Aspirin Resistance: Prospective evaluation of increased cardiovascular risk in aspirin nonresponsive patients

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A. Introduction

a. Rationale
Aspirin works by binding to and acetylating the serine-530 residue in the active site of the cyclooxygenase 1 (COX1) enzyme and thereby blocking the synthesis of thromboxane A2 (TXA2). TXA2 is a potent platelet agonist and vasoconstrictor that is implicated in acute arterial thrombosis. This deactivation is irreversible and maintained for the life of the platelet. The benefit of aspirin therapy alone, or in conjunction with other antiplatelet agents, has been broadly demonstrated in reducing the number of cardiovascular events. Recently, the concepts of clinical and pharmacologic aspirin resistance have been forwarded to explain persistent cardiovascular morbidity despite adequate aspirin use. However, depending on the mode of assay employed, estimates of aspirin resistance range from 5-45%.

Furthermore, there is little prospective data analyzing the clinical implication of documented aspirin resistance. Using a commercially available point-of-care platelet function assay, this large international multicenter prospective trial aims to identify the incidence of aspirin resistance in patients with stable cardiovascular disease and to correlate aspirin non-responsiveness to adverse clinical events.

b. Literature Review
Aspirin is widely used in both primary and secondary prevention of cardiovascular disease. In the Physicians Health Study, when aspirin therapy was compared to placebo, a 44% reduction in the risk of myocardial infarction was observed in the treatment group. When used in conjunction with thrombolytic therapy, aspirin use is associated with a nearly 25% reduction in mortality following an MI. Similarly, the large Antiplatelet Trialists' Collaboration (ATC) meta-analysis showed that aspirin therapy was associated with a 48% reduction in vascular graft and arterial occlusion. Despite the impressive body of evidence that has amounted of the past 25 years supporting aspirin's role in preventing cardiovascular disease, patients treated with aspirin continue to suffer from ischemic events. Moreover, even with continuous aspirin therapy, laboratory evidence of continued platelet aggregation can be demonstrated in at risk individuals. Recently, numerous investigators have forwarded the emerging concept of aspirin resistance to explain this relative treatment failure.

Alternative anti-platelet agents, such as the thienopyridines (clopidogrel and ticlopidine), have been shown to provide additional benefit when used in conjunction with aspirin therapy. These agents work by blocking the ADP receptor on platelet membranes leading to the blockade of the ADP-dependent activation of the glycoprotein IIb/IIIa complex. Experience from the CAPRIE and CURE trials have shown the additional benefit of ADP receptor blockade with clopidogrel; when clopidogrel was added to aspirin therapy, individuals at risk demonstrated a 7% reduction in relative risk for recurrent stroke, MI, or vascular death and a 20% reduction in post-MI vascular events (cardiovascular death, non-fatal MI, and stroke). The added benefit of thienopyridines is likely a multifactorial process that is likely to include aspirin non-compliance, TXA2 independent platelet activation (i.e. serotonin and thrombin), and quite possibly aspirin resistance. However, what is not immediately evident in these clopidogrel studies is the narrow therapeutic risk to benefit ratio. With every two individuals prevented from reaching the primary endpoint, one additional serious bleeding episode will occur. Moreover, the cost of adding these additional antiplatelet agents to standard anti-thrombotic therapy is not insignificant. Tailoring this therapy to individuals at risk for thrombotic events, such as those with documented aspirin resistance, would perhaps take advantage of the benefits of thienopyridines while improving upon the risk to benefit ratio.
Nonetheless, the thienopyridine studies provide compelling evidence that aspirin therapy alone is often inadequate anti-thrombotic therapy. This was particularly evident in the PURSUIT trial\textsuperscript{10}, which evaluated the role of glycoprotein IIb/IIIa inhibitors in acute non-ST segment elevation MI. In this trial, 64\% of individuals had been taking aspirin for at least 2 weeks prior to presenting with infarction. As of yet, it remains unclear what role aspirin resistance plays in these examples of treatment failure.

Aspirin resistance can be defined in both laboratory and clinical terms. Thrombotic disease in individuals despite aspirin use is considered clinical resistance. By contrast, in vitro analysis of aspirin defines laboratory aspirin resistance, of which there are two distinct operational definitions: pharmacologic failure (i.e. the continued production of Thromboxane A2) and continued platelet aggregation. Eikelboom et al\textsuperscript{11} recently demonstrated that a significant number of individuals given aspirin displayed systemic evidence of TXA2 production. However, it makes intuitive sense that laboratory definitions based on platelet function would be more appropriate surrogates for clinical aspirin therapy. For this reason, platelet aggregometry is the often considered the gold standard of laboratory platelet function\textsuperscript{12}. Classically, this is done by taking a platelet rich serum, adding a platelet agonist (such as ADP or arachidonic acid), and using optical density to assess for percentage of platelet aggregation. This technique is not readily available and usually reserved for experimental analysis. Moreover, it is largely regarded as non-physiologic. It ignores the flow and shear stress of in vivo thrombosis and ignores the contribution of erythrocytes on hemostasis\textsuperscript{13}. The Platelet Function Analyzer (PFA) is a promising technique for the rapid assessment of aspirin resistance. Its benefits include ease of use, rapid analysis, and reproducibility of results. Whole blood is passed through a capillary tube into an aperture on a collagen/agonist membrane. The time until platelet plug formation resulting in cessation of flow is the outcome measure. By using whole blood and approximating shear and flow forces, this method overcomes many of the limitations of optical density aggregometry. The utility of this assay was recently demonstrated by Wang et al\textsuperscript{5}. They reported the incidence of aspirin nonresponsiveness in 422 aspirin users by means of the commercially available point-of-care Ultegra Rapid Platelet Function Assay-ASA (RPFA-ASA) and found nearly one in four participants to be aspirin nonresponders (24\%).

Thus far, only one small prospective trial has attempted to look at aspirin response and future incidence of cardiovascular events. Using optical platelet aggregation, Gum et al\textsuperscript{2} looked at the incidence of aspirin resistance in 326 stable cardiovascular patients. Of the patients studied, 17 (5.2\%) were considered aspirin resistant. After a two-year follow-up, they reported that aspirin resistance was associated with an increased risk of death, MI, or CVA compared to their aspirin sensitive patients (24\% vs. 10\%). However, their sample size was small and their event rate even smaller (only 4 cases). Significance in this study would have been lost if there were one fewer events, making the possibility of committing an alpha error not impossible. Nonetheless, this study is an important first step in correlating aspirin resistance with actual clinical practice.

Aspirin resistance is clearly a multifactorial process. In addition to the possibility of medication noncompliance and inadequate dosing, there are three general categories that are theoretically involved: 1) Non-COX-1 mediated platelet activation 2) Increased platelet activity and 3) Increased platelet turnover. There are many known platelet agonists of which both serotonin and thrombin have been shown to activate platelets despite aspirin therapy. Additionally, although thromboxane A2 is irreversibly inhibited by aspirin, neighboring nucleated cells such as vascular endothelial cells (VECs) and smooth muscle cells are known to upregulate their own COX-2 expression during vascular injury\textsuperscript{14}. They convert AA to PGH2, which can be transported into platelets and converted to TXA2. Finally, catecholamine release during times of stress (injury, exercise) can serve as prothrombotic platelet agonists\textsuperscript{12}. Alternatively, the platelets themselves may be more susceptible to activation. This can be due to interactions with erythrocytes\textsuperscript{13}, polymorphisms in the IIb/IIIa receptor that lowers the threshold for activation\textsuperscript{15}, or increased sensitivity to collagen activation. All of these mechanisms have been shown to promote platelet aggregation even in the presence of adequate aspirin therapy. Finally, during periods of increased platelet turnover (major bleed or recent surgery) the fraction of circulating platelets with inactivated COX-1 may be temporarily altered promoting a pro-thrombotic state\textsuperscript{12}. 
While novel anti-platelet agents are continually being developed, a surmounting body of evidence is returning our attention to optimizing therapy with aspirin. A clinically significant determination of aspirin nonresponsiveness must first be reliably established. Only then can the question of proper therapy be addressed. This investigation, a large, multicenter prospective trial, is indicated to corroborate early suggestions of a connection between aspirin resistance and cardiovascular event outcome, thereby assisting the risk stratification, and tailored anti-thrombotic therapy.

c. Hypothesis
A substantial percentage of individuals are aspirin nonresponsive, and therefore, at risk for clinically significant cardiovascular events (death, MI, or cerebrovascular events). The identification of the individuals in a rapid and reliable fashion will allow for improved risk stratification. It will assist in tailored thienopyridine therapy and help clinicians improve upon the risk benefit of adding clopidogrel to prevent ischemic disease.

B. Methods

a. Conceptual and Operational Definitions
The primary outcome is the composite of death, myocardial infarction (MI), and cerebrovascular accident (CVA). Secondary endpoints will be the individual events of death, MI, and CVA. For the purpose of this study, death is defined as all-cause mortality due to MI, ischemic CVA, and other vascular and non-vascular causes. Myocardial infarction is defined as the presence of at least two of the following criteria: (1) prolonged angina for ≥20 minutes (2) elevation in troponin I or CK-MB of at least two times the upper limit of normal (3) ischemic changes on electrocardiogram (ST segment elevation of ≥0.1 mV in two contiguous leads, new significant Q waves). CVA is defined as an acute neurological event with focal signs for more than 24 hours.

Definition of aspirin nonresponsiveness is directed by the predetermined values of the Ultegra RPFA-ASA assay (Accumetrics®), as validated by Wang et al. Extent of platelet aggregation is reported in aspirin reaction units (ARUs). An ARU greater than 550 is consistent with no platelet dysfunction, while values less than 550 are indicative of platelet dysfunction. For a patient taking aspirin, an ARU greater than 550 is consistent with aspirin nonresponsiveness. Using platelet aggregometry as the comparative gold standard, Malinin et al. determined the sensitivity and specificity of the Ultegra system to be 92% and 85% respectively, by testing aspirin naïve healthy individuals 2 and 30 hours after ingestion of 325mg of aspirin.

b. Study Design
This is a multicenter, prospective, blinded observational study. Eligible participants will be recruited from outpatient clinics or upon presentation for elective cardiac catheterization. To be eligible for this study consenting adults ≥ 21 years of age, with a known history of cardiovascular disease, who, by self-report, have been taking aspirin for ≥7 days prior to study entry. Informed consent at the time of study enrollment will be obtained. Demographic information (age, gender, cardiac risk factors) and aspirin dosage will be recorded. Three tubes of whole blood will be collected from each participant; the first tube containing 3.2% sodium citrate will be used for the RPFA-ASA analysis. A second tube is anticoagulated with EDTA for assessment of baseline hemoglobin and platelet counts. A third tube will be taken for serum analysis of salicylate level and creatinine. Follow up for a period of two years via telephone interview and query of the Social Security Death Index will be performed for all participants at 3-month intervals. Interviewers will be blind to the results of the aspirin resistance testing. Chart review on all participants meeting study endpoint will be done to corroborate that study criteria were in fact achieved.

c. Statistical Analysis
Categorical data (participant demographics, aspirin resistant vs. sensitive, presence vs. absence of study endpoint) will be reported as percentages and frequencies. Fisher’s exact test will be used to
compare categorical variables. Continuous data are presented as means ± standard deviations (SD). If a normal distribution is obtained for continuous data, Student’s t test will be used to compare means. If data are not normally distributed, the Wilcoxon two-sample test will be used. Kaplan-Meier curves will be computed for freedom from the composite endpoint of death, MI, or CVA and for each of the secondary endpoints, with respect to aspirin resistance status. Finally, both univariate and multivariate logistic and linear regressions will be performed to determine significant predictors of aspirin non-responsiveness. The following variables will be entered into the model: age, gender, race, and history of tobacco use, diabetes, hypertension, hyperlipidemia, revascularization, MI, hemoglobin, platelet count, and creatinine.

d. Sample Size
With 250 participants in each group, the study will be 80% powered to detect a significant difference of 10% between the aspirin resistance and responsive individuals. Given the 25% prevalence in Wang et al’s study using the Ultegra system, we would have to screen roughly 1000 individuals to identify the requisite number of aspirin resistant participants. Therefore, enrollment will continue until 250 aspirin resistant individuals identified, or 1000 participants enrolled, whichever is achieved first.

C. Subject Selection

Inclusion criteria
- To be eligible for the study participants must be at least 21 years of age
- a prior history of cardiovascular disease (defined by previous cardiac catheterization documenting stenosis ≥ 60%)
- previous history of MI or CVA
- or previous invasive revascularization procedure
- and reported taking 325mg of aspirin for at least 7 days prior to study enrollment.

Exclusion criteria
- use of ticlopidine, dipyridamole, systemic steroids or other NSAIDs
- administration of heparin or low molecular weight heparin within 24 hours of study enrollment
- major surgical procedure within one week of study enrollment, malignant paraproteinemia
- family or personal history of bleeding disorder
- platelet count < 150,000 or > 450,000 per microliter
- hemoglobin < 8g/dl
- and history of either myeloproliferative disorder or heparin induced thrombocytopenia.

D. Miscellaneous

Informed consent will be obtained prior to enrollment in the study. All data will be kept strictly confidential. No minors will be enrolled in this protocol. There will be no financial compensation to the study participants and no cost to participate.

E. References


