 (36)	0.6
Prior PCI (%)	4 (24)	105 (34)	0.4
Prior MI (%)	5 (29)	118 (38)	0.5
Prior CVA (%)	1 (6)	15 (5)	0.4
CHF (%)	0 (0)	17 (6)	1.0
Laboratory values			
Hemoglobin (g/dl)	13 ± 2	14 ± 2	0.02
Platelet count ($\times 10^3/\mu$l)	223 ± 69	217 ± 68	0.9
Medication use baseline			
β-blocker (%)	8 (47)	162 (52)	0.9
ACE inhibitor (%)	7 (41)	121 (39)	0.7
Statin (%)	8 (47)	166 (54)	0.6
Medication use follow-up			
No ASA for ≥1 month (%)	2 (12)	24 (8)	0.6
β-blocker (%)	10 (59)	160 (52)	0.5
ACE inhibitor (%)	7 (42)	116 (38)	0.8
Statin (%)	9 (53)	186 (60)	0.5
Ticlopidine/clopidogrel (%)	2 (12)	10 (3)	0.1

ACE = angiotensin-converting enzyme; ASA = aspirin; CABG = coronary artery bypass grafting; CHF = congestive heart failure; CVA = cerebrovascular accident; MI = myocardial infarction; PCI = percutaneous coronary intervention.

were aspirin resistant were more likely to be women and had a slightly lower hemoglobin count compared with patients who were not aspirin resistant. The two groups had similar rates of previous cardiovascular events, including history of prior revascularization procedures, MIs, or neurologic events. Classic atherosclerotic risk factors were similar between the two groups.

Additional medication use both at baseline and during follow-up was not significantly different between the two groups. Of the patients documented at baseline to be aspirin resistant, 12% discontinued aspirin for ≥1 month during follow-up compared with 8% of those documented to be aspirin sensitive (p = 0.6). Use of medications well proven to reduce clinical events by other previous studies, including beta-blockers, angiotensin-converting enzyme inhibitors, statins, and thienopyridine inhibitors, was similar between the two groups as well.

Table 2. Analyses of Time-to-Event Among Patients According to Aspirin Sensitivity

	HR (95% CI)	p Value
Univariate analyses		
Death/MI/CVA	3.12 (1.10–8.90)	0.03
Death	2.98 (0.68–13.14)	0.15
MI	1.91 (0.25–14.72)	0.54
CVA	5.44 (0.60–49.49)	0.13
Multivariate analyses		
Aspirin resistant	4.14 (1.42–12.06)	0.009
Age	1.05 (1.02–1.08)	0.003
Platelet count	1.01 (1.00–1.01)	< 0.001
History of CHF	3.04 (1.15–8.03)	0.025

CI = confidence interval; HR = hazard ratio. Other abbreviations as in Table 1.

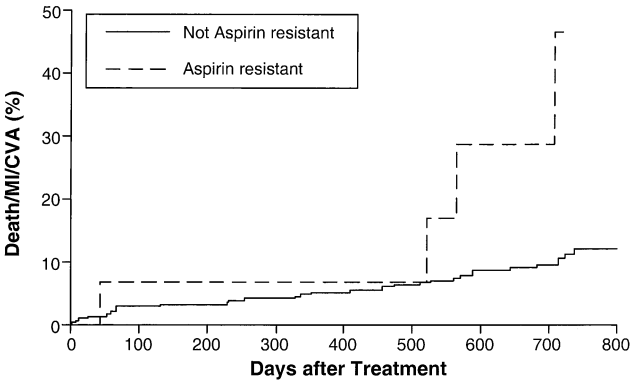

Figure 1. Time-to-event curves, log rank chi-square = 5.05, p = 0.03. CVA = cerebrovascular accident; MI = myocardial infarction.

Clinical events. Major events occurred in 34 (10%) of the 326 patients. Among the patients who were aspirin resistant, 4 of 17 (24%) experienced death, MI, or CVA, compared with 30 of 309 (10%) patients who were not aspirin resistant (p = 0.03). Hazard ratios (HRs) for both univariate and multivariate analyses are outlined in Table 2. Patients who were aspirin resistant were more likely to experience a major clinical event compared with patients who were not aspirin resistant. Although the association for aspirin resistance and adverse individual outcomes did not reach statistical significance, there was a consistent unfavorable trend associated with aspirin resistance for these end points as well (death 12% aspirin resistant vs. 5% not aspirin resistant, p = 0.13, MI 7% aspirin resistant vs. 4% not aspirin resistant, p = 0.54, and CVA 12% aspirin resistant vs. 1% not aspirin resistant, p = 0.09). Figure 1 depicts the Kaplan-Meier time-to-event curves for event-free survival based on aspirin sensitivity with log-rank test results. After adjustment for other clinical factors, aspirin resistance remained an independent predictor of long-term adverse events (HR 4.14, 95% confidence interval [CI] 1.42 to 12.06), p = 0.009). Other factors identified as correlates of poor outcome were history of congestive heart failure, elevated platelet count, and increasing age.

Although the primary purpose of this study sought to

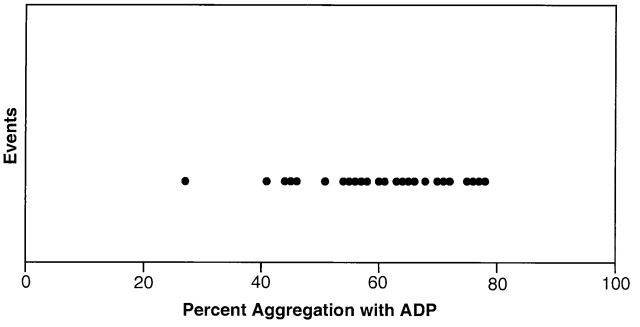

Figure 2. Events (death, myocardial infarction, or cerebrovascular accident) by mean percent platelet aggregation with adenosine diphosphate (ADP).

evaluate clinical risk associated with a predefined set point of platelet aggregation despite aspirin therapy, we further evaluated events on a continuous basis. Figure 2 depicts all events (death, MI, or CVA) in relation to the mean percent platelet aggregation with ADP. Although the results do not reach statistical significance, a distinct gradation of increased risk for clinical events with increased platelet aggregation despite aspirin therapy can be seen.

DISCUSSION

In this blinded, prospective study, we demonstrated aspirin resistance as documented by optical platelet aggregation testing to be negatively associated with long-term outcomes in a population of stable cardiovascular patients. Previous studies have demonstrated aspirin resistance by both clinical evidence of unresponsiveness to aspirin (8) and ex vivo platelet function testing (5,9–13). To date only three studies have evaluated the clinical consequence of aspirin resistance in select populations (14–16). Grotemeyer et al. (14) evaluated 180 acute stroke patients for evidence of aspirin's effect on platelet reactivity. Patients with elevated platelet reactivity despite aspirin were more likely to experience vascular death, MI, or CVA. Mueller et al. (15) reported an association between failed inhibition of platelet reactivity by aspirin and risk of reocclusion after peripheral vascular angioplasty in patients with claudication. Most recently, Eikelboom et al. (16) reported an increased risk for MI, CVA, or cardiovascular death associated with aspirin resistance as documented by urinary concentrations of 11-dehydro thromboxane B_2 in patients with cardiovascular disease or diabetes and one other risk factor for cardiovascular disease.

The current study extends these previous findings in several important respects. Our study was performed in a prospective, blinded fashion. Those performing the optical aggregation testing were blind to clinical histories, and follow-up was done in a blinded manner without regard to aspirin sensitivity. We prospectively set out to measure the incidence of aspirin resistance in a stable population and to determine its clinical significance. We utilized optical platelet aggregation, the current gold standard of platelet aggregation, to measure aspirin resistance. We established normal values via 40 in-house normal samples. With respect to clinical significance, we used "real-world" end points, including all-cause mortality, MI, and CVA, rather than vascular death or vessel reocclusion. Furthermore, this study evaluated the clinical significance of aspirin resistance in a stable cardiovascular population that had not experienced a recent acute event before enrollment. The natural history of aspirin resistance has not previously been evaluated in this population in a fully prospective manner.

Recent trials have demonstrated the superior clinical benefit of clopidogrel and the combination of clopidogrel with aspirin compared with aspirin alone (17–19). These studies make it apparent that alternative antiplatelet agents will likely play a significant role in the treatment of cardiovascular disease. It is plausible that the clinical benefit of clopidogrel and agents similar to it observed in these trials would be even more pronounced in patients who are aspirin resistant and therefore not benefiting from adequate antiplatelet inhibition. Thus, future treatment of aspirin resistance with additional antiplatelet agents such as clopidogrel may significantly improve the poor prognosis we found associated with this diagnosis.

The late divergence of the event curves that we observed is intriguing. It is not entirely clear why this occurred. That our patient population was composed of patients who were clinically stable upon enrollment and therefore less likely to have acute events during early follow-up may have influenced the timing of events. Alternatively, the small number of patients enrolled may have contributed to skewing the curves. This clinical finding needs to be further investigated with additional, larger trials.

There remain other significant areas in this field that need further investigation. The findings of our study should be confirmed with larger trials in various populations. Although possible explanations of aspirin resistance include the presence of Pl^{A2} polymorphism, degree of cyclooxygenase-2 expression, and the role of erythrocytes, the definitive mechanism of aspirin resistance has not yet been elucidated. This will ultimately be important to allow prospective diagnosis of aspirin resistance, which will help guide the selection of antiplatelet therapy as well as potential pharmacogenomic therapy. Likewise, clinical studies evaluating the possible benefit from alternative antiplatelet agents in aspirin-resistant patients will be important. Finally, the possibility of thienopyridine resistance also warrants investigation.

Study limitations. There are some inherent limitations to our study. Aspirin use was based on answers to questionnaires. Salicylate levels or pill counts were not performed. Aggregation studies were performed only at baseline, and it is possible that response to aspirin is variable. Although our study demonstrated an association between aspirin resistance and long-term clinical events, the overall number of events is small. Our estimate of the clinical consequences of aspirin resistance may therefore be under- or overestimated.

Conclusions. Aspirin resistance is an important and real clinical diagnosis. Our study demonstrates it to be significantly associated with major adverse events during long-term follow-up. The availability of safe, alternative long-term antiplatelet agents makes screening for aspirin resistance in cardiovascular patients a potentially important and useful diagnostic test. Further investigation to confirm our findings, evaluate possible treatments, and elucidate the precise biologic mechanism of aspirin resistance should be performed.

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