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STATE-OF-THE-ART PAPER

Platelet Biology and Response to Antiplatelet Therapy in Women

Implications for the Development and Use of Antiplatelet Pharmacotherapies for Cardiovascular Disease

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Women are underrepresented in cardiovascular studies, even as their preponderance in the aging population steadily increases. Although concerns have been raised about the differential benefit of antiplatelet medications for women, the propensity for increased bleeding among women has also been recognized. A better understanding of the factors contributing to the observed sex-related differences in platelet biology is warranted. These factors include differences in the frequency and expression of genetic polymorphisms affecting platelet responsiveness to agonists (with and without antiplatelet therapies), which might be obtained through population-based studies and in large controlled clinical trials; inflammatory marker levels and their influence on atherothrombotic risk, and the role of specific hormones in mediating platelet activation and function. Knowledge gained about these mechanistic factors might inform the development of sex-specific antithrombotic treatment regimens that confer optimized safety and efficacy. (J Am Coll Cardiol 2012;59:891–900) © 2012 by the American College of Cardiology Foundation

Although randomized clinical trials have shown the benefit of antiplatelet therapy for patients with cardiovascular disease, their findings cannot reliably be generalized to subgroups if not sufficiently represented in study populations. Because women are underrepresented in cardiovascular trials (1,2), sex differences in the response to antithrombotic therapies, in terms of thrombosis and bleeding risks, remain unclear. Similarly, the link between possible sex differences in platelet biology and the response to antithrombotic therapies is unknown. A better understanding of these differences could lead to the development of optimized therapies for the prevention and treatment of ischemic events in women, while minimizing bleeding risk. The focus of the meeting was the

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Abbreviations and Acronyms

ACS = acute coronary syndromes
ADP = adenosine diphosphate
CAD = coronary artery disease
CI = confidence interval
CRP = C-reactive protein
GP = glycoprotein
MI = myocardial infarction
PCI = percutaneous coronary intervention

translation of mechanistic constructs in platelet biology into safe and effective patient care.

Epidemiology of Thrombotic Disorders in Women

Ischemic heart disease and stroke account for nearly 25% of all deaths among women worldwide (3). In the United States, coronary artery disease (CAD) accounted for 15.6% of deaths in women in 2007, and cerebrovascular disease accounted for another 6.7% (4). These atherothrombotic disorders also account for substantial morbidity and resource use. In 2007, 607,000 American women were hospitalized for CAD; among those, nearly 1 million cardiac catheterization, percutaneous coronary intervention (PCI), stenting, or coronary artery bypass graft procedures were performed, and the average length of hospital stay was 5.3 days for acute myocardial infarction (MI) alone (5). Another 458,000 women were hospitalized for cerebrovascular disease that same year, staying an average of 5.4 days in the hospital (5).

The prevalence of these disorders in women varies greatly with age. As shown in Figure 1, the incidence of CAD, stroke, and peripheral artery disease all increase substantially as women become older (6). In particular, the incidence of CAD more than triples around the time of menopause (4). Further, given the longer life expectancy of women relative to men worldwide (3), increasing proportions of the direct and indirect costs of CAD and cerebrovascular disease, currently estimated at $505 billion in the United States alone (7), will occur among women.

Sex Differences in Platelet Biology

Platelet reactivity. Differences in platelet reactivity across populations have been described using several methods and in response to varying stimuli (Fig. 2) (8). Platelets from women without CAD are more reactive than those of men in response to standard concentrations of agonists such as adenosine diphosphate (ADP) (2 to 20 μmol/l) and thrombin receptor agonist protein (50 μmol/l) (9,10). One study showed that the number of glycoprotein (GP) IIb/IIIa receptors that could bind PAC-1 antibody in response to ADP or thrombin receptor agonist protein stimulation was 50% to 80% greater in women than in men (10). Among asymptomatic patients, platelets from women, in particular, white women, bound more fibrinogen in response to low and high concentrations of ADP and showed more spontaneous aggregation compared with men after adjustment for risk factors such as smoking, hypertension, diabetes, hyperlipidemia, and aspirin use (11). In a population-based study of asymptomatic, premenopausal women, high platelet reactivity was associated with an increased risk of downstream MI (2- to 3-fold increased risk compared with those with normal platelet reactivity) (12). A recent study of signaling proteomes in platelets showed significantly higher levels of signaling cascade proteins expressed on platelets from male versus female donors (13). Although this concept warrants further investigation, these early results suggest that sex-specific platelet proteomic signaling mechanisms might contribute to differences in platelet reactivity and outcome disparities between women and men.

GeneSTAR (Genetic Study of Aspirin Responsiveness, which studied healthy men and women, also demonstrated higher platelet reactivity among women compared with men in response to varying concentrations of arachidonic acid, ADP, or epinephrine after adjustment for age, risk factors, race, menopausal status, and hormone therapy (14). However, after low-dose aspirin therapy, men and women showed similar degrees of platelet inhibition in response to arachidonic acid (Fig. 3). After aspirin therapy, women's platelets remained significantly more reactive than those of men in response to collagen or ADP stimulation; for example, platelet aggregation in response to 5 μg/ml collagen was 31.8% for women and 27.2% for men after aspirin therapy ($p < 0.001$), which, according to the investigators, likely reflects "platelet activation pathways indirectly related to cyclooxygenase-1" (14). In addition to platelet-related sex differences, thromboelastography-based studies also show a greater prothrombotic tendency (increased speed and strength of platelet-fibrin clotting) among women compared with men (15,16).

Inflammation. Activated platelets, through the release of cytokines and immunomodulatory ligands, mediate an inflammatory response that further amplifies platelet response and endothelial activation during plaque rupture (17). On

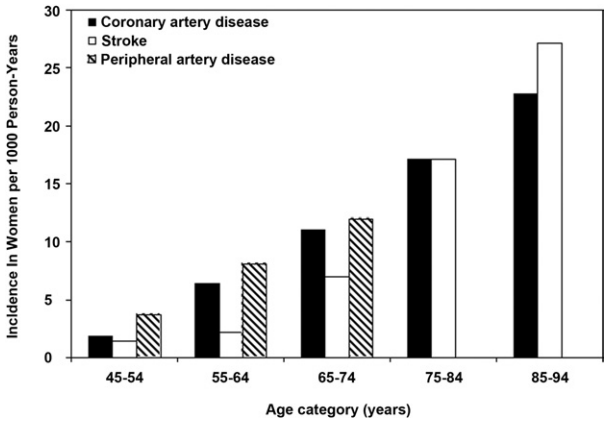

Figure 1 Incidence of Atherothrombotic Disease in Women by Age Category

Data for coronary artery disease and stroke are from the Framingham original and offspring cohort, and data on peripheral arterial disease are from the ARIC (Atherosclerosis Risk In Communities) cohort (per 1,000 person-years) (6).

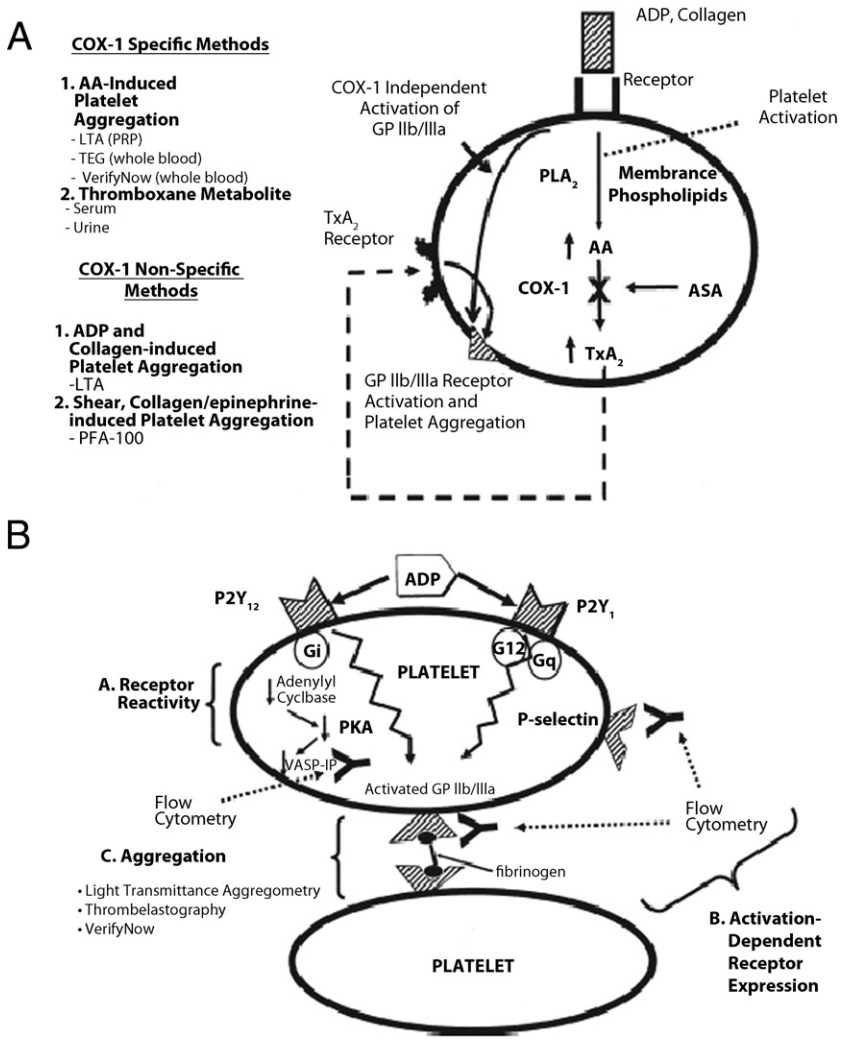

Figure 2 **Platelet Responsiveness to Aspirin and Clopidogrel**

Laboratory measurement of platelet responsiveness to aspirin **(A)** and clopidogrel **(B)**. AA = arachidonic acid; ADP = adenosine diphosphate; ASA = acetylsalicylic acid (aspirin); COX = cyclooxygenase; GP = glycoprotein; LTA = light transmittance aggregometry; PFA = platelet function analyzer; PKA = protein kinase A; PLA_2 = phospholipase A_2; PRP = platelet-rich plasma; TEG = thrombelastography; VASP = vasodilator-stimulated phosphoprotein; Y = monoclonal antibodies. Reprinted with permission from Gurbel et al. (8).

acute plaque rupture, exposure of its core contents promotes adhesion of platelet receptors and integrins to von Willebrand factor and collagen in the subendothelium (18,19). Activated platelets also release inflammatory factors, bind to leukocytes to form platelet-leukocyte coaggregates, facilitate the release of proinflammatory cytokines, and may promote an inflammatory response within the vessel wall (19,20).

Among healthy postmenopausal women in the Women's Health Study, a higher C-reactive protein (CRP) level, the most commonly studied marker of inflammation, independently predicted the risk of cardiovascular death, nonfatal MI, stroke, or need for coronary revascularization (relative risk: 1.5 for the highest quartile versus the lowest; 95% confidence interval [CI]: 1.1 to 2.1) (21). Although the Cardiovascular Health Study showed that CRP added to risk prediction only for intermediate-risk men, and not women (22), a recent meta-analysis showed a risk ratio for CAD of 1.41 (95% CI: 1.13 to 1.75) among women in 5 studies whose CRP level was >3.0 mg/l versus <1.0 mg/l (23). Other inflammatory markers, such as leukocyte count and P-selectin expression, may be higher among healthy women and predictive of future cardiovascular events (24,25).

Antiplatelet therapies have been associated with pleiotropic anti-inflammatory effects beyond their direct platelet-mediated effects (26). Aspirin showed synergistic effects with statins in lowering CRP levels in a population-based, longitudinal stroke study in which 50% of the participants were women, and this synergy did not differ by sex (27). Clopidogrel therapy was associated with reduced levels of inflammatory markers, even after adjustment for sex, in 1

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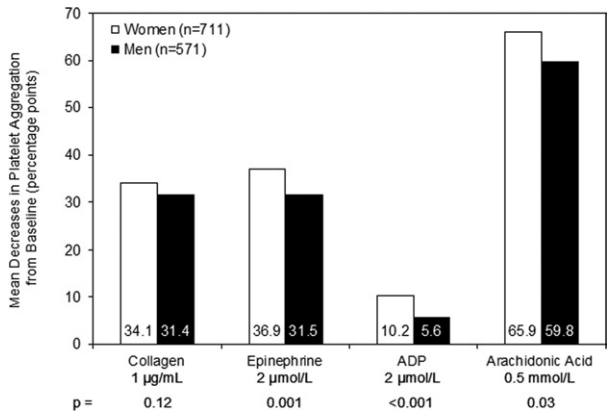

Figure 3 **Platelet Aggregation Before and After Aspirin Treatment**

Decrease in mean platelet aggregation in platelet-rich plasma in response to various concentrations of agonists after aspirin therapy in 1,282 apparently healthy children of parents with early coronary artery disease. ADP = adenosine diphosphate. Data from Becker et al. (14).

nonrandomized study (28). In another small study in which 50% of the participants were women, CRP levels increased 1 month after clopidogrel cessation without significant sex differences (29). Whether greater reduction in inflammation with newer, more potent antiplatelet agents can improve clinical outcomes in women, men, or both, is worthy of further investigation.

Role of hormones in platelet biology and inflammation. Megakaryocytes and platelets express the estrogen receptor β (30) and androgen receptor (30,31), and platelet nitric oxide synthase release and thromboxane A_2 generation can be modulated by estrogens and/or androgens (31,32). In addition, transcripts from both estrogen receptor β and androgen are upregulated during megakaryocyte differentiation (31). Thus, genomic effects in megakaryocytes, signaling properties in platelets, or both, might contribute to sex differences in platelet function (31).

There is conflicting evidence of the effect of female menstrual-cycle hormones on platelet biology. One study reported that platelets bound more fibrinogen during the luteal phase than the follicular phase, suggesting that hormones may regulate platelet GP IIb/IIIa activation (11). Platelet adhesion to vascular collagen also demonstrates biphasic peaks during the menstrual cycle (30). In contrast, other studies found no significant relationship between platelet aggregability and menstrual cycle phase, oral contraceptive use, or menopausal state (13,33,34). Both oral contraceptive and perimenopausal hormonal agents alter expression of proteins in the coagulation-fibrinolytic pathways, increasing both factor VII levels and activity, promoting fibrinogen binding to platelets, and increasing levels of fibrinogen and plasminogen activator inhibitor-1 (35). These changes may contribute to increased thrombotic risk.

Systemic inflammation also mediates the relationship between hormones and atherothrombotic risk. Randomized (36) and cross-sectional (37) studies have found increased CRP levels among post-menopausal women treated with estrogen. However, in a nested case-control analysis from the 2 randomized, controlled studies of the Women's Health Initiative, baseline CRP did not show a significant statistical interaction with hormone therapy in predicting CAD risk (38).

Several studies have suggested a link between genetic polymorphisms for platelet glycoproteins and the risk of atherothrombotic events (39–41), but relatively little is known about whether sex-specific differences influence the impact of these polymorphisms on platelet biology, treatment response, and patient outcomes. Among women receiving placebo in the HERS (Heart Estrogen–Progestin Study), heterozygotes and homozygotes for the GP Ibα-5C allele had a significantly higher incidence of the composite endpoint (death, MI, or unstable angina) at 5.8 years of follow-up compared with women homozygous for the GP Ibα-5T allele (42). Postmenopausal hormone treatment was associated with a 46% lower adjusted cardiovascular risk in women with the -5C allele versus the -5TT genotype ($p < 0.001$ for interaction) (42). Hormone therapy was associated with significant harm in the 21.2% of women who had the GP Ibα-TT and GP VI-TC/CC genotypes, and with significant benefit in the 16.6% of women with the GP Ibα TC/CC and GP VI-TT genotypes (42). These data suggest a role for pharmacogenomic testing in determining underlying cardiovascular risk in postmenopausal women, which could alter the risk-benefit ratio for proposed antiplatelet treatment. Further investigation is warranted, however.

Clinical Evidence of Sex Differences in Response to Antiplatelet Therapy

Aspirin. In a meta-analysis of 6 randomized primary prevention trials, aspirin therapy was associated with significantly reduced stroke risk in the 51,342 women studied over a mean 6.4 years of follow-up, but no significant effect was shown for MI (43). In contrast, for men, MI risk was significantly reduced with aspirin therapy, but no effect was seen for stroke. (Tests for heterogeneity of treatment effect were not reported.) The ATT (Antithrombotic Trialists) Collaboration meta-analysis noted significant reductions in major coronary events and serious vascular events with aspirin therapy in men, but not women, in both primary and secondary prevention studies (Fig. 4) (44). After adjustment for multiple comparisons, however, no heterogeneity of treatment effect was noted by sex for any of the endpoints assessed (44). The ISIS-2 (International Study of Infarct Survival-2) trial likewise found that the reduction in vascular mortality with aspirin versus placebo therapy after acute MI was 22% for men, but only 16% for women ($p > 0.05$ for heterogeneity) (45).

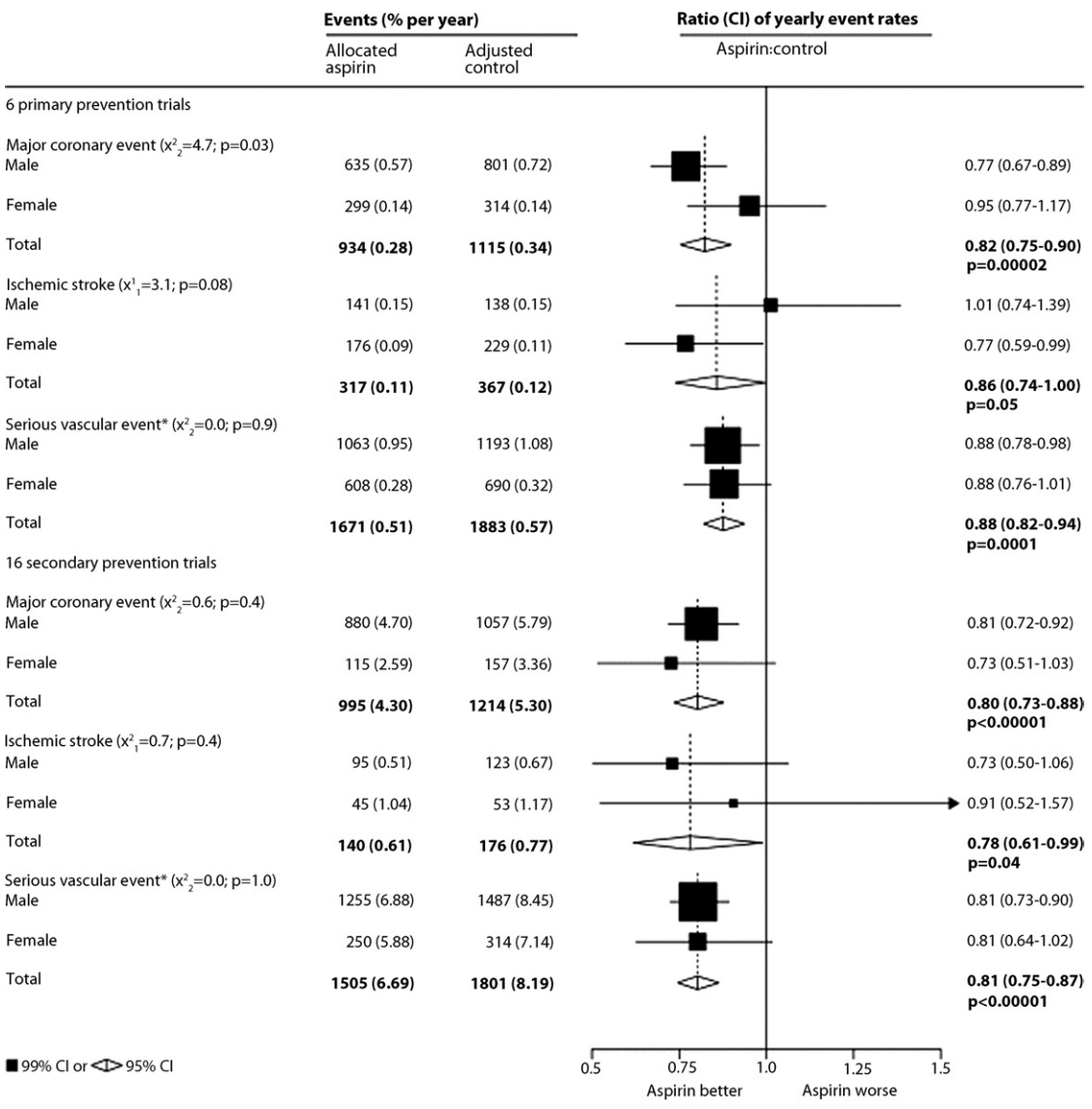

Figure 4 **Selected Outcomes in Primary and Secondary Prevention Trials of Aspirin, by Sex**

CI = confidence interval. Reprinted with permission from the ATT (Antithrombotic Trialists) Collaboration (44).

Secondary analyses from previous clinical trials showed no clear overall benefit with aspirin doses >100 mg compared with lower doses (46–48). Further, although women have been shown to demonstrate higher platelet reactivity (9,10,11,14), these secondary studies found no particular benefit associated with a higher aspirin dose among women. Based on sex differences in the epidemiology of cardiovascular disease and available evidence supporting therapy, the U.S. Preventive Services Task Force recommends low-dose aspirin for the prevention of MI among men age 45 to 79 years and for prevention of stroke among women age 55 to 79 years, when the potential benefit outweighs the potential risk of gastrointestinal bleeding (49).

Similarly, the American Stroke Association and American Heart Association recommend aspirin therapy for persons whose 10-year cardiovascular risk is at least 6% (50).

Thienopyridines. In a meta-analysis of 5 large randomized, controlled trials, clopidogrel treatment was associated with a significant reduction in the risk of cardiovascular events (cardiovascular death, MI, or stroke) overall in patients with known risk factors for CAD (51). However, among the 23,533 women evaluated, only the risk of MI was significantly reduced with clopidogrel (relative risk: 0.81; 95% CI: 0.70 to 0.93) and not that for stroke or all-cause mortality. (The 56,091 men evaluated showed significant reductions in all 3 endpoints.) In the pivotal randomized trials of the antiplatelet agents prasugrel (52)

and ticagrelor (53), although the reductions in the primary endpoint were relatively smaller in women than in men, neither study noted a significant interaction between sex and treatment assignment. Of note, these studies were not powered to examine treatment interactions among subgroups.

Polymorphisms of the cytochrome P450 (CYP) 2C19 allele have been independently associated with variable responses to clopidogrel, specifically, clopidogrel prodrug metabolism to its active metabolite. Higher risks of both major bleeding (54) and stent thrombosis (55) have been observed, depending on the presence of a gain-of-function and loss-of-function genotype, respectively. Because these polymorphisms are similarly distributed among men and women, genotype-driven differences in outcome between men and women would not be expected. However, studies of this question could be important because other gene-gene or gene-environment interactions might drive possible sex differences.

GP IIb/IIIa inhibitors. A significant interaction between treatment and sex was observed for trials of GP IIb/IIIa inhibitors with respect to cardiovascular events. Although GP IIb/IIIa inhibitor use was associated with a significantly reduced incidence of death or MI at 30 days versus placebo among men with acute coronary syndromes (ACS), women had worse outcomes with such treatment (Table 1) (56). Important differences in clinical characteristics may explain this difference in treatment effect—women were older, had more comorbid conditions, and more frequently had larger infarctions compared with men. When risk was further stratified by troponin level, no sex differences were seen. More recent studies have not shown sex-related differences in outcome, possibly because of the concomitant use of clopidogrel (57).

Sex Differences in Bleeding Associated With Antiplatelet Therapies

The Women's Health Study reported that serious gastrointestinal bleeding requiring transfusion occurred more commonly among women randomized to receive aspirin for primary prevention of cardiovascular disease versus placebo (relative risk: 1.40; 95% CI: 1.07 to 1.83) (58). However, in a meta-analysis by Berger et al. (43), aspirin use over an average 6.4 years was associated with 2.5 major bleeding events per 1,000 women treated versus 3 major bleeding events per 1,000 men treated. For clopidogrel treatment, the odds ratio for bleeding was numerically higher among women than men (1.43; 95% CI: 1.15 to 1.79 vs. 1.22; 95% CI: 1.05 to 1.42), but there was no evidence of heterogeneity of effect between women and men for major bleeding ($p = 0.24$) (43).

In ACS, sex-related differences in bleeding risk are consistently demonstrated. In a multivariable risk model, Subherwal et al. (59) identified sex as an independent predictor of in-hospital major bleeding (odds ratio: 1.31; 95% CI: 1.23 to 1.39) among patients with ACS. In the CRUSADE (Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines?) registry, women who underwent invasive treatment with PCI had significantly higher rates of in-hospital major bleeding compared with men (14.1% vs. 5.9%, $p < 0.001$) (60). Women undergoing PCI tend to have more access-site complications (e.g., access site bleeding, retroperitoneal bleeding) (61), whereas gastrointestinal bleeding after MI is more common in men (62). Bleeding-site differences may relate in part to the smaller blood vessels in women (63), but other sex-specific differences, such as in vascular reactivity (64), might also contribute to differences in bleeding risk. The question of weight-based dosing has been raised for antiplatelet agents such as clopidogrel (65), which might provide a mechanism for variations in antiplatelet response, particularly among women with less body mass.

The CRUSADE registry identified a significant interaction between sex, GP IIb/IIIa inhibitor use, and bleeding risk ($p = 0.014$) (Fig. 5) (60). Some of the higher bleeding risk in women receiving antithrombotic therapy reflects inappropriate dosing of these agents. An estimated 15% of the major bleeding observed in CRUSADE could be

Table 1 Meta-Analysis of 6 Trials of GPI in Acute Coronary Syndromes: Outcomes by Sex

	Women			Men			
	GPI	Control	OR (95% CI)*	GPI	Control	OR (95% CI)	p Value†
Overall	n = 6,410	n = 4,603		n = 11,886	n = 8,502		
Death	3.9%	3.6%	1.08 (0.89–1.33)	3.2%	3.8%	0.83 (0.71–0.96)	0.030
Death or MI	11.5%	10.4%	1.15 (1.01–1.30)	10.4%	12.6%	0.81 (0.75–0.89)	<0.001
Baseline cardiac troponin I or T <0.1 μg/l	n = 1,548	n = 1,003		n = 2,095	n = 1,449		
Death	2.3%	2.0%	1.20 (0.69–2.10)	2.3%	2.1%	1.07 (0.67–1.71)	0.84
Death or MI	6.2%	5.3%	1.29 (0.91–1.83)	7.6%	6.9%	1.10 (0.84–1.43)	0.65
Baseline cardiac troponin I or T ≥0.1 μg/l	n = 939	n = 567		n = 2,174	n = 1,284		
Death	6.2%	7.6%	0.80 (0.53–1.21)	4.1%	5.2%	0.75 (0.54–1.04)	0.88
Death or MI	12.7%	13.6%	0.93 (0.68–1.28)	9.3%	11.3%	0.82 (0.65–1.03)	0.48

†For heterogeneity. Adapted with permission from Boersma et al. (56).
CI = confidence interval; GPI = GP IIb/IIIa inhibitor; MI = myocardial infarction; OR = odds ratio.

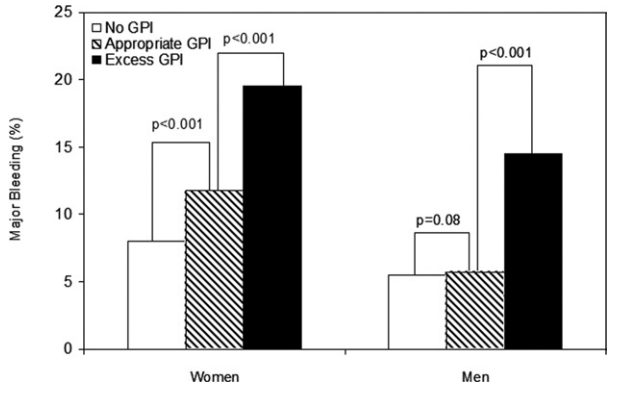

Figure 5 **Major Bleeding by Sex and GPI Dosing**

Incidence of in-hospital major bleeding among women and men with acute coronary syndromes who did not receive a GP IIb/IIIa inhibitor (GPI), those who received appropriate GPI dosing, and those who received excess GPI dosing in the CRUSADE registry. Probability values represent unadjusted comparisons. Adapted with permission from Alexander et al. (60).

explained by excess dosing of unfractionated heparin, low molecular weight heparin, or GP IIb/IIIa inhibitors (66). Even after adjustment for age, weight, and renal function, women remained at higher risk of excess dosing and bleeding than did men. Among women, excess dosing of these agents accounted for 72% of the increased bleeding risk compared with only 27% of the risk in men (60). In the ISAR-REACT (Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment) (67) and ACUITY (Acute Catheterization and Urgent Intervention Triage strategY) trials (68), rates of bleeding complications were also higher among women compared with men receiving appropriately dosed bivalirudin.

Bleeding has implications beyond the event itself. Patients who have bleeding in the setting of ACS are more likely to have a heightened systemic inflammatory response (69) and are less likely to be prescribed antiplatelet therapies at discharge (70), possibly because reinitiation of such therapies is deferred until the patient is deemed "safe" from additional bleeding. However, this treatment "gap" can persist for as long as 6 months after the initial event (70). Given the higher incidence of in-hospital bleeding among women compared with men, this phenomenon has major implications for their risk of future ischemic events.

Implications and Knowledge Gaps

The cumulative evidence highlights a conundrum in which women are at higher risk of both thrombosis and bleeding compared with men (60). Excess antithrombotic drug dosing, an important culprit, can be reduced by implementation of quality-improvement measures (71), but sex differences in bleeding risk persist even with appropriate dosing. These differences might be explained by innate differences in platelet reactivity and contributing influences from inflammatory and hormonal processes, as outlined earlier. What, then, can be done to reduce cardiovascular risk without increasing bleeding risk?

One consideration might be the use of platelet function testing or pharmacogenomics to guide drug selection and dosing (72). The recent GRAVITAS (Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety) trial showed that women had significantly higher residual platelet reactivity after standard clopidogrel dosing compared with men when a point-of-care ADP-specific platelet aggregation assay was used (72). However, in this study, an empirical, higher clopidogrel dosing strategy in response to platelet testing results did not improve clinical outcomes (73). In contrast, a small study by Cuisset et al. (74) found that patients with high platelet reactivity while taking clopidogrel who were randomized to routine (vs. provisional) GP IIb/IIIa inhibitor therapy during PCI had improved 30-day cardiovascular event rates without a significant increase in bleeding.

Sex-specific implications are difficult to draw from these small studies. Targeting other platelet pathways might present another therapeutic option. Cilostazol, which increases intracellular cyclic adenosine monophosphate via selective phosphodiesterase inhibition, has shown additional platelet inhibition among patients with high on-treatment reactivity to clopidogrel. Cilostazol, in addition to aspirin and clopidogrel therapy, has reduced long-term cardiovascular risk without an increase in bleeding among patients undergoing PCI (75) and has shown substantial benefit among women that has not been observed among men (76). Although tailored therapy is an attractive concept, the lack of an accepted standardized test and uncertainty about alternative therapeutic options to optimize outcomes currently limits recommendations for the use of these tests in routine practice (77).

A systematic knowledge gap exists regarding sex differences in platelet biology, genomics, and response to therapeutics. Women remain underrepresented in both early and later phase studies (2). One reason may reflect their preponderance in older populations, which are underrepresented by approximately 50% relative to their prevalence in the general ACS population alone (78), when not excluded from research altogether (79). It could also reflect physician underestimation of risk, misinterpretation of symptoms, and bias against referral for interventional treatment of cardiovascular disease in women (80). Finally, women who are candidates for inclusion in cardiovascular trials might be less willing than men to participate because of a greater perceived risk of harm and adverse events (81).

Although higher baseline levels of platelet reactivity have been observed in women, we are in need of a better understanding of the responsible factors, including the relative frequency and expression of genetic polymorphisms affecting platelet responsiveness, differences in inflammatory marker levels and their influence on atherothrombotic risk,

and the role of hormones in mediating platelet effects. These mechanistic underpinnings will form the basis of sex-specific treatment regimens aimed at optimizing the safety and efficacy of antithrombotic therapy. Analyses of sex-specific differences can provide insight into the scientific basis for individual therapy differences and provide future directions for research. Dedicated trial designs for genetic, in vitro, and clinical studies are needed to balance female representation in platelet-related research.

Conclusions

Although it has been known for decades that women respond differently from men to antithrombotic therapies—showing a greater propensity for bleeding, even with appropriate dosing, and differential benefit in terms of prevention of ischemic events—only a minority of comparative studies have provided insights into the biology underlying sex-related differences in platelet function and response. It is imperative for the scientific community to embark on carefully designed investigations to fill an existing knowledge gap that affects patient care.

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Key Words: bleeding ■ inflammation ■ platelets ■ sex ■ thrombosis ■ women.

APPENDIX

For a list of the participants in the 2010 Platelet Colloquium, please see the online version of this article.