European Heart Journal (2014) **35**, 2213–2223
doi:10.1093/eurheartj/ehu279

REVIEW

Clinical update

Platelet function and long-term antiplatelet therapy in women: is there a gender-specificity? A 'state-of-the-art' paper

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Received 22 March 2014; revised 27 May 2014; accepted 18 June 2014; online publish-ahead-of-print 14 July 2014

Although the female gender is generally less represented in cardiovascular studies, observational and randomized investigations suggest that—compared with men—women may obtain different benefits from antiplatelet therapy. Multiple factors, including hormonal mechanisms and differences in platelet biology, might contribute to such apparent gender peculiarities. The thrombotic and bleeding risks, as well as outcomes after a cardiovascular event, appear to differ between genders, partly in relation to differences in age, comorbidities and body size. Equally, the benefits of antiplatelet therapy may differ in women compared with men in different vascular beds, during primary or secondary prevention and according to the type of an antiplatelet agent used. This document is an attempt to bring together current evidence, clinical practices and gaps of knowledge on gender-specific platelet function and antiplatelet therapy. On the basis of the available data, we provide suggestions on current indications of antiplatelet therapy for cardiovascular prevention in women with different clinical features; no strong recommendation may be given because the available data derive from observational studies or *post hoc*/subgroup analyses of randomized studies without systematic adjustments for baseline risk profiles.

Keywords Platelets • Antiplatelet therapy • Thrombosis • Gender differences • Women • Men

Introduction

Gender differences have long emerged in the incidence and presentation of cardiovascular events and in the rates of bleeding following medical or invasive interventions.[1] Previous studies suggested that women may not derive the same benefit from antithrombotic therapy as men;[2–4] pathophysiological mechanisms causing these disparities are not entirely clear, and multiple factors, including differences in platelet biology, may contribute.

Women are generally less represented than men in cardiovascular trials for reasons that include: (i) underestimation of cardiac risk and misinterpretation of symptoms, with lesser

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referral for cardiac testing, lower rates of appropriate diagnosis or treatment and lesser rates of referral to coronary angiography for acute coronary syndromes (ACS);[4] (ii) lower prevalence of cardiovascular diseases in women below the age of 65.[1] On the other hand, women included in antithrombotic drug trials are on average older and have more comorbidities and risk factors than men, and are thus at a higher risk of adverse outcomes, including thrombotic and bleeding events.[1] Moreover, because women are more prone to bleeding complications than men owing, at least in part, to lower body weight, lower glomerular filtration rates, and more frequent overdosing of antithrombotic drugs,[5] the net clinical benefit of antiplatelet agents tends to be generally smaller in women than in age-matched men.

On this basis, we here analyse potential reasons and clinical evidence for possible gender-specific disparities in indications of oral antiplatelet therapy. This document is based on a comprehensive review of the available literature. PubMed and EMBASE databases were searched through December 2013 for articles in English reporting platelet function and antiplatelet therapy in women. The following search terms were used: 'women', 'gender', 'platelet function', 'antiplatelet therapy', 'cardiovascular diseases', 'bleeding', 'diabetes', and 'pregnancy'. Identified references were hand-searched to locate other potentially useful references. Clinical randomized trials, prospective cohort studies and retrospective analyses were included, as well as meta-analyses; abstracts were excluded. Discordance regarding inclusion was resolved by discussion. Experimental animal studies were excluded unless considered highly relevant. The flow-diagram of the selection process leading to the final choice of the included references is indicated in *Figure 1*.

Gender differences in platelet function

That gender may influence platelet biology was put forward over 30 years ago.[6–9] Sites of potential gender differences in molecular mechanisms of platelet adhesion/aggregation are depicted in *Figure 2*. Recent works have confirmed that platelet count differs significantly by age and gender[10] (with higher values in women vs. men and in younger vs. older subjects),[10–12] and that platelets in women have a higher number of surface receptors and to bind a greater amount of fibrinogen.[7,11,12] However, other investigations have suggested that platelet count and surface expression of glycoprotein (GP) Ib-IX-V (responsible for initiating adhesion through a von Willebrand factor) and GP IIb/IIIa receptors (responsible for initiating aggregation mainly through fibrinogen) may not accurately reflect overall platelet reactivity.[9]

Regarding platelet aggregation, some reports have highlighted an enhanced platelet reactivity in women,[13,14] both with and without antiplatelet therapy,[15] but other investigations have not confirmed these results.[16,17] A possible laboratory confounder here is the higher haematocrit in men than in women, with relatively less plasma and greater *in vitro* dilution by added anticoagulant solutions, leading to falsely lower platelet counts and plasma concentrations of fibrinogen in men compared with women.[18] Some,[18] but not all,[19] investigations have indicated a greater extent of platelet adhesion to injured vessels in males, but greater agonist-induced platelet activation and aggregation in females.[20–22] Various platelet agonists have been documented to activate the GP IIb/IIIa receptors to a larger extent in women than in men[11,13,18,20] and higher adenosine diphosphate- or collagen-induced reactivity has been observed in

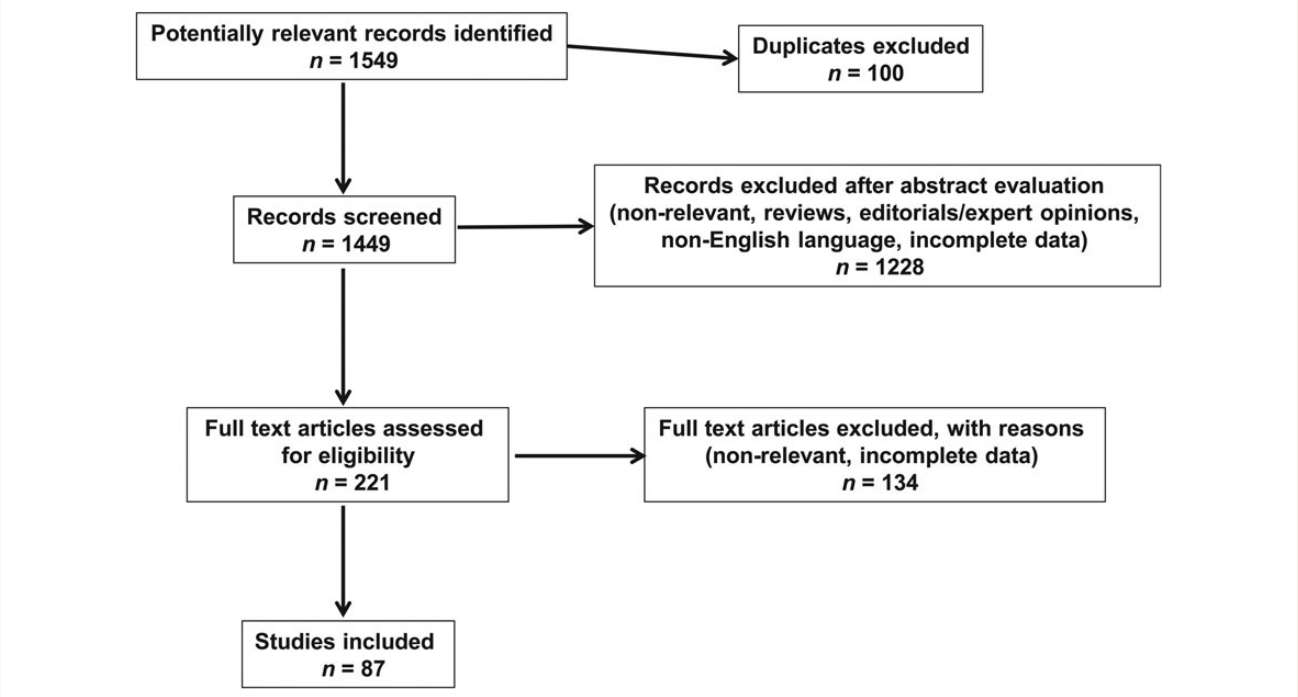

Figure 1 Flow-diagram of the selection process leading to the final choice of references included in the paper.

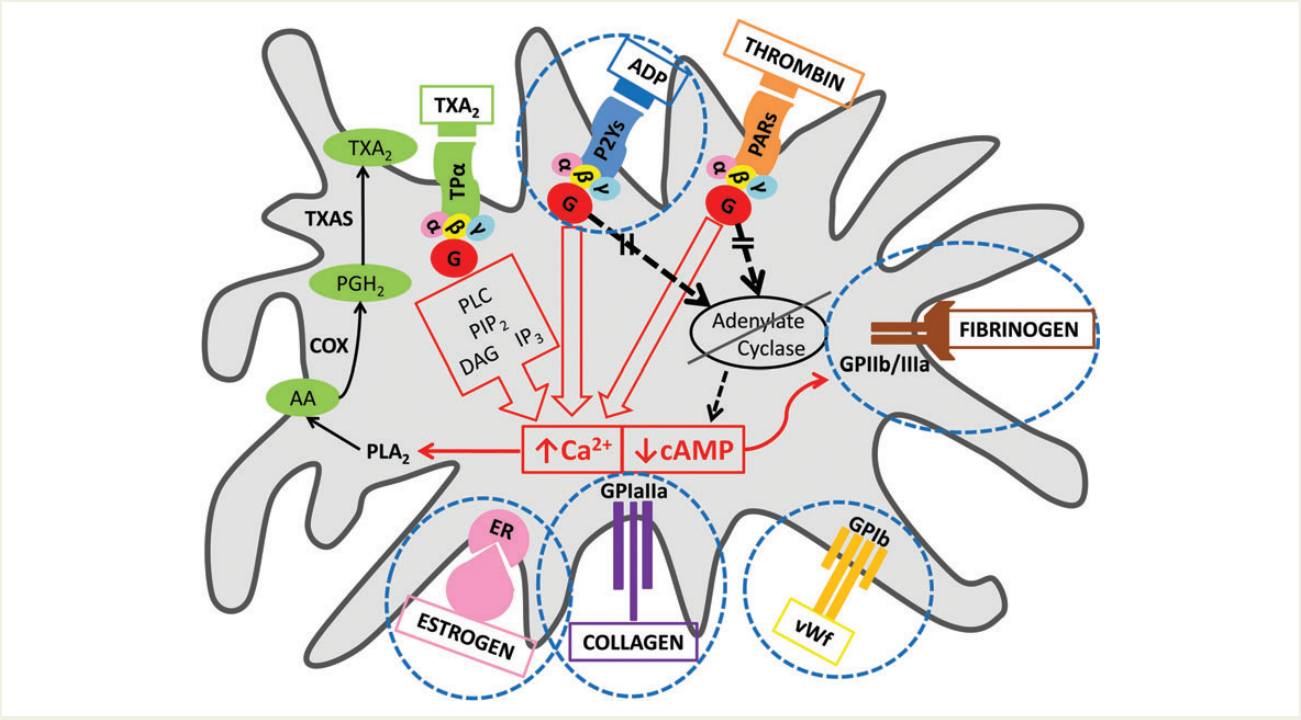

Figure 2 Scheme of agonists, receptors, and effector systems participating in platelet activation. Receptor/ligands probably accountable for gender differences in platelet function are highlighted by dotted blue circles: Reduced platelet reactivity in pre-menopausal women has been related to the presence of oestrogen receptors on the platelet surface. Platelets in women appear to have a higher surface expression of glycoprotein Ib-IX-V and glycoprotein IIb/IIIa. Glycoprotein IIb/IIIa receptors appear more activable in women. Higher adenosine diphosphate- or collagen-induced reactivity has been observed in female vs. male subjects, independently of the expression of surface receptors.

female vs. male subjects (*Table 1*).[13] This increased platelet aggregability in women has been found to be independent of platelet size and of the expression of surface adhesion molecules.[22] However, when bleeding times have been used to assess overall platelet competence, women feature 20–25% longer *in vivo* bleeding times than men,[6,8] in contrast with *ex vivo* data suggesting enhanced platelet function. Moreover, relative increases will be greater starting from lower absolute baseline levels and, conversely, will be lower starting from higher absolute baseline values, for a given absolute value of response.[23] Finally, the role of soluble, blood-borne inhibiting or activating factors on platelets is generally not considered by tests performed *ex vivo*.

Thus, caution is warranted when interpreting data on gender differences in platelet function, given the potential for selection bias, laboratory artefacts and heterogeneity of *in vitro*, *ex vivo*, and *in vivo* studies in different settings (rodents, healthy subjects, patients, pregnancy, pre-/post-menopausal states). Available data seem overall to suggest increased activity of primary haemostasis (i.e. enhanced platelet adhesion and shorter bleeding times) in males, but more reactive platelets in response to *ex vivo* agonists in females.

Thrombotic and haemorrhagic burden in women

Thrombotic risk

Gender-based differences in the epidemiology, pathophysiology, and treatment of athero-thrombotic events have been the object of a growing body of recent literature. Men are affected by coronary heart disease (CHD) more than women until the age of 39, and almost equally between 40 and 79 years;[24] conversely, more women than men have CHD by 80 years or above.[24] Recent data [25,26] demonstrated that the prevalence of myocardial infarction (MI) has increased in midlife women (35–54 years), that especially younger women have taken up smoking and that women have shown an increase in the prevalence of diabetes and hypertension compared with men, with an expected impact on the overall incidence of cardiovascular diseases.

Women[23] experience frequent fluctuations of pro-thrombotic activity during their lifetime related to menstrual cycles, use of oral contraceptives, pregnancy, menopause, and hormone replacement therapy, with potential impact on the clinical manifestations of atherosclerotic disease. It has been hypothesized that gender differences in *ex vivo* platelet function could be the result of direct effects of oestrogens, progesterone, or androgens on platelets, or could be an indirect effect of sex hormones on the vasculature.[27] The role of endogenous oestrogen status in delaying the onset of athero-thrombotic events in women is, however, still under debate,[28,29] although oestrogens induce the synthesis of prostacyclin, increase nitric oxide bioavailability and directly inhibit platelet aggregation;[21] of note, the reduced platelet reactivity in pre-menopausal women has been related to the presence of oestrogen receptors on the platelet surface.[30]

Bleeding risk

Bleeding episodes in patients with a cardiovascular event impact on later survival and gender-related differences in bleeding risk have

Table 1 **Gender differences in platelet function**

Reference	Experimental setting	Platelet assay	Main findings
O'Brien[8]	Male and female volunteers standardized skin incision	Bleeding time	Longer bleeding time in women
Stevens and Alexander[9]	Male and female blood donors citrated blood	Platelet count	Higher platelet count in women
Bain and Forster[6]	Male and female volunteers Standardised skin incision	Bleeding time	Longer bleeding time in women
Lawrence *et al.*[18]	Male and female donors Citrated blood	Platelet spreading, adherence and aggregability	Greater spreading and adherence in men Greater ADP-aggregability in women
Faraday *et al.*[11]	Male and female volunteers Washed platelets	Platelet binding of fibrinogen or PAC-1 in response to ADP or TRAP	More activatable glycoprotein IIb/IIIa in women independently of platelet count
Caulin-Glaser *et al.*[21]	Female human umbilical vein endothelial cells (HUVEC)	Release of the platelet inhibitor nitric oxide by HUVEC	Endothelial nitric oxide synthase activity induced by 17 beta-estradiol
Haque *et al.*[13]	Male and female volunteers platelet-rich plasma	Platelet aggregability by light transmission/scatter	Greater ADP-induced aggregability in women
Leng *et al.*[22]	Male and female littermate mice—washed platelets	Agonist-induced fibrinogen binding and aggregation	Greater fibrinogen binding and aggregability of female mouse platelets
Becker *et al.*[20]	Healthy men and women Citrated blood and plasma	Aggregability in response to collagen, arachidonic acid, ADP and epinephrine	Greater ADP and collagen-induced aggregability in women
Eshel-Green[19]	Male and female inbred littermate mouse platelets	Platelet responses to fibrinogen, thrombin, or collagen	No gender differences in platelet adhesion, spreading or aggregation under flow

ADP, adenosine diphosphate; PAC-1, antibody against glycoprotein IIb/IIIa; TRAP, thrombin receptor activating peptide.

been reported. Data from 24 045 patients of the Global Registry of Acute Coronary Events (GRACE) indicated that women vs. men had a 43% increased risk of bleeding during hospitalization;[31] the risk was even higher in the setting of ST-segment elevation MI (odds ratio, OR: 1.71). Women undergoing percutaneous coronary intervention (PCI) also showed a significantly higher incidence of in-hospital major bleedings, including access-related complications, compared with men;[32] this increased bleeding risk appears at least in part related to inappropriate dosing of antithrombotic drugs.[33] The implications of bleedings extend beyond the acute phase of a coronary event, because patients who suffer bleeding events are less likely to be prescribed antiplatelet therapies during the follow-up and the re-initiation of treatment is often indefinitely delayed, with major implications for the risk of future ischaemic events.

Antiplatelet therapy in primary cardiovascular prevention

Possible gender differences in response to antiplatelet therapy

The Women's Health Study (WHS)[34] evaluated the efficacy and safety of aspirin in 39 876 initially healthy women ≥45 years of age randomly allocated to receive either 100 mg of aspirin on alternate days or placebo, and then monitored for 10 years; here aspirin did not reduce the overall risk of major cardiovascular events, but decreased the risk of stroke (relative risk 0.83; $P = 0.04$); there was no difference in the incidence of haemorrhagic stroke in the two arms, but a 1.4-fold higher risk of gastrointestinal bleedings requiring transfusion with aspirin. Pooling data with other trials for a total of 51 342 women, there was a borderline significant prevention of cardiovascular events and ischaemic stroke by aspirin, no significant benefit on MI or cardiovascular mortality and an increased risk of overall bleeding (OR: 1.68; $P = 0.01$).[35] The 0.3% absolute risk reduction of cardiovascular events was small, i.e. aspirin prevented only three cardiovascular events (two strokes) per 1000 women followed for 6.5 years, while causing 2.5 major bleeding events. The Antithrombotic Trialists' (ATT) collaboration meta-analysis[35] of six primary prevention randomized trials (of which three conducted in men only and one in women only, therefore only two allowing within-trial gender comparisons) on individual participant data from 95 000 subjects demonstrated a significant decrease of major coronary events with aspirin in men, which was not apparent in women (*Table 2*). Several hypotheses have been put forward to explain this possible gender-related divergences in cardioprotection afforded by aspirin: differences in platelet biology and in aspirin metabolism, as well as 'aspirin resistance', which, although variably defined, tend to be more common in women than in men;[36] however, the play of chance in such reported gender differences cannot be excluded, as suggested by the overlapping confidence intervals (CIs) for the risk ratios in men and women (*Table 2*). In a *post hoc* analysis of the WHS, age was positively related to treatment effect, whereas a baseline cardiovascular risk profile was not:[37] selective treatment of women >65 years of age yielded more net benefit than treating none, providing a 10-year number needed-to-treat to prevent one cardiovascular events >50.[38]

A recent trial[39] evaluated 30-day clinical outcome in 10 010 patients (47% women) at risk for vascular complications (two-thirds of whom without clinically evident atherothrombotic disease) randomized to aspirin or placebo before non-cardiac surgery and throughout the early post-surgical period; use of aspirin was not associated with reduction in death or MI but increased the risk of major bleeding. Outcome results according to gender were not reported.

In summary, although gender differences have been claimed for cardiovascular protection by aspirin, an overall analysis of the literature does not allow to draw firm conclusions on this point. Suggestions aimed at guiding clinical decision-making of antiplatelet therapy for primary cardiovascular prevention in women are listed in Supplementary material online, *Summary Box 1*

Specific subsets

Some female-related conditions deserve consideration in view of possible aspirin administration for primary prevention of cardiovascular events.

The increased incidence of cardiovascular diseases in post-menopausal vs. pre-menopausal women has been related to the decline in oestrogen levels, although the results of trials evaluating the effects of hormone replacement therapy on cardiovascular outcome have been contradictory; this discrepancy might be ascribed to the type of oestrogen component and/or the timing of replacement therapy. Although no final consensus has been reached, aspirin might balance the postulated increased thrombotic risk of post-menopausal women on hormone replacement therapy. Further trials are welcomed to evaluate the benefit–risk ratio of such a strategy.

Breast cancer is the most common cancer in women worldwide and radiotherapy in the early stage reduces the rates of recurrence and premature death. However, with radiotherapy the heart receives an average dose of 5 Gy and a linear relationship has been documented between mean radiation dose and future cardiovascular events.[40] The proportional increase in risk was 7.4%/Gy, with no apparent

Table 2 Selected outcomes in primary cardiovascular prevention in men and women, modified from the Antithrombotic Trialist collaboration meta-analysis[35]

Events (% per year)	Males on aspirin	Male controls	RR (CI)	Females on aspirin	Female controls	RR (CI)
Major coronary event	0.57%	0.72%	0.77 (0.67–0.89)	0.14%	0.14%	0.95 (0.77–1.17)
Ischaemic stroke	0.15%	0.15%	1.1 (0.74–1.39)	0.09%	0.11%	0.77 (0.59–0.99)

RR, relative risk; CI, confidence interval.

Table 3 Descriptors of main studies on antiplatelet therapy for primary and secondary prevention of stroke

Drug	Women/total patients	Follow-up (years)	Clinical end-points and treatments	Effects on stroke in women	Effects on stroke in men	Bleeding events
ASA						
WHS[34]	39 876/39 876 (100%)	10	First non-fatal MI, non-fatal stroke or CV death ASA vs. placebo	17% reduction IS; non-significant increase in HS (RR 1.24) with ASA	NA	1.4-fold increase in major GI haemorrhages with ASA
ATT Collaboration meta-analysis[35]	NR/95 000 participants in primary prevention trials + 17 000 participants in secondary prevention trials	5	First serious vascular event ASA vs. placebo	OR 0.77 for IS (primary prevention) OR 0.73 for IS with ASA (secondary prevention)	OR 1.01 for IS (primary prevention) OR 0.91 for IS (secondary prevention)	OR 1.32 (primary prevention) with ASA OR 1.67 (secondary prevention) with ASA
Ticlopidine						
CATS[54]	407/1072 (48%)	2	Subsequent stroke, MI or vascular death after an atherothrombotic stroke ticlopidine vs. ASA	34.2% RRR of the combined endpoint; 33.5% RRR of fatal stroke in the overall population (20.5% at the ITT analysis) with ticlopidine		34 bleeding events reported in the ticlopidine arm (2 severe) vs. 16 non-severe bleeding events in placebo
TASS[55]	1104/3069 (36%)	2–6	Death or recurrent stroke in patients with recent minor stroke or TIA receiving ticlopidine or ASA	RRR of 21% at 3 years in the ticlopidine arm in both genders		1% GI bleeding reported with ticlopidine
Clopidogrel						
PRoFESS[58]	7319/20 332 (36%)	2.5	Recurrent stroke in patients with previous ischaemic stroke receiving ASA-ERDP vs. clopidogrel	Recurrent stroke: 9.0% in the ASA-ERDP vs. 8.8% in the Clopidogrel arm (HR: 1.01) Better results reported for clopidogrel in women than in men		Major haemorrhagic events: 4.1% in the ASA-ERDP vs. 3.6% in the clopidogrel arm (HR 1.15) Net risk of stroke or major bleeding similar in the two groups (11.7 vs. 11.4%; HR 1.03)
ASA/dypiridamole						
ESPS-2[56]	2772/6602 (42%)	2	Stroke, death or combination of stroke/death in patients with prior stroke randomized to ASA, ASA-ERDP, ERDP or placebo	Combination therapy reduced the risk of stroke by 23% vs. ASA alone and by 25% vs. placebo.		All-site and GI bleeding more common in ASA recipients
ESPRIT[57]	958/2739 (35%)	3.5	Vascular death, stroke, MI or major bleeding in patients with prior stroke receiving ASA vs. ASA-ERDP	Event rates: 13% in the combination (HR 0.84 for recurrent stroke) vs. 16% in the ASA arm without significant differences between genders		Major bleeding event rate: 2% in ASA-ERDP recipients vs. 4% in ASA recipients (HR: 0.67)

ASA/Clopidogrel					
MATCH[59]	2821/7599 (37%)	1.5	IS, MI, vascular death or rehospitalization for any vascular ischaemia in patients with prior stroke receiving placebo + clopidogrel vs. ASA + clopidogrel	Non-significant 7.1% RRR of IS with combination therapy, without significant differences between genders	1.3% absolute increase in life-threatening and major bleeding in the combination therapy arm
Cilostazol					
CSPS[60]	373/1067 (35%)	2	Recurrent stroke in patients with prior cerebral infarction treated with cilostazol vs. placebo in association with an antiplatelet or an antithrombin agent	41.7% RRR of the primary end-point with cilostazol No gender-related differences reported	No increase in GI or intracranial haemorrhages with cilostazol
CSPS -2[61]	NR/2672	2.5	Recurrent stroke in Asian patients with prior cerebral infarction treated with cilostazol vs. ASA	HR 0.74 , $P = 0.03$ indicating non-inferiority of cilostazol vs. ASA No gender-related differences reported	0.77%/year bleeding in the cilostazol arm vs. 1.78%/year in the ASA arm

ASA, aspirin; ASA-ERDP, Aspirin – extended release dypiridamole; CV, cardiovascular; GI, gastrointestinal; HR, hazard ratio; HS, haemorrhagic stroke; IS, ischaemic stroke; ITT, intention-to-treat; MI, myocardial infarction; NR, not reported; OR, odds ratio; RRR, relative risk reduction; TIA, transient ischaemic attack.

lower threshold, starting <5 years after exposure and continuing for at least 20 years. Of note, the absolute increase of future cardiovascular events for a given dose was larger for women with pre-existing risk factors. Thus, women with breast cancer should optimize their risk factor profile and prophylactic use of antiplatelet drugs might be advocated in those undergoing radiotherapy.

Antiplatelet therapy in secondary cardiovascular prevention

Efficacy

In the ATT meta-analysis, there was no convincing evidence of interaction between gender and the effects of aspirin vs. placebo for the secondary prevention of cardiovascular disease: in particular, the relative risk reduction of major coronary events during the follow-up with aspirin was 19% in males and 27% in females (P for interaction = 0.4) and of serious vascular events (MI, any stroke, or vascular death) 19% in both genders (P for interaction = 1.0).[35]

Berger *et al.* performed a meta-analysis of five randomized trials comparing clopidogrel vs. placebo in addition to aspirin and involving a total of 79 613 patients with CHD (the large majority with ACS) or at a high risk for the recurrence of cardiovascular disease.[41] Among the 23 533 women studied here, the cardiovascular event rates over the long term were 11.0% in the clopidogrel group vs. 11.8% in controls (OR: 0.93; 95% CI: 0.86–1.01); the decrease in cardiovascular events with clopidogrel was significant in men (7.8 vs. 9.0%; OR: 0.84; 95% CI: 0.78–0.91). There was a trend towards statistical heterogeneity based on gender ($P = 0.09$), but the authors hypothesized that most of those gender differences could be explained by chance. This analysis, mainly focused on secondary prevention, nicely exemplifies how the absolute risk of events during the follow-up is higher in women than in men and how the relative benefit by clopidogrel therapy appears attenuated in women (7% relative reduction) vs. men (16% reduction), while gender differences in the absolute benefit are not striking (0.8% in women vs. 1.2% in men).[41] In the TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet iNhibition with prasugrel–Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38), comparing prasugrel vs. clopidogrel in aspirin-treated ACS patients receiving PCI,[42] there was no significant interaction between treatment and gender, although men again showed higher absolute (2.4 vs. 1.6%) and relative (21 vs. 12%) reductions in major cardiovascular events at 15 months with prasugrel than women. Similarly, in the PLATelet Inhibition and patient Outcomes (PLATO) study comparing ticagrelor vs. clopidogrel on the top of aspirin therapy in ACS patients, no significant gender difference was observed in the absolute and relative reductions of adverse events at 1 year by ticagrelor.[43]

Finally, a recent investigation[44] on patients receiving oral anticoagulants (69% for atrial fibrillation) and undergoing PCI compared triple (anticoagulant plus clopidogrel and aspirin) or dual antithrombotic therapy (anticoagulant plus clopidogrel alone); the latter strategy decreased 1-year incidence of bleeding complications (HR: 0.36, 95% CI: 0.26–0.50) and this was irrespective of gender. No increase of thrombotic events was observed in the clopidogrel-anticoagulant arm, but the study was not powered for ischaemic endpoints.

Suggestions regarding the use of antiplatelet therapy in women for secondary cardiovascular prevention are reported in Supplementary material online, *Summary Box 2*.

Safety

Berger *et al.*[41] documented in a large meta-analysis that the use of clopidogrel vs. placebo on the top of aspirin therapy was associated with more frequent major bleedings over the long term in both men (OR: 1.22, 95% CI: 1.05–1.42) and women (OR: 1.43, 95% CI: 1.15–1.79). In the TRITON-TIMI 38 trial, multivariate analysis indicated that female gender was the strongest predictor of non-bypass-related major bleeding during the follow-up (hazard ratio, HR: 1.77).[45] Also in the PLATO study, female gender was independently associated with a greater risk of bleeding in the context of PCI (HR: 2.3), but this association was no longer statistically significant for non-PCI-related bleeding.[46]

Antiplatelet therapy and stroke

Stroke is a growing health concern especially among women; in females aged <75 years, the prevalence of stroke is higher than that of CHD, women between 45 and 54 years are more than twice as likely as men to have a stroke,[47] and, since women have a greater life expectancy, their lifetime risk of stroke is more pronounced. Besides a higher prevalence of systemic hypertension, women have unique risk factors for stroke and hormone replacement therapy may independently increase the risk of ischaemic stroke in post-menopausal women.[48,49] Of note, women present a worse outcome after a stroke compared with men, with increased post-stroke mortality, disability, depression, and dementia.[50]

Primary prevention of stroke

In the WHS, there was a 17% risk reduction (*Table 3*) in the incidence of stroke with the use of aspirin, especially of ischaemic stroke, and the most consistent benefit was found in women >65 years old and in those with multiple risk factors or a >10% cardiovascular risk at 10 years;[34] in this study, there was only a trend towards decreased rates of any stroke (ischaemic or haemorrhagic) with aspirin, probably due to a somewhat increased incidence of haemorrhagic stroke. In large meta-analyses, the risk of intracranial bleeding with the use of aspirin in individuals without overt cardiovascular disease was 0.03% per year (vs. 0.02% in controls) and the risk of extracranial bleeds was 0.08% per year (vs. 0.05%).[35]

Secondary prevention in patients with stroke

Four antiplatelet drugs have been approved for the prevention of vascular events in patients with stroke or transient ischaemic attacks (TIA): aspirin, ticlopidine, the combination of aspirin and dipyridamole, and clopidogrel. These agents overall reduced the relative risk of stroke, MI, or death by 22%, but differences between the effects of such drugs were observed, with implications for therapeutic selection.[51] We now summarize the differential role of the various antiplatelet agents in the secondary prevention of stroke (see also *Table 3*):

Aspirin

Among patients with a recent stroke or TIA, in randomized trials the use of aspirin was associated with a 15% relative reduction in the overall risk of a new cerebrovascular event.[35,51,52] The reduction of ischaemic stroke risk was greater than the increase of haemorrhagic stroke risk, thus resulting in a net clinical benefit. Benefits on ischaemic stroke were observed with daily doses ranging from 50 to 1500 mg, without significant differences, while side effects, particularly gastro-intestinal bleeding, increased with higher doses.[51–53] Thus, the recommended doses range from 50 to 325 mg/day.

Ticlopidine

The randomized Canadian American Ticlopidine Study (CATS) compared ticlopidine (250 mg twice a day) with placebo for the prevention of stroke, MI or vascular death in patients with a recent ischaemic stroke;[54] after a mean follow-up of 2 years, the event rates among patients assigned to ticlopidine therapy were approximately one-fourth lower than in those assigned to placebo, and this benefit was similar in men and women. The Ticlopidine Aspirin Stroke Study (TASS) assessed the recurrence of stroke, as well as mortality, in patients with a recent minor stroke or TIA treated with ticlopidine 250 mg twice a day or aspirin 650 mg twice a day. Here, about one-third of subjects enrolled were women and 3-year event rates in both genders were reduced in the ticlopidine arm;[55] however, the overall safety profile of ticlopidine was poorer than that of aspirin, with a higher incidence of side effects, including neutropenia, skin rash, and diarrhoea (35 vs. 15%).

Dipyridamole plus aspirin

Dipyridamole inhibits adenosine reuptake and, at higher concentrations, inhibits phosphodiesterases, enhancing prostacyclin-induced suppression of platelet function. In patients with previous TIA or stroke, the association of dipyridamole (400 mg daily) with low-dose aspirin (50 mg daily) was compared with low-dose aspirin (50 mg daily) in the European Stroke Prevention Study (ESPS-2)[56] and with higher-dose aspirin (30–325 mg daily) in the European/Australasian Stroke Prevention in Reversible Ischemia (ESPRIT) trial.[57] In these two studies such combination therapy significantly reduced the risk of both stroke and cardiac/cerebral events. No significant difference in the outcome was found between genders, although a trend towards lower benefit with combination therapy was seen in women. However, headache and gastro-intestinal symptoms were an important cause of dipyridamole discontinuation, and this represents a major limitation for the use of such an agent in clinical practice.

Clopidogrel

No specific comparison is available between clopidogrel and placebo in secondary stroke prevention. In the Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial, patients with ischaemic stroke were randomly allocated to clopidogrel (75 mg per day) or aspirin (25 mg twice daily) plus extended release dipyridamole (200 mg twice daily) and followed for 2.5 years.[58] The study showed similar rates of recurrent stroke in the two arms. The incidence of bleeding events was higher in the aspirin/dipyridamole group, but the net risk of recurrent stroke or major bleeding was not different.[58] Although the trial was not powered to determine

gender-related effects, a better treatment effect with clopidogrel was reported in women vs. men (0.5% absolute reduction of recurrent stroke vs. 0.1% absolute increase).

Combination of aspirin and clopidogrel

The effectiveness of associating clopidogrel 75 mg/day and aspirin 75 mg/day vs. clopidogrel alone in patients with a recent TIA or ischaemic stroke was evaluated in the Management of AtheroThrombosis with Clopidogrel in High-risk Patients with Recent Transient Ischaemic Attacks or Ischaemic Stroke (MATCH) trial.[59] A total of 7599 patients were followed for 1.5 years for the occurrence of the primary composite outcome including ischaemic stroke, MI, vascular death, or re-hospitalization for any central or peripheral ischaemic event. No significant benefit was found in patients allocated to the combination therapy, in whom a significant increase in the risk of major bleedings was observed. Similar results were reported in the subgroup of women.

Other antiplatelet agents

A largely studied agent in Asian populations (Japan, China) is cilostazol, a phosphodiesterase inhibitor. In patients with previous non-cardioembolic cerebral infarction the Cilostazol Stroke Prevention Study (CSPS) compared cilostazol with placebo[60] and two studies evaluated cilostazol vs. aspirin.[61,62] Similarly to other antiplatelet trials, in CSPS[60] women were ~35%, while in CSPS-2 no data about gender composition of the included population were provided.[61] Overall, in comparison with placebo, cilostazol was effective in reducing the incidence of secondary stroke, especially in patients with lacunar infarction, suggesting a pivotal role in small vessel disease. Compared with aspirin, the use of cilostazol was associated with a significantly lower risk of vascular events and haemorrhagic stroke, but at the price of an increased occurrence of side effects.

Antiplatelet agents in patients with diabetes

Gender may contribute to the platelet hyper-reactivity or low response to antiplatelet agents in patients with diabetes mellitus; Park *et al.*[63] evaluated a large South-Korean population including 1658 women and interestingly found that platelet count was significantly higher in women with metabolic syndrome compared with those without, whereas mean platelet volume was lower. High platelet volume has been associated with an increased risk of adverse cardiovascular events in diabetic subjects, indicating that women with diabetes might have some protection.[64]

Primary prevention

Current knowledge of gender-related effects of aspirin for cardiovascular prevention in patients with diabetes is essentially based on subgroup analyses of trials designed to evaluate its effects in general populations, which increases the risk of bias.[65] While randomized trials on aspirin for primary cardiovascular prevention have insufficient data at the individual patient level to stratify the estimates of aspirin effects in men and women with diabetes, recent observational evidences suggest that these effects may differ between genders, with women consistently deriving less benefit than men. In a large population-based cohort study of 18 646 diabetic patients free of cardiovascular disease followed for a mean of 3.9 years, the benefit–risk ratio associated with aspirin treatment was less favourable in women:[66] in particular, overall bleeding rates were 2.4/1000 patients/year in women and 2.3/1000 patients/year in men, whereas the HR for mortality vs. no aspirin use was 1.07 (95% CI: 0.81–1.40) in women and 0.81 (0.64–1.02) in men.

Secondary prevention

The American Diabetes Association (ADA) recommends using low-dose aspirin (75–162 mg/day) for the secondary prevention of both cerebrovascular and cardiovascular events in all diabetic patients irrespective of gender.[67] This is supported by an ATT collaboration meta-analysis on major secondary prevention trials, demonstrating a 1% absolute and 7% relative reduction of death, MI, and stroke in patients with diabetes mellitus, irrespective of gender.[51]

Robust data on the efficacy of clopidogrel alone for secondary cardiovascular prevention are lacking; however, in the subgroup analysis of the Clopidogrel vs. Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial[68] for every 1000 diabetic patients treated, clopidogrel prevented 21 vascular events (38 in insulin-treated patients). Moreover, the use of clopidogrel instead of no clopidogrel use after MI was associated with lesser reduction in the risk of cardiovascular death in patients with diabetes compared with those without (HR: 0.93 vs. 0.77, P for interaction 0.01), irrespective of gender.[69] Of note, it is well known that the prevalence of impaired response to clopidogrel is significantly lower in non-diabetic vs. diabetic subjects and this may explain the reduced degree of clinical benefit with this agent in the latter.[70] In the subgroup of diabetic patients with ACS enrolled in the TRITON-TIMI 38 trial, the relative risk reduction of major adverse cardiac events at 15 months with prasugrel vs. clopidogrel was numerically higher in men than in women (36 vs. 15%), but *P* for interaction was not significant.[71] Finally, no interaction between gender and the efficacy of ticagrelor vs. clopidogrel was evaluated in the diabetic subgroup of the PLATO trial.[72] Suggestions regarding the use of antiplatelet therapy for primary and secondary cardiovascular prevention in diabetic women are reported in Supplementary material online, *Summary Box 1*.

Antiplatelet therapy in pregnancy

Pregnancy is associated with an increased incidence of thrombotic events. In particular, cardiovascular diseases complicate 0.4–4% of all pregnancies and ACS may occur in 0.6 to 1.0 cases per 10 000 deliveries, with a maternal mortality ranging from 5 to 37%.[73,74]

The higher occurrence of thrombotic events in pregnancy is mainly related to a pro-thrombotic state, physiologically useful to reduce bleeding at delivery.[75] Such haemostatic changes are more pronounced in the third trimester, have been attributed to increased oestrogen levels, and include higher platelet aggregation and concentration of coagulation factors together with reduced fibrinolytic capacity.[75] During pregnancy, platelet count slightly decreases, leading to a benign gestational thrombocytopenia due to increased platelet consumption in the utero-placental circulation;[76] this accelerated platelet turnover and the consequent higher number of immature platelets explain the higher platelet aggregability. Pregnancy also induces changes in the platelet membrane, with consequent

enhanced activity of calcium adenosine triphosphatase.[77] Finally, a significant increase in α-granule-released proteins, such as beta-thromboglobulin and platelet factor-4, has been observed in the circulation of pregnant women, suggesting more pronounced platelet activation.[77]

Data concerning the efficacy of antiplatelet drugs in pregnancy are limited, as pregnant women are usually excluded from randomized trials, and in this particular setting the safety profile of drugs is a crucial concern. Regarding the level of risk to the foetus, drugs are classified in five categories, as indicated in *Table 4*.[78]

Aspirin (risk category C for low dose and D for high doses)

Experimental data on aspirin treatment in the first trimester of pregnancy have shown an increased incidence of birth defects, i.e. fissure of spine and skull, facial and eye defects, central nervous system malformations and abnormalities of visceral and skeletal development (Supplementary material online, *Summary Box 1*)[79,80] however, these data are not consistent with clinical findings on pregnant women receiving aspirin.[81] While high-dose aspirin was associated with a premature closure of the ductus arteriosus and with foetal and maternal haemorrhages,[78] previous reports have shown efficacy and safety of low-dose aspirin in pregnant ACS patients.[74] Although the use of aspirin in the third trimester should be avoided (as it may cause premature closure of the ductus), in isolated observations low-dose aspirin showed a potential benefit in reducing the incidence of pre-eclampsia and the occurrence of pre-eclampsia-related pre-term delivery (absolute reduction of 2.5 per 100 women treated); according to available data, use of low-dose aspirin may be justified in women judged to be at a greater risk of early pre-eclampsia.[82] Finally, no adverse effect was reported with aspirin therapy during breastfeeding,[74,78] but the use of high doses may cause rashes, platelet abnormalities, and bleeding in nursing infants.

Table 4 Drug classification regarding the level of risk to the foetus.[78]

Category	Definition
A	Well-controlled studies failed to demonstrate a risk to the foetus in the first trimester of pregnancy and there is no evidence of foetal risk in later trimesters
B	Animal reproduction studies failed to demonstrate a risk to the foetus and there are no well-controlled studies in pregnant women
C	Animal reproduction studies showed an adverse effect on the foetus and there are no adequate studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential foetal risks
D	There is positive evidence of human foetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential foetal risks
X	Animals or humans studies demonstrated foetal abnormalities and/or there is positive evidence of human foetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits

Clopidogrel (risk category B)[79]

Reproduction studies on animal models at doses of up to 300 and 500 mg/kg/day (65 and 78 times the recommended daily human dose, respectively) have shown no foetal toxicity with clopidogrel. As for other antiplatelet drugs, there are no extensive data from clinical studies on clopidogrel in pregnant women. Most available findings derive from isolated case reports,[83,84] in which the general outcome was favourable for both the mother and the foetus. Only in one case was there a 3.6 g/dL drop in haemoglobin levels after delivery, requiring blood transfusion, but here the source of bleeding was not identified.[85] In another case, the baby presented a patent foramen ovale, a muscular ventricular septal defect, and moderate mitral regurgitation.[84]

Prasugrel (risk category B)[79]

No extensive clinical data on pregnant women are available; this agent should be used only if the potential benefit to the mother overcomes the potential risk to the foetus. According to the prasugrel prescribing information, pregnant rats and rabbits received maternally toxic oral doses equivalent to >40 times the human exposure: no structural malformations were observed, but there was a slight decrease in pup body weight. A successful pregnancy and delivery was described in one patient treated with prasugrel plus low-dose aspirin after drug eluting stent implantation.[86]

Ticagrelor (risk category C)[79]

In animal studies, ticagrelor caused structural abnormalities of the foetus at maternal doses five to seven times the maximum recommended human doses. However, no clinical study has investigated the safety of ticagrelor during pregnancy; thus, according to drug's prescribing information, ticagrelor should be used in pregnant women only if the potential benefit overcomes the potential deleterious effects to the foetus.

Studies in animal models have shown that all oral P2Y12 inhibitors are excreted in milk, but it is not known whether such drugs are excreted in human milk. Because of the potential for adverse reactions in nursing infants, these agents should be used during breastfeeding only if the potential benefit to the mother exceeds the potential risk to the nursing infant.

Conclusions

Data from observational and intervention studies, for instance in primary cardiovascular prevention, do not exclude gender-specific effects on clinical outcomes with antiplatelet agents; differences in platelet function, vascular factors, and coagulation mechanisms in different vascular beds, partly related to hormonal status, might contribute, although strong evidence is lacking. For secondary cardiovascular prevention, although no significant gender-related differences in the efficacy of antiplatelet agents emerge, special attention should be paid to age, renal function, body weight, and dosing strategies when treating women in order to optimize benefits and minimize bleeding complications.

On the basis of the available data, we provide suggestions on current indications of antiplatelet therapy for cardiovascular prevention in women with different clinical features (see Supplementary material online, *Summary Boxes*). As the mean percentage of women included in randomized trials evaluating cardiovascular outcomes with the use of antiplatelet drugs has not changed in the last 20 years (~30%), it is urgent to include more women in such trials in order to produce strong evidence-based recommendations on the topic.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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