Prevalence of High Platelet Reactivity in Aspirin-Treated Patients Referred for Coronary Angiography

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Abstract

Background: Aspirin (ASA) reduces adverse events in coronary artery disease (CAD) patients by inhibiting platelets. Some CAD patients have high platelet reactivity (HPR) despite ASA therapy and these individuals have increased risk of adverse events.

Objective: The purpose of this study was to determine the prevalence of HPR in ASA-treated patients referred for coronary angiography and to assess whether the HPR correlates with the severity of CAD.

Methods: This single center investigation enrolled 115 consecutive ASA-treated patients with stable CAD. ADP- and collagen-induced platelet reactivity were evaluated by light transmittance aggregometry (LTA). Patients with greater than 70% ADP- and collagen-induced aggregation were determined to have HPR and, in this group, ASA compliance was assessed by examining blood salicylate levels. Mean age was 60.9 years and average ASA dose was 164.2 mg.

Results: Smoking and DM were present in 28.7% and 31.5% respectively. HPR was found in 14 patients (13%) however 7 of the 14 patients (50%) with HPR had low serum salicylate levels (< 2.0 μg/mL) suggesting medication noncompliance. Of the entire cohort, 6.5% of patients had HPR and detectable serum salicylate levels suggesting reduced ASA efficacy. HPR correlated with number and severity of coronary stenosis (p = 0.04).

Conclusion: In a general population of ASA-treated patients referred for coronary angiography, elevated platelet reactivity is prevalent (13%) with 50% related to noncompliance and 50% related to reduced aspirin efficacy.(Arq Bras Cardiol. 2013;100(1):29-36)

Keywords: Coronary disease; platelet aggregation; aspirin / therapeutic use; coronary angiography.

Introduction

Aspirin (ASA) is a primary therapy for the treatment of cardiovascular (CV) disease because it reduces major adverse CV events (MACE) by 25% in acute coronary syndrome (ACS) and stable coronary artery disease (CAD) patients[1]. Despite treatment with optimized medical regimens that include ASA, MACE rates are greater than 10% in stable CAD patients and greater than 28% in ACS patients[2]. Pharmacologic variability and reduced therapeutic efficacy may contribute to relatively high rates of recurrent CV events in CAD patients, and several studies have demonstrated that subsets of aspirin-treated patients have elevated residual platelet reactivity (ASA hyporesponders){Gum, 2003 #24}{Angiolillo, 2009 #711}{Helgason, 1994 #26}. Following percutaneous coronary intervention, ASA-treated patients with elevated platelet reactivity are at increased risk for periprocedural myocardial infarction (M.I.), MACE, and stent thrombosis[3,4].

The prevalence of high platelet reactivity (HPR) in ASA-treated patients varies between 0.4 and 34% depending on the patient population (healthy, stable CAD, ACS, diabetic), the type of platelet function test used, and the parameter or cutoff value which is chosen to define HPR[5,6]. The lack of standard laboratory methods and definitions to define aspirin hyporesponsiveness, also called resistance, has resulted in confusion about the clinical incidence of this phenomenon[7,8].

The purpose of this study was to determine the prevalence of HPR in aspirin-treated patients referred for coronary angiography with the goal of demonstrating the proportion of HPR related to medication noncompliance vs. reduced aspirin efficacy.

Methods

The clinical investigation protocol was approved by the Institutional Review Board of the Instituto de Cardiologia do Rio Grande do Sul and all patients provided informed consent. This single center study enrolled consecutive

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patients with stable CAD who were referred for coronary angiography between January 2006 and July 2007. Inclusion criteria required ongoing ASA use for more than 2 weeks, age > 18 years, and willingness to participate with informed consent. Exclusion criteria are listed in Table 1. Patients were excluded if they were taking concomitant medications (anticoagulants or non-steroidal anti-inflammatory drug -NSAID) that could inhibit platelet function or if they had medical conditions that influence hemostasis. Blood samples were collected from femoral artery or vein after sheath insertion and before administration of heparin or IIbIIIa inhibitors. Samples were immediately placed in evacuated tubes containing 3.2% citrate or EDTA 1.8%. Blood samples were obtained prior to administration of aspirin in preparation for coronary angiography, and platelet function testing occurred within 2 hours of obtaining samples. Platelet reactivity was assessed using light transmittance aggregometry (LTA) of platelet rich plasma on a dual channel Net Lab 2020 aggregometer using standard methods. Whole blood was centrifuged at 162g for 6 minutes to prepare platelet rich plasma (PRP). An aliquot of PRP was further centrifuged at 838g for 15 minutes to prepare platelet poor plasma that represented the baseline turbidity value equivalent to 100% aggregation. Platelet activity was measured by assessing aggregation in response to activation with 20μmol/mL of ADP or 10μg/mL of collagen. HPR was defined as 70% or greater aggregation for both ADP- and collagen-induced activation at 5 minutes. Plasma samples were stored at -80ºC to enable assessment of compliance in patients that had HPR by measuring blood salicylate levels using a commercial assay (Sigma Chemical Co., USA). Patients with blood salicylate levels less than 2 μg/mL were determined to not be taking chronic aspirin therapy. The extent and severity of coronary artery disease was assessed by two independent interventional cardiologists who were blinded to the results of the platelet function testing. Coronary stenoses were counted if both cardiologists assessed severity to be greater than 50%. Statistical analyses were performed using SPSS 15.0 statistical analysis software and Stata 9.0 (StataCorp, College Station, TX). Data are reported as mean ± standard deviation and normally distributed data were assessed using the Student *t* test. For correlation between platelet aggregation and severity of CAD, Pearson correlation coefficient was used, and for categorical variables, the Fischer exact test was used. The correlation between aspirin hyporesponsiveness and the number of diseased arteries was evaluated with logistic regression analysis. Findings with $p < 0.05$ were considered significant.

Results

Of the 125 patients enrolled in the study, 115 were included in the final analysis; 3 patients were excluded due to evidence of coagulation in the blood samples and 7 patients were excluded because there was greater than a 2 hour delay in the time required to perform aggregation studies. The baseline characteristics of the patients are listed in Table 2. The mean dose of aspirin was 164.7 ± 60.3 mg. A representative LTA curve is shown in Figure 1. For individual patients, ADP- and collagen-induced aggregation showed significant correlation when assessed by Pearson's coefficient (r = 0.68) (Figure 2). The extent of ADP- and collagen-stimulated aggregation did not vary across a spectrum of ASA doses (Figure 3), and specific indices of ADP- and collagen-initiated platelet aggregation are listed in Table 3.

Results of the platelet aggregation studies demonstrated that 101 patients (87%) met criteria for adequate ASA response (<70% collagen- or ADP-induced aggregation), while 14 patients (13%) had HPR with aggregation values greater than 70%. When we analyzed the blood from the 14 patients with HPR, 7 patients (50%) had blood salicylate levels less than 2 μg/mL (0.57 ± 0.24 μg/mL), and this group was determined to be noncompliant with ASA therapy. The remaining 7 patients had both HPR and a plasma salicylate level greater than 2 μg/mL. These 7 patients were determined to be ASA hyporesponders, and had a mean salicylate level of 18.4 ± 6.1 μg/mL. When ASA hyporesponsive patients were compared to those with normal responses, the groups did not differ in age, gender, presence of diabetes mellitus, history previous myocardial infarction or aspirin dose (Table 4). The rates of smoking and diabetes were higher numerically in the ASA hyporesponsive group (Diabetes OR 3.16, 95% CI 0.66 to 14.9, p = 0.2; Tobacco 3.61, 95% CI 0.75 to 17.24, p = 0.19) but these differences did not meet statistical significance. When the presence of CAD, (defined as > 50% stenosis of any epicardical heart artery) was evaluated, 85.7% of the ASA hyporesponders had significant CAD compared to 39.6% of the ASA responsive patients (OR 9.15, 95% CI 1.06 to 78.8). Aspirin hyporesponsiveness was associated with a higher number of diseased coronary arteries (OR 3.58 per vessel, 95% CI 1.69 to 7.61, $r^2 = 0.24$), with the ASA hyporesponders having an average of 1.85 ± 0.89 diseased coronary arteries per patient compared to 0.43 ± 0.79 in

Table 1 - Exclusion Criteria

History of recent surgery	Clopidogrel therapy
Bleeding diathesis	Ticlopidine therapy
Thrombophilia	Non-steroidal Anti-inflammatory (NSAID) therapy
History of cancer	Heparin or oral anticoagulant therapy

Table 2 - Baseline Characteristics

Mean age, years	60.94
Mean ASA dose, mg	164.7 ± 60.3 mg
Male gender	53.7%
Diabete Mellitus	31.5%
Smoking	28.7%
Dyslipidemia	62%

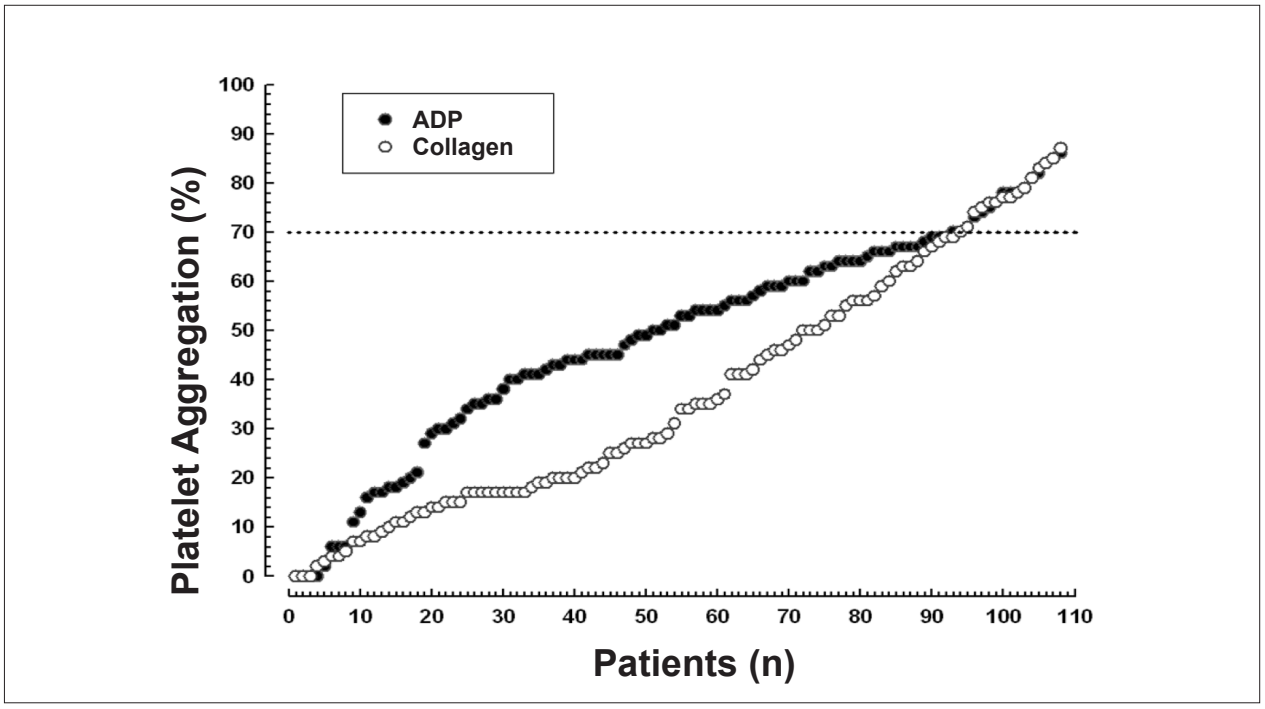

Figure 1 - *Representative platelet aggregation curve for ADP- and Collagen-stimulated platelet aggregation. High platelet reactivity was defined as 70% or greater aggregation for both ADP- and collagen-induced activation at 5 minutes.*

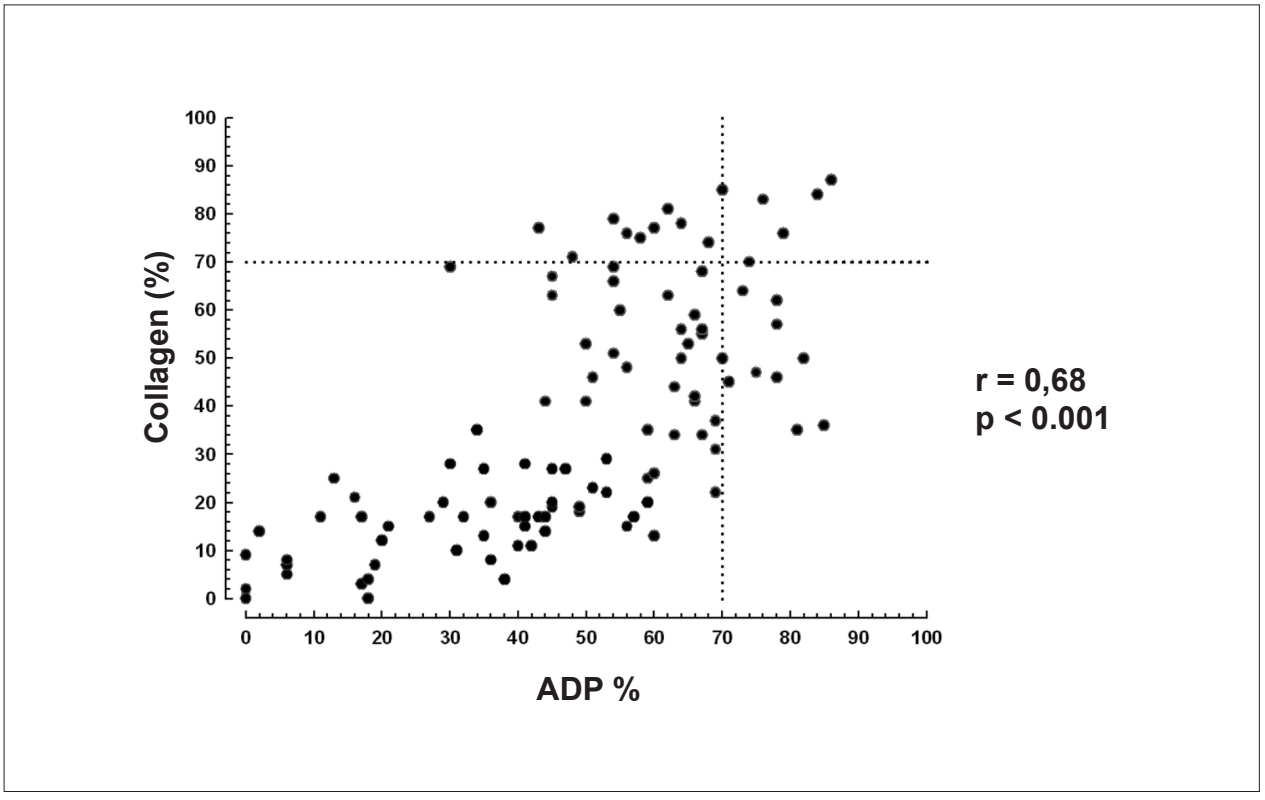

Figure 2 - *Correlation between ADP- and Collagen-stimulated platelet aggregation (Pearson's coefficient, r = 0.68).*

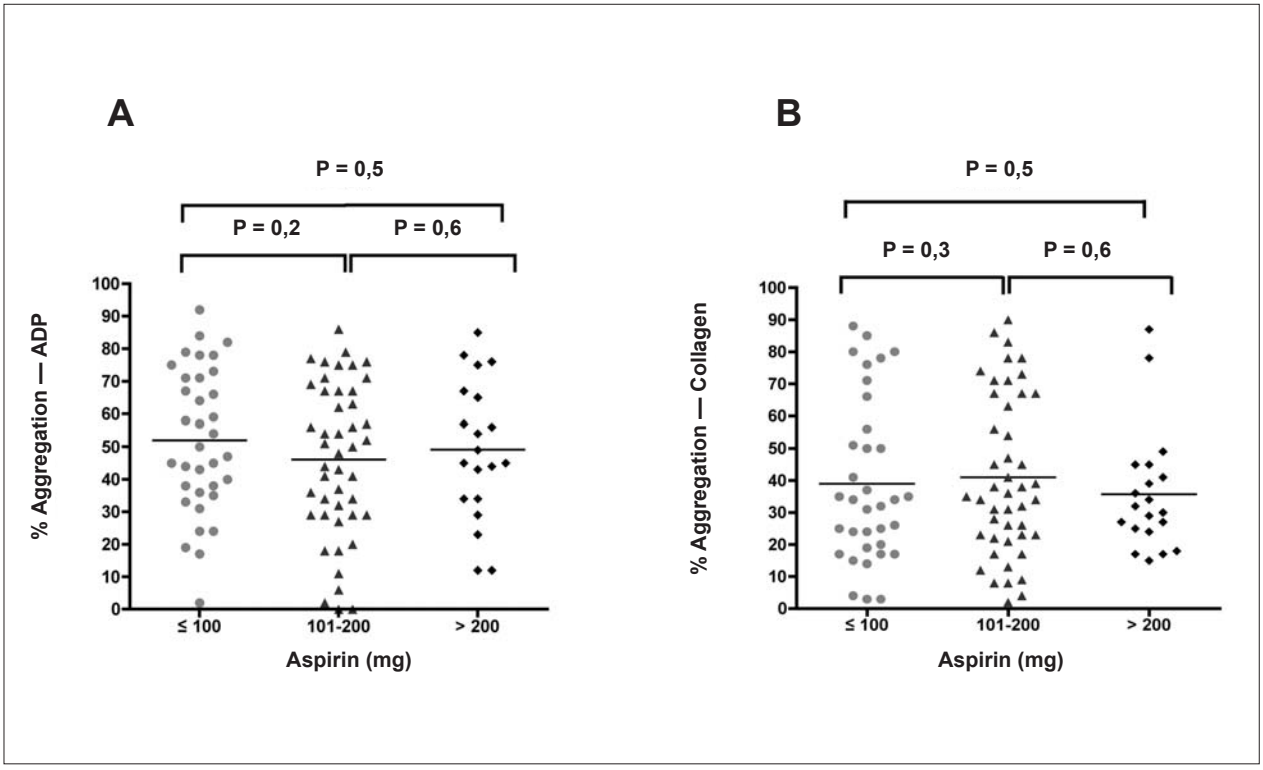

Figure 3 - *ADP- and Collagen-stimulated platelet aggregation and ASA dose. (A) ADP- stimilated aggregation. (B) Collagen-stimulated aggregation.*

Table 3 - Indices of ADP- and Collagen-Stimulated Platelet Aggregation

	ADP	Collagen
Mean ± SD	48.5 ± 22.0	37.1 ± 24.8
Median	52	32.7
Minimum	0	0
P 25	35.3	17
P 75	52.8	56.8
Maximum	86	87

SD : standard deviation; P25 : 25th percentile of platelet aggregation; P75 : 75th percentile of platelet aggregation.

the ASA sensitive group (p = 0.04) (Table 4). When patients were stratified by the number of coronary arteries with >50% stenosis, the incidence of ASA resistance increased with number of diseased arteries (Figure 4). There was no difference among aspirin responders and hyporesponders in the location of coronary stenoses (left main, left anterior descending, left circumflex, right coronary arteries).

Discussion

There is increasing scientific evidence supports the premise that there is significant interindividual variability in the degree of platelet inhibition achieved with common antiplatelet therapies. Antiplatelet response variability is an important clinical problem because CAD patients that have HPR in the setting of ASA or platelet P2Y12-ADP receptor antagonist therapy have increased risk of CV events[9]. The greatest risk of antiplatelet therapy hyporesponsiveness occurs following PCI and stenting where HPR in the setting of ASA and ADP-receptor antagonist dual antiplatelet therapy (DAPT) is associated with a 2-fold increase in the risk of stent thrombosis[3,10]. Stent thrombosis is an infrequent but serious complication, which results in myocardial infarction rates exceeding 60%, case fatality rates ranging from 10 to 45%[11-14], and increased long term MACE[15]. Several clinical factors including ACS presentation, body mass index (BMI) > 30 kg/m^2, diabetes, and congestive heart failure (CHF) are associated with hyporesponsiveness to antiplatelet therapies[16]. The prevalence of hyporesponsiveness to antiplatelet therapy varies greatly (0.4 to 83.3%) depending on the drug under examination, and the clinical and ethnic population assessed[17]. The prevalence of aspirin or ADP-receptor antagonist hyporesponsiveness also varies significantly depending on the method used to assess platelet activation and on the cut-point chosen to determine responsiveness[5]. More than five methods for assessing platelet function are available, and the specific tests differ in their ability to predict CV MACE[4,18]. LTA was used in this investigation because it is a well-validated method that accurately identifies HPR patients who are at increased risk of CV events and stent thrombosis[10,18].

Table 4 - Clinical Characteristics of ASA Hypo-responders and ASA Sensitive groups

	ASA hyporesponders n = 7	ASA sensitive n = 101	OR	CI 95%	p
Age, years	61.7 ± 10.1	60.75 ± 11.7	-	-	0.8
ASA Dose	164.3	158.5	-	-	0.4
Male Gender	4 (57.1%)	54 (53.5%)	1.16	-	0.99
Smoking	4 (57.1%)	27 (27.0%)	3.61	0.75 - 17.24	0.19
DM	4 (57.1%)	30 (29.7%)	3.16	0.66 - 14.9	0.2
Previous MI	1 (14.3%)	36 (36.4%)	0.29	0.034 - 2.5	0.4
Dyslipidemia	5 (71.4%)	62 (62%)	1.5	0.29 - 8.3	0.7
CAD	6 (85.7%)	40 (39.6%)	9.15	1.061 - 78.8	0.04
Diseased Vessels	1.85 ± 0.89	0.43 ± 0.79	3.58	1.69 – 7.61	< 0.01

OR : Odds Ratio; CI : confidence interval; DM : diabetes mellitus, MI : myocardial infarction; CAD : coronary artery disease with >50% stenosis in 1 or more coronary arteries.

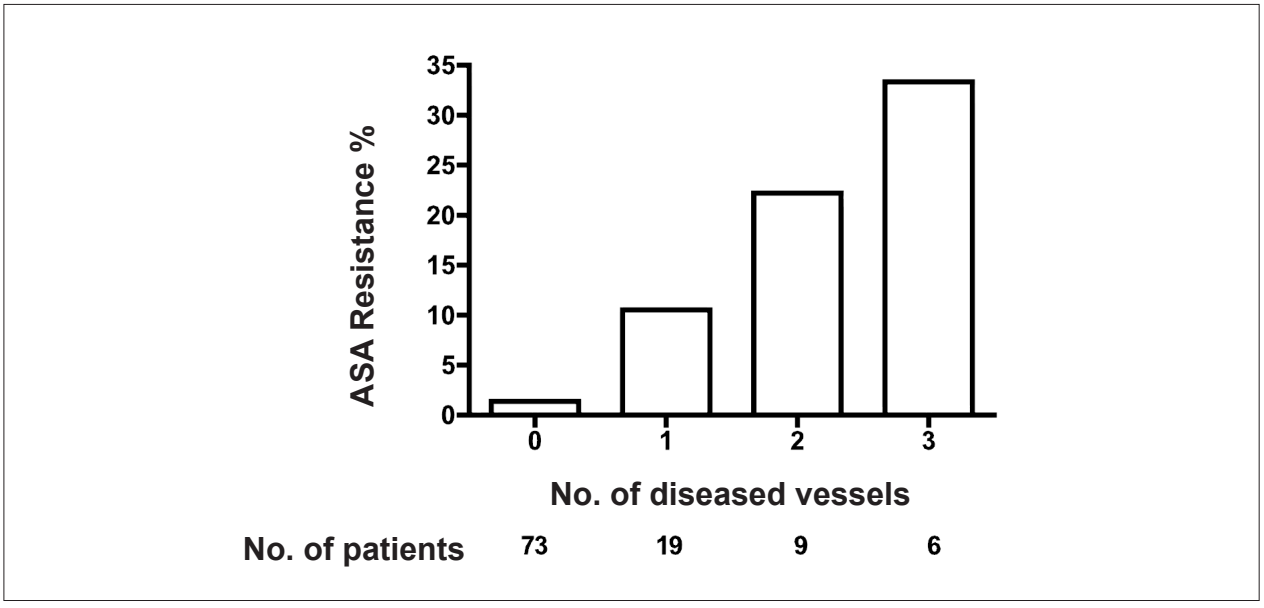

Figure 4 - *Aspirin resistance as function of the number of coronary arteries with >50% stenosis. Graph depicts the percentage of patients with 0, 1, 2, or 3 vessel CAD who were found to be ASA hypopresponders. The number of patients in each category is listed in the bottom column.*

It is increasingly evident that PCI patients who are hyporesponders to both aspirin and the ADP receptor antagonist clopidogrel are at markedly increased risk for CV events[3]. Recently, the mechanisms of clopidogrel hyporesponsiveness have been the focus of several clinical studies which have demonstrated that clopidogrel hyporesponsiveness results in part from reduced-function genetic polymorphisms in the cytochrome P450 enzyme pathways that convert clopidogrel, which is a prodrug, to its active form[10]. Additional mechanisms of clopidogrel hyporesponsiveness have been reviewed previously[9].

Aspirin inhibits platelet activation by acetylating the platelet cyclooxygenase (COX) enzyme resulting in irreversible inactivation and suppression of COX-mediated production of thromboxane (TX), which is a potent platelet activating agent. Compared to clopidogrel, the mechanisms of ASA hyporesponsiveness are less clear. Noncompliance is a very important factor that contributes to reduced medication efficacy and elevated platelet reactivity. A meta-analysis demonstrated that aspirin non-adherence or withdrawal is common, and is associated with three-fold higher risk of major adverse cardiac events in CAD patients[19].

The mechanisms of ASA hyporesponsiveness are possibly related to pharmacodynamic and pharmacogenetic variability in several physiological processes. ASA bioavailability can be affected by alterations in intestinal absorption; ASA can be hydrolyzed and inactivated by intestinal mucosal esterase and there is potential for interindividual variability in the

expression and activity of these enzymes. In addition, aspirin dosing can potentially affect the degree of platelet inhibition and several clinical studies have examined the optimal dose of ASA with the goal of maximizing CV benefit while minimizing bleeding risk. Although some studies have shown that ASA doses as low as 30 mg per day will fully inhibit COX-1 activity in platelets[20], recommended dosing for CV therapy varies between 75 mg and 325 mg[21]. Early reports raised the possibility that ASA doses lower than 100 mg per day could result in increased rates of high platelet activity and increased CV MACE in patients with stable coronary artery disease[22]. However, despite this initial concern, large-scale clinical outcome studies have demonstrated equivalent CV efficacy for doses over 100 mg/day[1,23,24].

Drug-drug interactions also have the potential to alter ASA efficacy. In particular, several NSAID have been shown to compete with ASA for binding to the active site of COX-1. Because these NSAIDS are non-covalent inhibitors with short half-lives (6-12h), concomitant use with ASA results in allosteric blockade of the COX active site. This NSAID-mediated blockade of the COX active site prevents ASA-mediated irreversible inactivation of COX and results in rapid recovery of COX function and increased TX formation. NSAID use in the setting of ASA therapy for CAD has been shown to increase the risk of M.I. in clinical studies[7]. Proton pump inhibitors (PPI) could also potentially alter ASA bioavailability by potentiating gastric hydrolysis of ASA[20]. PPIs are frequently prescribed in combination with antiplatelet therapy in order to prevent gastrointestinal bleeding. Despite concern for reduced efficacy of ASA and clopidogrel in the setting of use with concomitant PPI's, clinical data have failed to demonstrate that PPI use increases MACE in DAPT-treated patients following PCI[25].

Elevated Body Mass Index (BMI) and diabetes mellitus (DM) are associated with HPR[4,26] and patients with ACS consistently have elevated baseline platelet activity in the setting of DAPT[26]. Although obesity, DM, and ACS are associated with ASA hyporesponsiveness, the exact molecular mechanism involved in ASA resistance in these patients has not been clearly established[6,27]. Increased inflammation in obesity, diabetes, and ACS likely promotes platelet activation and contributes to reduced antiplatelet efficacy by increasing activation of inflammatory pathways that increase platelet COX activity[28]. Although ASA doses >100 mg/day can effectively block COX-1 function, COX-2 has been identified as potential additional source of TX that might contribute to elevated residual platelet responsiveness[29,30]. Until recently, COX-1 was thought to be the only isoform expressed in platelets, but it is now recognized that the platelets of patients with active inflammation (surgery, infection, and disorders with increased platelet turnover) express COX-2[31]. The degree to which COX-2 contributes to TX production *in vivo* is unclear[22]. However, the presence of elevated inflammatory mediators in ACS and diabetes may increase COX activity resulting in chronic baseline platelet activation. Recent data also support the premise that inflammation increases platelet activation pathways, demonstrating that platelets of patients with ACS have increased megakaryocyte-derived proinflammatory mRNA transcripts[32].

Genetic polymorphisms of the proteins involved in platelet activation pathways could also contribute to mechanisms of ASA hyporesponsiveness. Single nucleotide polymorphisms (SNPs) in COX-1 have the potential to impact enzyme activity and/or efficacy of inhibitors such as ASA, thereby contributing to ASA hyporesonsiveness[29].

The prevalence of ASA hyporesponsiveness in our investigation (6.5%) agrees with reported values. In our study, we chose LTA to assess platelet function because LTA is a well-validated method which has proven ability to identify patients with HPR who are at risk for MACE[10,33]. In order to assess compliance, we used a standard assay for measurement of plasma salicylate[27]. In previous studies, blood plasma salicylate levels were 19μg/mL +/- 3μg/mL after ingestion of 320 mg of aspirin[34]. In our study, aspirin users with high residual platelet activity had similar plasma salicylate levels (18.4 ± 6.1 μg/mL) which confirms medication compliance. Urinary TX measurement is another method which can be utilized to asses ASA compliance[35], although this method is less direct and has the potential to be influenced by non-platelet COX-2 activity as discussed above[36].

For our study, we specifically chose a stringent cutoff of >70% ADP- and collagen-stimulated platelet aggregation to identify ASA hyporesponders, as this methodology is consistent with other investigations examining ASA compliance[36-38]. Although a cut-point of 50% aggregation has been used by some groups to assess ASA and/or clopidogrel hyporesponsiveness in the setting of activation with either 5μM/mL or 20μM/mL ADP[10,18], striving to obtain stringent specificity, we elected to use 10μg/mL collagen and 20μM/mL ADP doses. The aggregation results obtained with ADP and collagen showed strong correlation in the extent of platelet activation (Pearson's coefficient, r = 0.68). This correlation between ADP- and collagen-activated platelet aggregation demonstrates similar state of platelet inhibition following stimulation through two distinct molecular activation pathways. Although we did not directly interrogate COX-dependent platelet activation pathways with the platelet agonist arachidonic acid, the current study does provide meaningful readout of ASA inhibition of ADP- and collagen-mediated platelet responses (Figure 3). In addition, these investigations demonstrate a clear correlation between the extent of coronary artery disease and ASA hyporesponsiveness (Table 4, Figure 4). This correlation between ASA hyporesponsiveness and CAD severity is consistent with previous reports demonstrating persistent formation of TX in ASA-treated patients with CAD requiring surgical revascularization[39] and in patients with known severe CAD[40]. Mechanistically, a higher inflammatory milieu in patients with extensive CAD might promote platelet activation and thus contribute to ASA hyporesponsiveness.

In the current study, it was not possible to ascertain differences in ASA response as a function of salicylate levels because the study was constrained to testing salicylate levels only in patients with HPR. In addition, the small sample size limits conclusions on the role of clinical factors such as tobacco use and DM on ASA response. Despite the small sample size, smoking and DM trended toward significance for association with ASA hyporesponsiveness as has

been shown in previous studies. All the physicians were informed about the results of the residual platelet activity. However, so far there is no data suggesting that changing the antiplatelet therapy on patients with high residual platelet activity would decrease the cardiovascular risk.

Conclusion

In a cohort of stable coronary artery disease patients referred for angiography, 6.5% of patients had HPR despite ASA therapy. Future research should focus on understanding the mechanisms of ASA resistance, and on determining if alternative ASA dosing strategies or adjunctive pharmacotherapy can reduce the risk of CV events in ASA hyporesponsive patients.

Potential Conflict of Interest

Author Rogério Sarmento-Leite reports receiving consulting fees or paid advisory board fees from Lilly Brazil, and Julio F. Marchini reports receiving consulting fees or paid advisory board fees from Lilly Brazil and Daiichi Sankyo.

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Study Association

This study is not associated with any post-graduation program.

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