

Gender and Responses to Aspirin and Clopidogrel: Insights Using Short Thrombelastography

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There is significant variability in both baseline clotting tendency and response to antiplatelet therapy. Responses are associated with outcome. We have investigated whether differences could explain the increased risk observed in women presenting with coronary artery disease. We have utilized short thrombelastography to assess (i) baseline clotting responses, (ii) response to aspirin and clopidogrel, and (iii) post-treatment platelet reactivity in 48 young volunteers, 22 older patients and 18 patients with previous stent thrombosis. Baseline responses were significantly higher in young women than in men. While there was no difference in response to aspirin, platelet reactivity on aspirin remained higher in women (area under curve at 15 min [AUC15] of arachidonic acid channel 332 ± 122 vs. 172 ± 80 , $P = 0.04$). Young women had less response to clopidogrel (% reduction in AUC15 with adenosine diphosphate [ADP] 36.4 ± 12.4 vs. 64.0 ± 13.2 , $P < 0.01$) in addition to higher post-treatment reactivity (AUC15 of ADP 714 ± 161 vs. 311 ± 146 , $P < 0.01$) compared to men. There were no such differences between male and female patients over 50. However, young women with previous stent thrombosis had among the highest platelet reactivity observed. Compared to men, young women have greater baseline clotting tendency, reduced response to clopidogrel, and greater post-treatment reactivity while on both aspirin and clopidogrel. Differences in clotting tendency and response to antiplatelet therapy may contribute to the excess risk observed in young women but are not observed in older female patients.

Background

There are well-established gender differences in both clinical presentation and outcome in ischemic heart disease. For example, women, while less likely to suffer from myocardial infarction (MI), have higher in-hospital mortality and are more likely to sustain complications than men [1,2]. While there are differences in presentation, anatomy, comorbidities, and provision of evidence-based therapies [3,4] between the sexes, significant clinical differences remain even when these factors are taken into account, particularly in women under the age of 50 who have double the mortality rate of men with the same risk profile [5,6].

It is possible that differences in propensity to blood clotting and response to antiplatelet therapy explain some of the observed gender differences. Baseline platelet hyperreactivity is more common in women, and mean levels of platelet aggregation in response to stimulation with platelet agonists are consistently greater in women than in men [7,8]. Responses to antiplatelet therapy may also differ between men and women. For example, aspirin therapy appears to be less effective in women. A randomized controlled trial of low-dose aspirin therapy as primary prevention in 39,876 high-risk women showed no significant reduction in cardiovascular events after 10 years when compared to placebo [9] in contrast to data from predominantly male study populations, which show

an average 32% reduction in risk of MI [10]. This could be partly explained by the finding that female gender is associated with higher adenosine diphosphate- (ADP) and collagen-induced platelet aggregation while on aspirin [11]. A strong association has also been shown between clopidogrel resistance and female gender in patients with coronary artery disease (CAD) using ADP-induced aggregometry [12].

It is unclear, however, whether these observed gender differences are due to differences in baseline reactivity or due to differences in response to antiplatelet agents. Using a newly validated modification of thrombelastography (TEG) PlateletMapping™, “short TEG” [13,14], we have sought to elucidate if there are gender differences in platelet reactivity in young women volunteers and in female patients undergoing percutaneous coronary intervention (PCI) that could potentially contribute to our understanding of these gender differences in clinical outcome.

TEG is uniquely suited to this role. Unmodified TEG provides a global assessment of coagulation incorporating the effects of platelets, thrombin, fibrin, and coagulation factors. Used in this way TEG has previously demonstrated greater coagulability in female trauma patients [15]. In addition, TEG can be modified to assess responses to antiplatelet therapy “TEG PlateletMapping™”, and such assays can now be completed as a 15-min test (“short TEG”), as previously described by this group [14]. Responses to both aspirin and clopidogrel assessed in whole blood with TEG PlateletMapping™ have been correlated with optical aggregation [16,17]. A single TEG assay can therefore provide information on both the overall tendency to thrombosis and the response to antiplatelet therapy.

The aim of this study was therefore to utilize short TEG to establish if there are gender differences in (i) clotting tendency, (ii) response to antiplatelet therapy, and (iii) post-treatment platelet reactivity in young volunteers and in patients undergoing PCI which could contribute to the high risk of mortality observed in young women post-MI.

Methods

Approval was obtained from the Southampton and South West Hampshire Research Ethics Committee B and the Isle of Wight, Portsmouth and South East Hampshire Research Ethics Committee prior to commencing the study. All subjects provided written informed consent. Power calculations determined that group sizes of 17 would detect a 10% difference in platelet function between men and women with 80% power. The following groups were studied.

Healthy Volunteers

Fifty-one volunteers (23 Males and 28 Females) all under the age of 50 were recruited. Individuals were excluded if they smoked, had taken antiplatelet medication or non-steroidal anti-inflammatory medication within 14 days, or if they had a history of peptic ulceration, bronchial asthma, or bleeding. All 51 individuals had a baseline blood test. Thirty-three had blood tests immediately before and 6 h after a witnessed administration of 300 mg of aspirin and 32 had blood tests immediately before and 6 h after 600 mg of clopidogrel.

Patients Admitted for Elective PCI

For comparison, a group of 22 patients (12 males and 10 females) attending for routine PCI were recruited. All patients were nonsmokers, not diabetic, on statin therapy, known to have CAD and between the ages of 50 and 80 years. All 22 patients reported compliance with aspirin maintenance therapy and received a 600-mg loading dose of clopidogrel prior to PCI. Blood tests were taken prior to, and 6 h after clopidogrel loading.

Patients with a History of Stent Thrombosis and Matched Controls

Data were also obtained from survivors of nonacute stent thrombosis (ST) while on maintenance aspirin and clopidogrel therapy from a consecutive cohort of PCI patients at this center and matched PCI patients without ST [18,19]. We studied responses in 18 patients with previous ST reporting compliance with dual antiplatelet therapy including aspirin and clopidogrel 75 mg daily (who were derived from a database search of 3004 consecutive PCI patients at this center) and an equal number of matched controls. Blood tests were taken from all patients at least 10 days after the latest coronary event or intervention.

Sample Analysis

In all subjects, venesection was performed from the antecubital fossa. The first 2 mL were discarded, and then blood was drawn into a 6 mL lithium heparin vacutainer.

The samples were analysed using TEG PlateletMapping (Haemoscope, IL, USA) utilizing four channels according to manufacturer's instructions. The four channels incorporate a “thrombin channel” with maximal platelet activation achieved through reversal of heparin with heparinase and maximal thrombin stimulation with kaolin

activation; a “fibrin channel” representing platelet independent clot formation (generated without thrombin generation by the addition of reptilase and factor XIIIa to a heparinized sample) and channels with reptilase, factor XIIIa and arachidonic acid (AA) or ADP stimulation. All channels were run until the maximum amplitude (MA; representing maximal clot strength, determined by the standard TEG software) was obtained or at least 60 min had elapsed. The area under the response curve at 15 min (AUC15; incorporating both clot strength and the rate of clot formation) was also calculated using a software program developed by this group using National Instrument Labview 7.0 (Areafinder 2:1) as previously described by this group [13,14].

For each sample, the MA and AUC15 were recorded for all channels. If subjects had more than one baseline sample only the first was included in analysis of baseline responses. In order to calculate the effect of treatment, the percentage change from baseline (taken immediately prior to drug administration) in the MA and AUC15 (of the AA for Aspirin, and the ADP channel for Clopidogrel) were calculated.

From the post-treatment samples, the percentage platelet inhibition (%PI_n) was calculated from the MA by comparing the fibrin channel with maximal platelet activation due to thrombin and platelet activation due to AA and ADP in the presence of fibrin stimulation [16]. The percentage clotting inhibition (%CI_n) was calculated from the AUC15 by comparing AA- or ADP-induced clotting responses with the response to thrombin [14].

The number of nonresponders in each gender group was calculated for both clopidogrel and aspirin. A “non-responder” to clopidogrel was defined using the AUC15 (less than 30% reduction in the ADP channel compared to baseline) and by the %PI_n (less than 30% inhibition). Nonresponse to aspirin was defined as less than 50% reduction in the AUC15 and by %PI_n of less than 50%.

Data are presented as the mean \pm 95% confidence interval of the mean. Significance between groups was determined using two-tailed, two group *t*-tests with a *P*-value of <0.05 considered to represent significance. Two-tailed Fisher’s exact tests were used to determine differences between numbers of responders.

Results

Healthy Volunteers

Demographics

There were no important differences between the gender groups. Mean age was 28.8 ± 2.6 years in men and 26.2 ± 2.7 years in women.

Table 1 Baseline TEG responses in male and female volunteers

	Female	Male	<i>P</i>
Thrombin	1141 \pm 93	983 \pm 94	<0.05
ADP	1090 \pm 55	812 \pm 112	0.0001
AA	1016 \pm 99	809 \pm 106	0.01
Fibrin	210 \pm 38	83 \pm 21	<0.0001

The AUC15 in the four channels of TEG PlateletMapping prior to the administration of antiplatelet medication. Data presented as mean \pm 95% confidence interval of the mean.

Baseline Responses

At baseline, both the MA and the AUC15 were significantly greater in females than in males in all four channels (Table 1).

Responses to Aspirin

In females, the MA and AUC15 of the AA channel were significantly greater after aspirin administration (Table 2). However, there were no significant differences between men and women in the percentage change from baseline (Fig. 1) or in the %PI_n or %CI_n. Two of 17 men (12%) and two of 15 women (13%) were nonresponders to aspirin assessed by change in the AUC15 of the

Table 2 Responses of male and female volunteers to aspirin and clopidogrel

	Female	Male	<i>P</i>
Aspirin 300 mg			
MA at 6 h	28.9 \pm 10.5	14.8 \pm 5.6	0.04
AUC15 at 6 h	350 \pm 122	193 \pm 80	0.04
% change in MA	57.6 \pm 14.6	74.8 \pm 14.9	0.11
% change in AUC15	67.0 \pm 10.8	74.5 \pm 14.9	0.44
%CI _n	63.9 \pm 15.5	78.6 \pm 10.2	0.13
%PI _n	71.8 \pm 22.7	82.5 \pm 13.2	0.43
Nonresponders (AUC15)	2 (13%)	3 (17%)	1.0
Nonresponders (%PI _n)	3 (20%)	2 (11%)	0.64
Clopidogrel 600 mg			
MA at 6 h	43.1 \pm 10.0	25.3 \pm 7.0	0.008
AUC15 at 6 h	663 \pm 187	353 \pm 112	0.01
% change in MA	35.1 \pm 13.7	56.2 \pm 11.0	0.03
% change in AUC15	40.7 \pm 14.2	61.0 \pm 10.1	0.02
%CI _n	37.5 \pm 18.3	61.4 \pm 12.3	0.02
%PI _n	40.6 \pm 16.0	65.3 \pm 11.7	0.04
Nonresponders (AUC15)	10 (63%)	1 (6%)	0.002
Nonresponders (%PI _n)	8 (50%)	0 (0%)	0.01

Responses to (i) aspirin 300 mg assessed with the TEG AA channel and (ii) clopidogrel 600 mg assessed with the TEG ADP channel. Data presented as mean \pm 95% confidence interval of the mean.

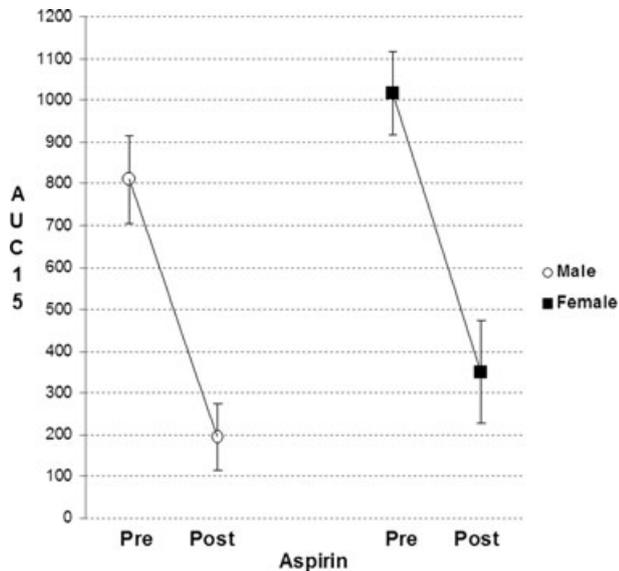


Figure 1 Responses to aspirin in male and female volunteers. Response to aspirin assessed with the AUC15 of the AA channel. There are significant differences from baseline in both males and females. There is no statistically significant difference in the change from baseline based on gender.

AA channel, and one (6%) man and three (20%) women when assessed using the %PI_n.

Responses to Clopidogrel

The MA and the AUC15 of the ADP channel were significantly greater in females after clopidogrel administration (Table 2). In addition, the percentage change from baseline was significantly less in females (Fig. 2) as was the %PI_n and %CI_n.

Ten of 16 females (63%) and one of 16 males (6%) met the criteria for nonresponse to clopidogrel using the change in the AUC15 of the ADP channel ($P = 0.002$). Eight women (50%) and no men (0%) were nonresponders as assessed by the %PI_n ($P = 0.01$).

Patients Undergoing Elective PCI

Demographics

There were no significant differences between the two groups in terms of age (68 ± 5 in men and 64 ± 5 in women, $P = 0.27$), medication (all were on statins, one in each group on Atorvastatin), or baseline hematological parameters. Males were significantly heavier (90 ± 8 kg vs. 73 ± 7 kg, $P = 0.004$).

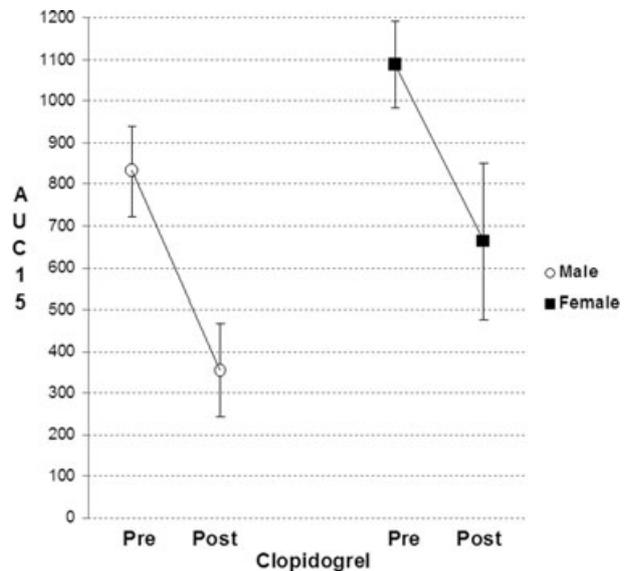


Figure 2 Responses to clopidogrel in male and female volunteers. Response to clopidogrel assessed with the AUC15 of the ADP channel. There are significant differences from baseline in both males and females. The change from baseline is greater in men.

Responses to Clopidogrel

There were no significant differences in the MA or the AUC15 between the gender groups after clopidogrel administration (Table 3). There were also no significant differences in the percentage change from baseline or in the number of nonresponders to clopidogrel therapy.

Patients with History of Stent Thrombosis

Demographics

There were 14 ST cases in men and four cases in women. Three of the four cases in women occurred in women

Table 3 Responses of male and female patients to a clopidogrel loading dose

	Female	Male	<i>P</i>
MA at 6 h	43.1 ± 10.0	25.3 ± 7.0	0.008
AUC15 at 6 h	663 ± 187	353 ± 112	0.01
% change in MA	35.1 ± 13.7	56.2 ± 11.0	0.03
% change in AUC15	40.7 ± 14.2	61.0 ± 10.1	0.02
%CI _n	37.5 ± 18.3	61.4 ± 12.3	0.02
%PI _n	40.6 ± 16.0	65.3 ± 11.7	0.04
Nonresponders (AUC15)	10 (63%)	1 (6%)	0.002
Nonresponders (%PI _n)	8 (50%)	0 (0%)	0.01

Response to a 600-mg clopidogrel-loading dose in patients with acute coronary syndromes, on 75 mg aspirin maintenance therapy assessed with the TEG adenosine diphosphate channel. Data presented as mean ± 95% confidence interval of the mean.

under the age of 52. The groups were closely matched. All patients had drug eluting stents. There were no significant differences in duration and dose of antiplatelet therapy (dose of clopidogrel 80.4 ± 10 mg in men vs. 75 ± 0 in women, duration of clopidogrel 287 ± 163 days vs. 276 ± 77 days, coronary risk factors 14% of men were diabetic vs. 0% of women; 21% of men vs. 25% of women were smokers) or procedure undertaken (1.5 ± 0.3 vs. 2 ± 2 stents; minimum stent diameter 2.75 ± 0.2 vs. 2.75 ± 0.2 mm; 14% vs. 25% emergency procedures).

Responses to Clopidogrel

The AUC15 of the ADP channel was 849 ± 188 in the ST group and 640 ± 128 in the control group. Two of the three young women with previous ST have among the highest platelet reactivity despite clopidogrel (AUC15 1159 and 1056, respectively).

Discussion

This study, employing a newly validated short TEG method, demonstrates that there is both an elevated baseline clotting tendency and a reduced response to clopidogrel in young healthy females compared to equivalent males. While these gender differences are not observed in a typical population of older and postmenopausal female patients, it is of interest that the small number of young women from our registry who survived ST did indeed exhibit reduced responsiveness to Clopidogrel.

It is now well established that there is significant variability in responses to antiplatelet therapy (particularly clopidogrel) between individuals [11–14,16,17] and that poor response is associated with adverse outcomes [19–23]. In addition, overall tendency to thrombosis (measured by unmodified TEG) has been associated with adverse outcome in PCI [20]. These observations stimulate speculation that such inter-individual variability could be a potential target for therapeutic intervention and manipulation. However, there is as yet no universally accepted, rapid, and reliable near-patient test of the effects of antiplatelet therapy and no widely accepted definition of resistance or poor response. Furthermore, currently available assays are limited by their inability to differentiate poor response to therapy from the effects of high baseline platelet reactivity. As a result, identifying individuals at risk of ST after PCI either (a) by virtue of an increased overall tendency to thrombosis or (b) through resistance to antiplatelet therapy is still not possible in routine clinical practice. A universal “one size fits all” strategy is still used for the administration of oral antiplatelet therapies to patients undergoing PCI.

In this study, we have used short TEG to determine (i) overall clotting tendency, (ii) response to platelet agonists at baseline, and (iii) as a 15-min test of response to antiplatelet therapy. In healthy volunteers, we have shown that young (premenopausal) women have a greater clotting tendency and increased response to platelet agonists (AA and ADP) at baseline. While men and women in this cohort of volunteers respond equally to aspirin (in terms of percentage reduction from baseline, %CIn, %PIn, and the percentage of “nonresponders”), post-treatment platelet reactivity remains higher in women. With Clopidogrel, baseline and post-treatment platelet reactivity is higher in women but, in contrast to aspirin, the percentage reduction from baseline, %CIn, and %PIn are also significantly reduced in women compared to men, and there are also more women demonstrating hyporesponsiveness (or resistance). This suggests that the diminished response to aspirin observed in young female volunteers is explained by increased baseline platelet reactivity alone, whereas for clopidogrel the diminished response in women is due to both increased baseline platelet reactivity and a diminished response to the drug.

Intuitively, one would expect that premenopausal women exposed to blood loss would have compensatory mechanisms to limit bleeding. Indeed, baseline platelet count and reactivity are known to be higher in women than men [7,8]. This effect may be influenced by female hormones, possibly including hormonal replacement therapy and contraceptives [24]. Investigating the exact etiology was however beyond the scope of this preliminary study and we did not specifically investigate the effect of female hormones. However, even the role of oral contraceptives on platelet function is likely to be complex, depending not only on the hormones administered but also on the stage of the menstrual cycle.

The gender differences in volunteers are interesting but we found no such important differences between male and female patients over the age of 50. This age group represents the vast majority of female patients in clinical practice. Thus, on the basis of these data it is clearly not possible to extrapolate from the physiological differences observed in our healthy volunteers to the female patient. However, it is possible that a high baseline clotting tendency and a diminished response to clopidogrel may render younger women treated with PCI and stents at increased risk of ST. Certainly, the data from the few survivors of ST who were younger women did seem to provide some support to this hypothesis. While the groups were very closely matched, the small number of patients included in this study does not ensure that a clinically important difference between male and female patients can be excluded.

The findings from this study highlight the importance of understanding the differences between assays of clotting tendency, assays of platelet reactivity, and assessment of response to antiplatelet therapy. Specifically, while comparison with a baseline sample demonstrates the effect of drug therapy it may not most accurately reflect residual risk while on treatment. Similarly, an assay of platelet reactivity while on antiplatelet therapy may not be able to differentiate treatment failure from high baseline (and therefore post-treatment) platelet reactivity. In this regard, TEG PlateletMapping has potential advantages as (i) the thrombin channel gives additional information on overall clotting tendency, itself a marker of risk and (ii) allows assessment of the mechanism of treatment failure, allowing differentiation between "true" poor response to treatment (as we found in young women administered clopidogrel) and an adequate response to treatment but residual high platelet reactivity due to high baseline clotting tendency (as we found in young women administered aspirin).

This study has some limitations. First, while the administration of all medication was witnessed we cannot exclude the use of other medications not reported by study participants that could have influenced baseline results. Second, as mentioned above, there is no specific investigation of the effects of hormonal contraceptives in women in this study.

Conclusions

This study demonstrates higher post-treatment platelet reactivity in young female volunteers after the administration of both aspirin and clopidogrel. There was also a reduced response to clopidogrel, but not aspirin, in women. As both high post-treatment reactivity and reduced response to clopidogrel have been correlated with outcome this may at least in part explain the worse outcome observed in young women. Consistent with this concept, we have found poor response to clopidogrel in two out of three young women with previous ST. This effect, however, does seem limited to young women and we found no differences in clotting tendency or response to antiplatelet therapy in older women who make up the vast majority of the patient population.

CAD is rare in premenopausal women. However, given the significantly increased risk associated with CAD in this group and the current data indicating an increased clotting tendency and diminished response to clopidogrel in young women volunteers we feel that this area warrants further study.

The strategy of giving standard doses of aspirin and clopidogrel to all PCI patients is almost certainly flawed.

This study also highlights how understanding the mechanisms of "poor response" could help guide treatment modification in these high-risk individuals.

Authors' Contributions

ARH participated in study design, data acquisition, and analysis, and drafted the manuscript. ZQ and PB performed data acquisition and analysis. NPC participated in study design and preparation of the final manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

Unrestricted research grants were obtained from Boston Scientific Corporation, Haemoscope Limited, and Medice Limited. Support was also obtained from Wessex Heartbeat Charity and Heart Research UK.

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