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ARTICLE

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Sex differences in platelet reactivity in patients with myocardial infarction treated with triple antiplatelet therapy - results from assessing platelet activity in coronary heart disease (APACHE)

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Abstract

)Several earlier studies have reported increased risk of bleeding in women with myocardial infarction, (MI) compared to men. The reasons for the observed difference are incompletely understood, but one suggested explanation has been excess dosing of antithrombotic drugs in women. The aim of this prospective observational study was to assess sex differences in platelet activity in patients treated with three different platelet inhibitors. We recruited 125 patients (37 women and 88 men) with MI, scheduled for coronary angiography. All patients received clopidogrel and aspirin. A subgroup of patients received glycoprotein (GP) IIb/IIIa-inhibitor. Platelet aggregation in whole blood was assessed at several time points, using impedance aggregometry. Soluble *P*-selectin was measured 3 days after admission. There were no significant differences between women and men in baseline features or comorbidities except higher frequency of diabetes, lower hemoglobin value, and lower estimated glomerular filtration rate, in women on admission. We observed significantly more in-hospital bleeding events in women compared to men (18.9% vs. 6.8%, $p = .04$). There were no differences in platelet aggregation using three different agonists, reflecting treatment effect of GPIIb/IIIa-inhibitors, clopidogrel, and aspirin, 6–8 hours, 3 days, 7–9 days, or 6 months after loading dose. Moreover, there was no significant difference in soluble *P*-selectin. The main finding of this study was a consistent lack of difference between the sexes in platelet aggregation, using three different agonists at several time-points. Our results do not support excess dosing of anti-platelet drugs as a major explanation for increased bleeding risk in women.

Keywords

Gender, myocardial infarction, platelet aggregation, sex

History

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Introduction

Platelet activation and clot formation play a very important role in the pathogenesis of myocardial infarction (MI). Dual antiplatelet therapy (DAPT), consisting of aspirin and a P2Y12-inhibitor, is a cornerstone in the treatment of patients with ACS, in the acute setting, as well as in secondary prevention during the first year of follow-up [1,2]. The more recently developed P2Y12-inhibitors ticagrelor or prasugrel have shown a lower risk of ischemic events and are therefore recommended in clinical guidelines. However, the decreased incidence of ischemic events comes at the price of increased rate of bleeding events [3–6]. Clopidogrel is still the most commonly used P2Y12-inhibitor worldwide, which is supported by a recent report from Wang et al. [7], partly because its lower price, but also because its association with lower bleeding risk compared to the newer drugs. Bleeding is the most common non-ischemic complication in patients with MI. This observation has gained much attention during the last years, due to the association between bleeding events and worse outcome, including prolonged hospitalization and increased mortality [8–10]. Previous studies have reported higher risk of bleeding in women with an ACS, compared to men, at least in the acute phase [11–13]. The reasons for the observed difference is incompletely understood, but clustering of other conditions associated with an increased risk of bleeding, such as age, low body weight, and chronic kidney disease, has been proposed [14]. Differences in dosage and effect of antithrombotic drugs and differences in platelet function has also been put forward as possible explanations [15]. Some studies on this topic have reported higher platelet reactivity in response to agonists in women as compared to men, which would point to an increased risk for ischemic events

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rather than bleeding events in women [16–19]. To our knowledge, there are no previous reports on platelet activity in an MI population treated with aspirin, clopidogrel, and a GPIIb/IIIa-inhibitor (GPI).

The first aim of the current study was to assess sex differences in platelet aggregation in MI patients treated with three different and commonly used platelet inhibition drugs. A secondary aim was to assess a soluble marker of platelet activity.

Methods

Study Population

The study protocol has been previously described in detail [20]. Briefly, between Jan 2009 and Aug 2011, 125 patients with ST-elevation myocardial infarction (STEMI) or non-ST-elevation myocardial infarction (NSTEMI), defined according to the Global definition of myocardial infarction [21], and scheduled for coronary angiography, were recruited at the Department of Cardiology, Heart Center, University Hospital, Linköping, Sweden. Exclusion criteria were: participation in an intervention study, treatment with warfarin before admission, short life expectancy (less than 6 months) or unwillingness to participate. All patients received 600 mg loading dose (LD) of clopidogrel, followed by 75 mg once daily. When the study was planned and initiated there were no third-generation P2Y12 inhibitors (prasugrel or ticagrelor) approved in Sweden. According to clinical routine, if a patient was not on chronic aspirin treatment on admission, a LD of 300 mg aspirin was given, followed by a maintenance dose of 75 mg daily. Also by clinical routine, patients with STEMI were treated with abciximab (0.25 mg/kg body weight as a bolus dose) and weight-adjusted heparin (50 units/kg body weight). There were no patients on direct oral anticoagulation (DOAC) on admission or at discharge. Coronary interventions were performed according to current guidelines. Choices of stents were made according to treating physicians´ discretion.

Blood Sampling and Platelet Reactivity Testing

Venous blood samples were collected on several occasions: 6–8 hours after LD, 3 days after admission and LD (as a clinically convenient time-point when most patients were still hospitalized), 7–9 days after LD (median 8 days, a time-point when steady state for aggregation was ascertained even with single doses), and 6 months after admission and LD of clopidogrel (as an end-of-trial value to assess aggregation value in stable patients). All samples for aggregation measurements were drawn into blood collection tubes containing hirudin as anticoagulant (Dynabyte Medical, Munich, Germany). According to the instructions from the manufacturer, blood samples were kept at room temperature for a minimum of 30 minutes and a maximum of 120 minutes before aggregometry analyses were performed. Platelet activity was measured in whole blood using a Multiplate® impedance aggregometer (Roche diagnostics, Mannheim, Germany, former Dynabyte Medical, Munich, Germany). The procedure is described in detail elsewhere [22]. In summary, whole blood was mixed in a 1:1 proportion with 0.9% saline in the test cuvette, and aggregation was initiated with adenosine diphosphate (ADP), arachidonic acid (ASPI), and thrombin receptor activating peptide (TRAP). The ADP test is used to measure the effect of ADP-receptor antagonists (e.g. clopidogrel), the ASPI test is used to assess the effect of cyclooxygenase inhibitors (like aspirin). TRAP is an activator developed primarily to measure the effect of very potent aggregation inhibitors (GPI), with limited sensitivity toward ADP-receptor inhibition by clopidogrel and cyclooxygenase inhibition by aspirin. Impedance is measured between two electrodes in the test cuvettes. Activated platelets adhere and aggregate on the electrodes, increasing the impedance. The impedance, as a function of time (the area under the curve [expressed as Arbitrary Units (AU)*min]) is proportional to the degree of platelet aggregation.

Platelet and Coagulation System Activation

Soluble *P*-selectin (sP-selectin) was measured as a surrogate marker of platelet activation. Blood samples were collected in vacutainer tubes (using citrate as anticoagulant) at time-points indicated above. Samples were centrifuged to separate plasma, which then was stored at −70°C until analyzed, using an enzyme-linked immunosorbent assay (ELISA) and commercial kits for the analysis (Human *P*-Selectin/CD62P, R&D Systems for sP-selectin (reference interval 18–40 ng/mL)).

Bleeding Risk Score

We also calculated a bleeding risk score. According to recommendations in clinical guidelines from the European Society of Cardiology [6], we used the CRUSADE score [23]. The score was slightly modified since we had no information on congestive heart failure on arrival. Sex was not added, because the aim of this analysis was to explore potential sex differences.

Outcome Definitions

Bleeding events were defined according to the TIMI definition [24]. Bleeding localization was not captured in the dataset.

Based on earlier studies and consensus document, high residual platelet reactivity (HRPR) on clopidogrel treatment was defined as ADP-stimulated aggregation >468 AUC*min and low residual platelet reactivity (LRPR) was defined as <188 AUC*min [25,26]. Values between 188 and 468 were regarded as optimal platelet reactivity (OPR). We calculated the proportion of HRPR and LRPR (with ADP stimulated aggregation) at 3 days and 7–9 days after LD.

Statistical Analysis

The sample-size calculations for the overall trial have been described in detail previously, and was based on expected clinical ischemic events [20]. The present subgroup analysis was pre-specified in the original statistical analysis plan, but no separate power calculation was performed, neither regarding ischemic events nor bleeding events. Thus, the results should be considered exploratory.

Baseline variables are presented as numbers and percentages for categorical variables and mean and standard deviations (SD) or medians with interquartile ranges (IQR) for continuous variables, as appropriate. The Shapiro-Wilk test was used to test whether data were normally distributed or not. Some of the aggregation measurements were not normally distributed. Therefore, we chose a conservative approach, presenting data with medians (IQR) and non-parametric statistical testing for all platelet activity measurements.

Differences between women and men were assessed with the chi-square test for categorical variables and with students T-test or Mann–Whitney U test (depending on if the variable had a normal distribution or not) for continuous variables. A p-value of <0.05 was regarded as significant.

A logistic regression model was built to assess sex differences in bleeding complications. In a crude model, only sex was added as explanatory variable. In a second model CRUSADE score and age was added. There was no difference if the score was used as a continuous variable or as a categorical variable.

Ethical Considerations

The study was performed according to good clinical practice, complies with the Declaration of Helsinki and was approved by the Regional Ethical Review Board in Linköping (Dnr M45-08). All patients gave written informed consent.

Results

We included 125 patients, 37 women and 88 men; median age was 67 years (67 years for women and 67.5 years for men).

A majority of the patients were admitted with STEMI, 67.6% of women and 54.5% of men, $p = .18$. There were no significant differences between men and women in baseline features or comorbidities except that diabetes was more prevalent in women (27% vs. 6.8%, $p < .01$). Women had significantly lower hemoglobin value (134 vs. 144 g/L, $p = <.001$) and estimated glomerular filtration rate (eGFR) according to the Cockroft-Gault equation (72 vs. 88 mL/min, $p < .01$), on admission. Women also had significantly higher platelet count (270 vs. 229 x10^9/unit, $p = .02$) on admission. There were no significant differences between women and men regarding medication on admission (Table I).

All but two were catheterized (37 women and 86 men), 81.5% underwent PCI (83.8% of the women vs. 80.5% of the men, $p = .66$). During PCI, 56.8% (64.9% women vs. 53.4% men, $p = .24$) were treated with a GPI (abciximab). In conjunction with angiography and/or PCI 84.8% were treated with heparin (86.5% women vs. 84.1% men, $p = .73$). At discharge 91.1% of the patients were treated with clopidogrel (89.2% of women vs. 92.0% of men, $p = .73$) and 100% with aspirin. There were no significant differences between women and men in medications at discharge, except that women were discharged more often with diuretics (27% vs. 11.4%, $p = .03$) and men were more often discharged with statins (100% vs. 94.6%, $p = .03$) (Table II).

Women had higher CRUSADE bleeding risk score 23 vs. 17, $p = .013$.

Bleeding Complications

Women experienced significantly more bleeding complications than men (18.9% vs. 6.8%, $p = .04$), during hospital stay. All but one of the in-hospital bleeds were defined as TIMI minimal.

From discharge, over 6-months follow-up, bleeding events occurred more often in women, but without statistical significance (8.1% vs. 2.3% bleeding events, $p = .13$). Follow-up bleedings

Table I. Baseline characteristics.

	All	Women	Men	*p*-value
	($n = 125$)	($n = 37$)	($n = 88$)	
Age, years, median (IQR)	67.0(15)	67.0(16)	67.5(15)	0.45
Body Mass Index, kg/m^2, mean(SD)	27(4)	27(5)	27(4)	0.57
Systolic blood pressure, mean(SD)	149(28)	148(28)	149(28)	0.87
Heartrate, bpm, mean(SD)	74(15)	74(13)	73(15)	0.66
STEMI	73(58.4)	25(67.6)	48(54.5)	0.18
Risk factors and comorbidity				
Previous MI	25(20.0)	6(16.2)	19(21.6)	0.49
Previous PCI	14(11.2)	3(8.1)	11(12.5)	0.48
Previous CABG	8(6.4)	0(0)	8(9.1)	0.06
Previous Stroke/TIA	3(2.4)	0(0)	3(3.4)	0.26
Hypertension	49(39.2)	19(51.4)	30(34.1)	0.07
Diabetes mellitus	16(12.8)	10(27.0)	6(6.8)	<0.01
Smoker	75(60)	22(59.5)	53(60.2)	0.12
Laboratory values, median(IQR)				
Hemoglobin,[a] g/L	141(15)	134(17)	144(14)	<0.001
Nadir hemoglobin,[b] g/L	136(21)	123(19)	139(15)	<0.001
Platelets[a] × 10^9/L	234(87)	270(114)	229(80)	0.02
eGFR,[a] mL/min	80(37)	72(33)	88(40)	<0.01
eGFR <60 mL/min	23(18.4)	10(27.0)	13(14.8)	0.11
eGFR<30 mL/min	6(4.8)	1(2.7)	5(5.7)	0.48
hsTroponin T, ng/L (at 6–8 hours)	659(1956)	925(1402)	569(2698)	0.40
Medication on admission				
Clopidogrel	3(2.4)	1(2.7)	2(2.3)	0.89
Aspirin	30(24.2)	9(25.0)	21(23.9)	0.89
Warfarin	3(2.4)	1(2.7)	2(2.3)	0.89
Betablockers	35(28.0)	13(35.1)	22(25.0)	0.25
ACE-I/ARB	32(25.6)	13(35.1)	19(21.6)	0.11
Statin	31(24.8)	9(24.3)	22(25.0)	0.94
Calcium antagonist	23(18.4)	7(18.9)	16(18.2)	0.92
Diuretics	18(14.4)	8(21.6)	13(14.8)	0.35
NSAID	6(4.8)	3(8.1)	3(3.4)	0.26
Proton pump inhibitor	21(16.8)	8(21.6)	13(14.8)	0.35
Hormone replacement therapy	na	4(10.8)	na	na

Data are presented as numbers (percentages) if not otherwise specified.
[a]Laboratory values on admission. [b] During hospital stay.
Abbreviations (in order of appearance) IQR, Interquartile range; SD, Standard Deviation; bpm, beats per minute; STEMI, ST elevation myocardial infarction; MI, Myocardial infarction; PCI, Percutaneous Coronary Intervention; CABG, Coronary Artery Bypass Grafting; TIA, Transient Ischemic Attack; eGFR, estimated Glomerular Filtration Rate (according to the Cockroft Gault equation); ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; NSAID, Non-Steroidal Anti-inflammatory Drugs and na, not applicable.

Table II. In-hospital treatments and discharge medication.

	All	Women	Men	
	(*n* = 125)	(*n* = 37)	(*n* =)	*p*-value
In-hospital medications				
Fondaparinux	44(35.2)	11(29.7)	33(37.5)	0.41
GP IIb/IIIa inhibitor	71(56.8)	24(64.9)	47(53.4)	0.24
GP IIb/IIIa inhibitor infusion	7(5.6)	3(8.1)	4(4.5)	0.43
Heparin	106(84.8)	32(86.5)	74(84.1)	0.73
Interventions				
Angiography	123(98.4)	37(100)	86(97.7)	0.36
Radial access	56 (45.5)	14(37.8)	42(48.8)	0.26
PCI	101(81.5)	31(83.8)	70(80.5)	0.66
Medication at discharge				
Clopidogrel	113(91.1)	33(89.2)	80(92.0)	0.73
Acetylsalicylic acid	125(100)	37(100)	88(100)	NA
Betablockers	116(92.8)	35(94.6)	81(92.0)	0.62
ACE-I/ARB	97(77.6)	26(70.3)	71(80.7)	0.20
Statin	122(98.4)	35(94.6)	87(100)	0.03
Calcium antagonist	22(17.6)	7(18.9)	15(17.0)	0.80
Diuretics	20(16.0)	10(27.0)	10(11.4)	0.03
NSAID	7(5.6)	3(8.1)	4(4.5)	0.43
Proton pump inhibitors	30(24.0)	11(29.7)	19(21.6)	0.33

Figures presented as numbers (percentages) if not otherwise specified. GP, Glycoprotein; PCI, percutaneous coronary intervention; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; NSAID, Non-Steroidal Anti-inflammatory Drugs.

were defined as TIMI major life threatening in two cases (one in each sex), TIMI major other in one (a male), and TIMI minimal in two (a female).

Women had significantly higher bleeding rate from admission to end of follow-up 24.3% vs. 9.1%, $p = .023$, Odds Ratio (OR) 3.21, 95% Confidence Interval (CI) *1.13–9.14; p = .029*. Adding the CRUSADE bleeding risk score and age to the model did not significantly change the result *HR 3.16, 95% CI 1.07–9.32; p = .038*.

Platelet Aggregation

There were no significant differences in impedance aggregation values at any of the prespecified time-points (the presented time-points are related to LD of clopidogrel) in women and men, respectively.

Among the 66 STEMI patients treated with the GPI abciximab, there were no differences in TRAP-induced aggregation 6–8 hours after LD (406 vs. 394 AU*min, $p = .87$), 3 days after LD (651 vs. 697 AU*min, $p = .76$) or 7–9 days after LD (938 vs. 865 AU*min, $p = .07$) (Figure 1a–c). In addition we did not find any statistical difference in TRAP-induced aggregation between bleeders and non-bleeders after LD (581 vs. 498 AU*min, $p = .97$) 3 days after LD (668 vs. 720 AU*min, $p = .61$) or 7–9 days after LD (968 vs. 890 AU*min, $p = .10$).

We assessed ADP-induced platelet aggregation 6–8 hours after LD (restricted to 13 women and 38 men not treated with GPI), and observed no difference (254 vs. 288 AU*min, $p = .67$). Similarly, there was no difference in ADP-stimulated aggregation 3 days after LD (189 vs. 195 AU*min, $p = .80$), 7–9 days after LD (306 vs. 232 AU*min, $p = .74$) or 6 months after LD (288 vs. 216 AU*min, $p = .24$) (Figure 2a–d).

We also measured ADP-induced platelet aggregation before LD (in patients not treated with GPI), (878 vs. 567 AU*min, $p = .08$), and change in AU*min from before LD to 6–8 hours after LD (507 vs. 242 AU*min, $p = .28$) without any statistical difference between women and men, respectively.

To further examine potential differences between women and men in effect of clopidogrel, we assessed the proportion of patients with low, optimal, and high platelet reactivity (LRPR, OPR, and HRPR). Again, we did not observe any difference between the sexes in LRPR, OPR, and HRPR, neither at 3 days (49%, 43%, and 9% vs. 43%, 49%, and 7% for women and men, respectively, $p = .81$) nor at 7–9 days (34%, 44%, and 22% vs. 43%, 43%, and 15% for women and men, respectively, $p = .60$) after LD (Figure 4).

Moreover, we found no significant difference in ADP-induced platelet aggregation between bleeders and non-bleeders after LD (182 vs. 206 AU*min, $p = .79$), 3 days after LD (204 vs. 195 AU*min, $p = .95$) or 7–9 days after LD (316 vs. 245 AU*min, $p = .58$).

Finally, we assessed ASPI-induced aggregation at four time-points. Again, we found similar aggregation levels at 6–8 hours after LD in 13 women and 40 men not treated with GP IIIb/IIIa (88 vs. 101 AU*min, $p = .37$), 3 days after LD (71 vs. 89 AU*min, $p = .19$), 7–9 days after LD (94 vs. 113 AU*min, $p = .29$) and 6 months after LD (95 vs. 100 AU*min, $p = .81$) (Figure 3a–d).

Soluble *P*-selectin

To further explore differences in platelet activity, we measured soluble *P*-selectin, which functions as a cell adhesion molecule on the surface on activated platelets. Three days after admission there was no difference in levels of sP-selectin between women and men (28 vs. 28 ng/mL, $p = .82$)

Discussion

The main finding of this study was that, in spite of higher bleeding incidence in women, there were no differences between women and men, in platelet activity measures, reflecting the effects of three commonly used antiplatelet medications.

There were no significant differences in baseline characteristics regarding age (67 years), proportion of patients with STEMI, history of MI, or medication on admission. In agreement with earlier observations, women more often had a history of diabetes and hypertension [27,28]. Also, women had lower hemoglobin and eGFR values, but higher platelet count [29–31].

In accordance with previous studies, we observed a higher incidence of in-hospital bleeding complications in women [28,32,33]. Even after adjustment for baseline differences in bleeding risk factors, women had higher bleeding risk. Previous studies have found female sex to be an independent predictor of bleeding after MI [12,13]. Although the reasons for the observed differences in bleeding incidence after MI are incompletely understood, several hypotheses have been put forward; among them, differences in platelet surface receptor expression [34,35], excess dosing of antithrombotic drugs [15], and differences in baseline characteristics, such as lower body weight, age, and eGFR [15].

We performed impedance aggregometry with three different agonists reflecting the treatment effect of GPI, P2Y12-inhibitors, and aspirin, to assess sex differences in the pharmacological response to three frequently used platelet inhibitors in the context of MI treatment. Previous reports have suggested excess dosing of GPI in women compared to men, and that this may explain at least some of the observed difference in bleeding rate [15,36]. Our data indicate similar effect of the drug in women and men, with no difference in TRAP-induced platelet aggregation (reflecting the effect of GPI) at several time points. Difference between our and previous results may depend on how excess dosing has been defined. While previous studies defined excess dosing based on given dose, body weight, and

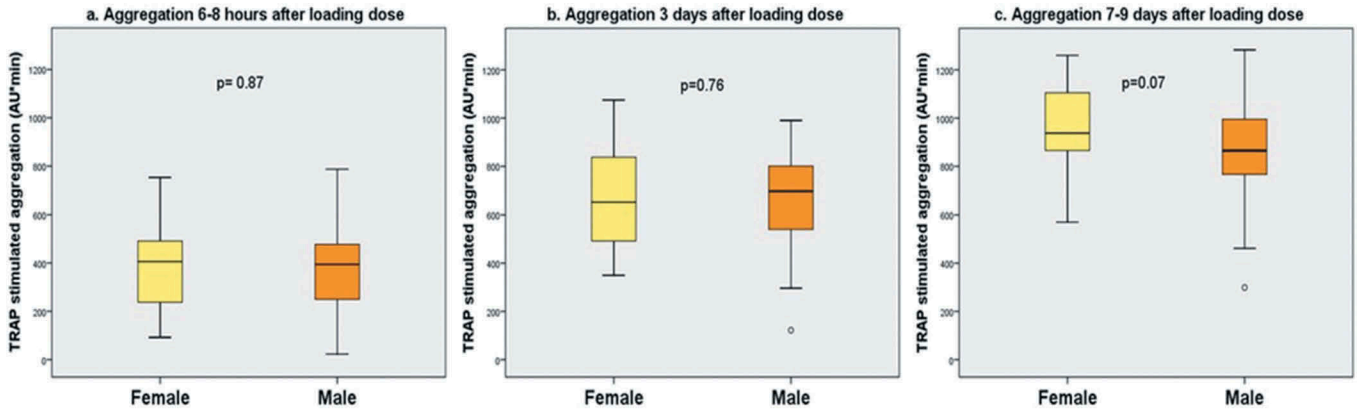

Figure 1. (a–c). Thrombin receptor activating peptide (TRAP) induced platelet aggregation 6–8 hours (a), 3 days (b), and 7–9 days (c) after loading dose, expressed as AU*min. The box indicates 25–75% quartiles with the lines as median and the whiskers 1.5 IQR. Statistical significance between females and males was tested with the Mann–Whitney U test.

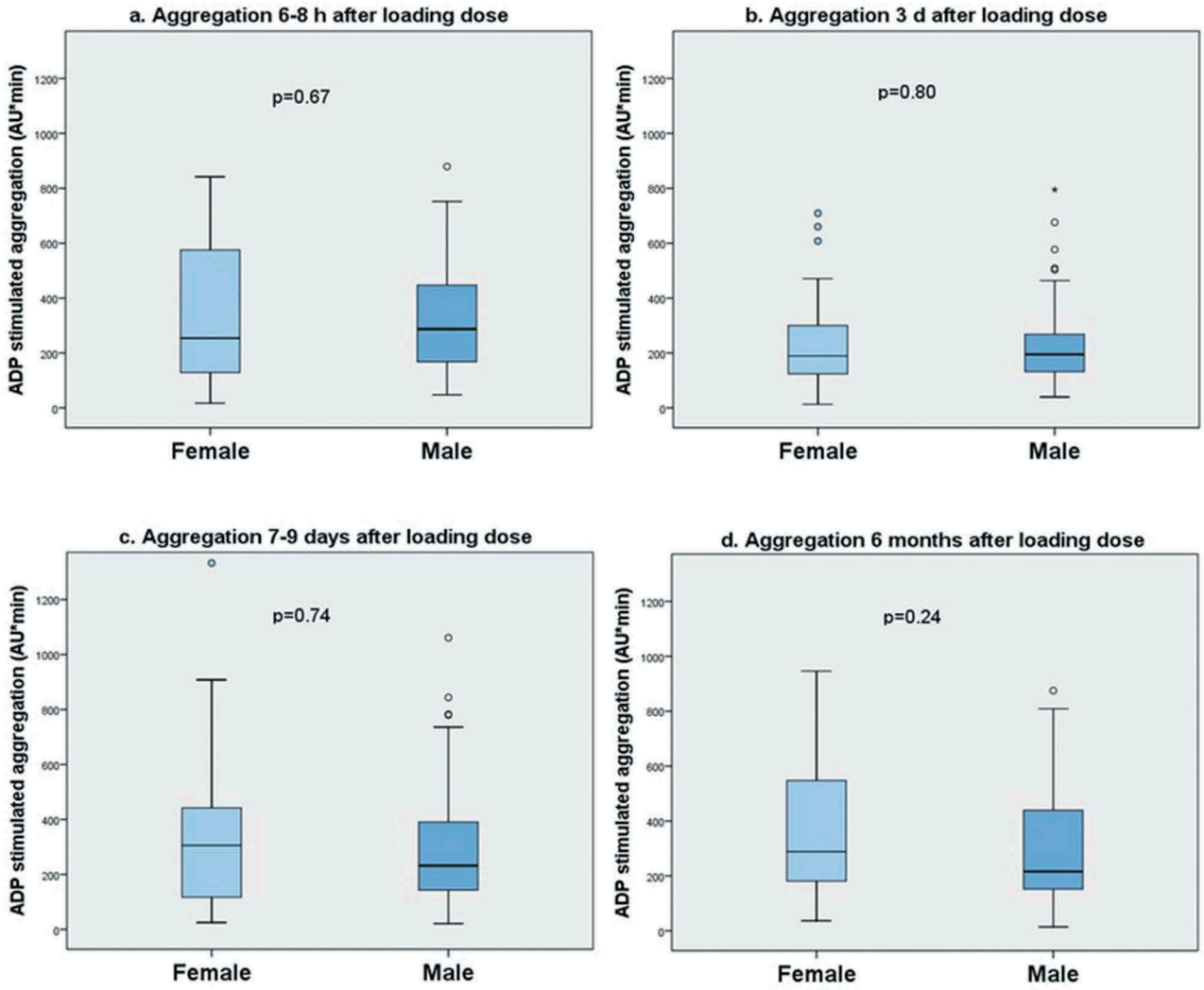

Figure 2. (a–d). Adenosine diphosphate (ADP) induced platelet aggregation 6–8 hours (a), 3 days (b), 7–9 days (c), and 6 months (d) after loading dose, expressed as AU*min. The box indicates 25–75% quartiles with the lines as median and the whiskers 1.5 IQR. Statistical significance between females and males was tested with the Mann–Whitney U test.

renal function; we assessed the effect on TRAP-induced aggregation, an established way to measure the effect of GPI. We believe that aggregation values may better reflect the individual effect than estimation of dosing based on weight and renal

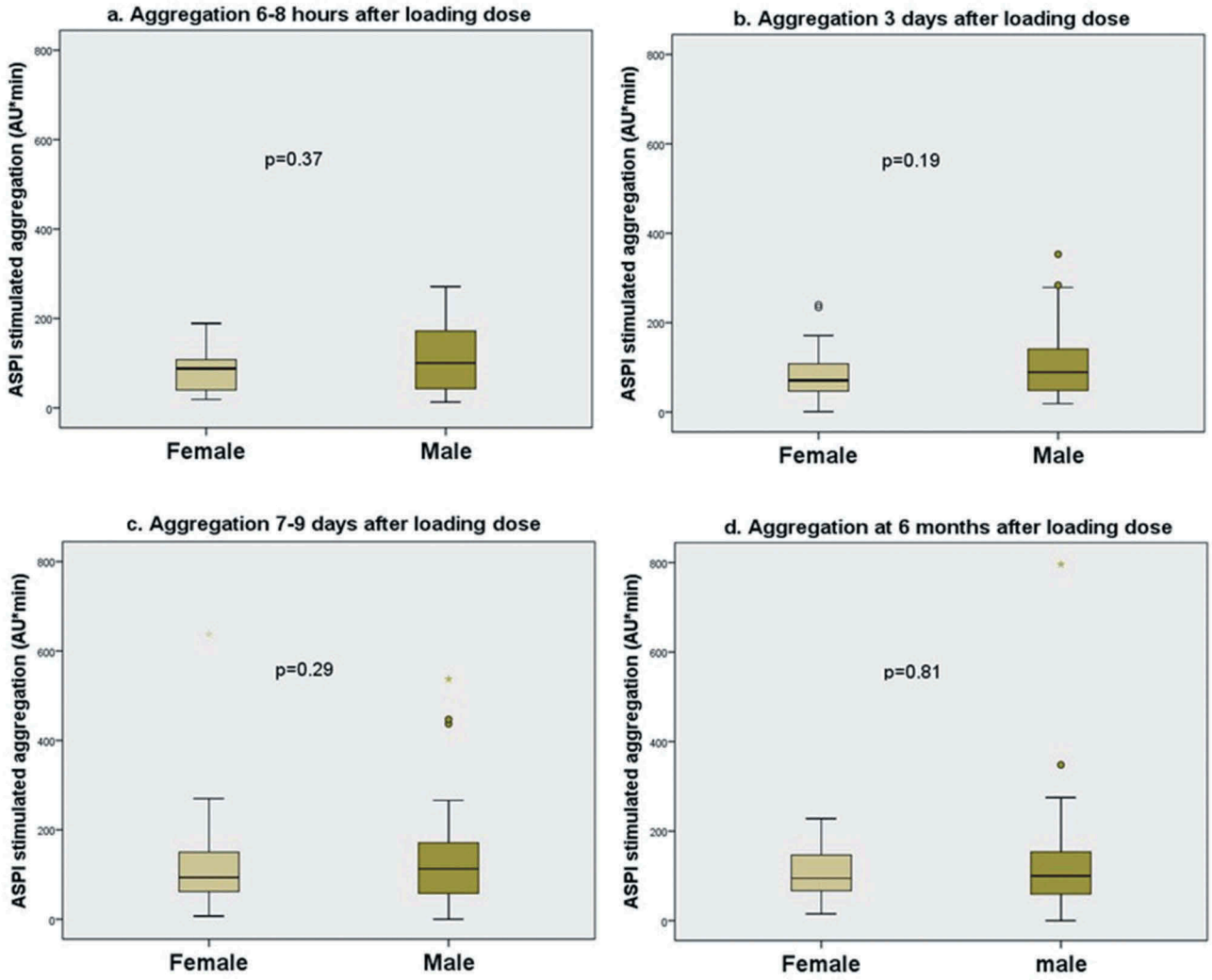

Figure 3. (a–d). Arachidonic acid (ASPI) induced platelet aggregation 6–8 hours (a), 3 days (b), 7–9 days (c), and 6 months (d) after loading dose, expressed as AU*min. The box indicates 25–75% quartiles with the lines as median and the whiskers 1.5 IQR. Statistical significance between females and males was tested with the Mann–Whitney U test.

function. Also, difference between our results and previous may be caused by differences in study populations, such as age, comorbid conditions (e.g. hypertension) and renal function.

We found no significant differences in ADP-stimulated aggregation. Some previous studies indicated higher ADP-induced aggregation in women among healthy volunteers [16,17] and in patients treated with DAPT after PCI [37]. Also, an integrated metaanalysis of pharmacodynamic studies on predominantly healthy subjects indicated lower inhibition of platelet activity (IPA) in women [19]. Other indicated no difference in patients treated with DAPT after PCI [38,39]. We observed numerically higher ADP-stimulated aggregation in women with MI, but it did not reach statistical significance. Our results are supported by one study on vascular patients and another study on patients with coronary artery disease, treated with DAPT post-PCI. Therefore, previous results, in agreement with our finding, do not support effect of platelet inhibitors as a major explanation for increased bleeding in women [40].

The aggregation tests used in this study were developed to assess antiplatelet drug effects and may be less accurate if they are used in a non-treated population. Also, sex differences in platelet aggregation may vary between an older population with MI and a healthier population including mainly pre-menopausal women, which may explain the apparently contradictory results.

HRPR on clopidogrel treatment has been associated with an elevated risk of new ischemic events as well as of stent thrombosis post-PCI [41], whereas LRPR has been associated with an increased risk of bleeding [26]. Therefore, to further explore sex differences in effect of P2Y12-inhibition, we calculated the proportions of men and women with HRPR, LRPR, and OPR 3 and 7–9 days after LD, and found that the proportions did not differ significantly between women and men at the two time-points [42].

Hence, in agreement with previous data, we found no evidence for increased effect of ADP inhibitors as an explanation for sex differences in bleeding rates. We further expand knowledge, showing lack of difference at several time-points [40].

For aggregation studies, we used a third agonist, arachidonic acid, to assess the effect of aspirin. Again, we did not observe any difference between men and women on several occasions. Although clinical trials have suggested possible differences in outcome between women and men treated with aspirin, little is known about sex differences in aggregation or other measures of platelet activity in aspirin-treated patients [43]. In one study, female platelets were found to be more reactive after arachidonic acid activation, compared to male platelets. However, after 14 days of low-dose aspirin treatment the levels were very low and the difference no longer statistically significant, which is in accordance with our results [44].

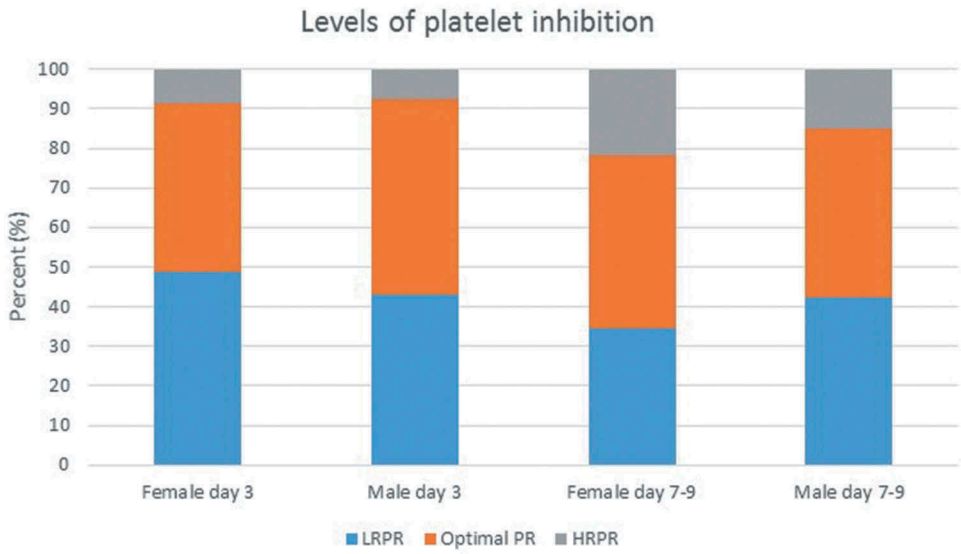

Figure 4. Proportion of patients with different degrees of residual platelet activity, on clopidogrel treatment. High residual platelet reactivity (HRPR) was defined as ADP-stimulated aggregation >468 AUC*min and low residual platelet reactivity (LRPR) was defined as <188 AUC*min. Values between 188 and 468 were regarded as optimal platelet reactivity (OPR). Two-sided Pearson Chi-Square tests for comparisons between female and male patients were non-significant at both time points ($p = .81$, and $p = .60$, 3 days and 7–9 days after LD, respectively).

Lack of difference between women and men in treatment effect of platelet inhibitors was further corroborated by lack of significant difference in levels of soluble *P*-selectin 3 days after admission. To our knowledge, this is the first report on sex differences in soluble *P*-selectin levels in an ACS context.

Explanations to why women have more bleeding complications are not completely understood. However, our results in addition to previous data may give some valuable insights. In this study, the majority of bleeding complications were defined as TIMI minimal. Major bleeding events may be more associated with excess dosing, as proposed in previous studies, explaining some of the difference between our results and previous [36]. Impaired renal function has been associated with bleeding events in several studies [45,46]. In accordance with previous data, we found lower eGFR in women compared to men, but at a relatively high level in both sexes. Adjustment for eGFR among other risk factors for bleeding did not eliminate the observed difference in bleeding complications in women vs. men. Some of the observed difference in bleeding associated with renal function may be associated with impaired drug metabolism, and excess dosing, but also impaired platelet function, not detectable with the aggregation method used in this study [47]. Lack of difference in TRAP-induced and ADP-induced aggregation supports that other factors than effect of GPI or ADP-inhibitors are driving bleeding complications in this ACS population.

We did not have information on bleeding localization, but previous studies have shown increased access-site bleeding complications among women [11,28]. Some reports have indicated that arterial access may be more challenging in women than in men, with smaller common femoral artery, being associated with increased bleeding incidence [48,49]. In this study, femoral access was more common than radial access, especially in women, which probably explain at least part of the observed difference in bleeding complications, even in the absence of difference in drug effect.

Limitations

There are some important limitations of this analysis. First, the small study size, with few clinical events, inevitably increases the risk of both type 1 and type 2 errors, and decreases the external validity. However, increased rate of bleeding complications has been shown in several other studies, and the consistent similarity in aggregation levels between the sexes (not only lack of statistical significance) makes a type 2 error unlikely. Moreover, there is a lack of data addressing variation in platelet reactivity between men and women, so these data add information to current knowledge, especially with different agonists. Second, a large proportion of our patients received GPI which may have had an impact of aggregation values day 3, but data from day 3 are in line with the results from other time-points. Also, GPIs are used less often today and mostly in bail-out situations. However, this analysis was an attempt to understand the mechanism behind previously reported increased bleeding incidence in women, when GPIs were used more often. In addition, subgroups without treatment with GPI are presented. Third, from a sex perspective, hormone replacement therapy, or menstrual phase in premenopausal women, may have impacted platelet activity. However, the results were very consistent, with similar results at different time points and with different agonists. Fourth, even if blood tests were scheduled to the morning we did not have an exact time, which may have impacted the aggregation values. Finally, we did not use pill count to assess compliance during follow-up.

Conclusion

The main finding of this study was that, in spite of a higher bleeding incidence in women, there was a consistent lack of difference between the sexes in platelet activity, using three different platelet agonists, at several time-points. The result does not support excess dosing of antiplatelet drugs as a major explanation to the commonly observed higher bleeding incidence in women with MI. The reason for the observed differences in bleeding risk is not known and further studies are needed.

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Declaration Of Interest

The authors report no conflict of interest.

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