Published in final edited form as:

Am J Cardiol. 2016 February 15; 117(4): 656–663. doi:10.1016/j.amjcard.2015.11.029.

Differences in Whole Blood Platelet Aggregation at Baseline and in Response to Aspirin and Aspirin Plus Clopidogrel in Patients with vs. without Chronic Kidney Disease

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Abstract

Thrombotic events while receiving anti-platelet agents (APA) are more common in individuals with vs. without chronic kidney disease (CKD). Data on anti-platelet effects of APA in CKD are scarce and limited by lack of baseline platelet function before APA treatment. We hypothesized individuals with stages 4–5 CKD vs. no CKD have higher baseline platelet aggregability and respond poorly to aspirin and clopidogrel. In a prospective controlled study, we measured whole blood platelet aggregation (WBPA) in 28 CKD and 16 non-CKD asymptomatic stable outpatients not on dual APA, frequency-matched for age, gender, obesity and diabetes mellitus. WBPA was re-measured after 2 weeks of each aspirin and aspirin plus clopidogrel. The primary outcome was percent inhibition of platelet aggregation (IPA) from baseline. The secondary outcome was residual platelet aggregability (RPA) (proportion with <50% IPA). Baseline platelet aggregability

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Clinical Trial Registration: NCT01768637

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was similar between groups except adenosine diphosphate (ADP)-induced WBPA, which was higher in CKD vs. non-CKD; median (IQR) =13.5 (9.5, 16.0) vs. 9.0 (6.0, 12.0) Ω , $p=0.007$. CKD vs. non-CKD participants had lower clopidogrel-induced IPA, 38% vs. 72%, $p=0.04$. A higher proportion of CKD vs. non-CKD participants had RPA after clopidogrel treatment (56% vs. 8.3%, $p=0.01$). There were no significant interactions between CKD and presence of CYP2C19 polymorphisms for platelet aggregability in clopidogrel-treated participants. In conclusion, CKD vs. non-CKD individuals exhibited similar platelet aggregation at baseline, similar aspirin effects and higher residual platelet aggregability on clopidogrel, which was independent of CYP2C19 polymorphisms.

Keywords

platelets; aspirin; kidney; thrombosis

Chronic kidney disease (CKD) patients are at higher risk for thrombotic cardiovascular events as compared to those without CKD.^{1, 2} Although there are several factors explaining increased thrombotic events in CKD patients, poor efficacy of antiplatelet agents (APA) is a well-known risk factor for increased thrombotic events.^{2, 3} However, previous studies reporting presence of residual platelet aggregability (RPA) were limited by the use of less physiological techniques, such as optical aggregometry, to measure platelet function,⁴⁻⁷ and the inclusion of primarily End Stage Renal Disease (ESRD) and not non-dialysis CKD patients,^{8, 9} where earlier intervention for secondary cardiovascular prevention may be more efficacious. These studies also suffered from failure to measure before and after-treatment effects of APA on platelet aggregability and lacked non-CKD controls.^{4, 6} Surprisingly, there are little data on how baseline platelet function may differ in stable CKD vs. non-CKD individuals.¹⁰ It is also unclear whether there are differences in platelet aggregation in response to APA based on the presence or absence of CKD¹¹ or as related to the presence of cytochrome P450 polymorphisms such as cytochrome P450 2C19 (CYP2C19), a major drug metabolic enzyme involved in the conversion of ingested clopidogrel pro-drug into its active metabolite.¹²

METHODS

We conducted the Whole Blood Platelet Aggregation in Chronic Kidney Disease on Aspirin (WICKD onASA) study, an open-label prospective clinical trial, to compare the degree of platelet inhibition from baseline by aspirin (81 mg/d) and by a combination of aspirin (81 mg/d) and clopidogrel (75 mg/d) in a frequency-matched cohort of asymptomatic non-dialysis CKD patients and controls without CKD. We hypothesized that as compared to those without kidney disease, those with CKD: 1) have higher platelet aggregability at baseline as measured by *ex vivo* whole blood impedance platelet aggregation; and 2) have a reduced response to aspirin and clopidogrel, as measured by lower inhibition of platelet aggregation (IPA) from baseline and higher RPA. Finally, we explored whether there was an interaction between CKD and presence of CYP2C19 polymorphisms for platelet aggregability in clopidogrel-treated patients. Institutional Review Board approval was obtained. Adults 21 years old were recruited from outpatient clinics at Parkland, Dallas

Veterans Affairs, and the University of Texas Southwestern (UTSW) Medical Centers in Dallas, Texas from December 10, 2012 to January 31, 2014. Non-CKD participants were included if there was no evidence of kidney disease as defined by estimated glomerular filtration rate (eGFR) ≥ 90 mL/min/1.73 m², urine albumin-to-creatinine ratio (UACR) <30 mg/g, and no detectable kidney disease by other methods defined by National Kidney Foundation guidelines.¹³ CKD patients were those with non-dialysis CKD stages 4–5, with an eGFR of <30 mL/min/1.73 m² for at least 3 months, calculated.¹³ Exclusion criteria were similar to those used in previous platelet studies:⁴ presence of ESRD, kidney transplantation, hospitalization or surgery within 3 months, cardiovascular event within 12 months, surgery within 3 months, history of blood dyscrasias, active bleeding or bleeding diathesis, gastrointestinal bleeding in the last 6 months, active malignancy or liver disease, ongoing use of APA or anti-thrombotic drugs, <30 days treatment with a glycoprotein IIb/IIIa antagonist, hematocrit $<25\%$, white blood cell count $>20,000/\mu\text{L}$, or platelet count $<50,000/\mu\text{L}$. Patients who were taking aspirin for primary cardiovascular prevention were included but asked to discontinue use for at least 2 weeks prior to baseline measurements. The 2 groups were frequency-matched for decade of age,¹⁴ gender,¹⁵ presence of diabetes⁴ and each 5 kg/m² of body mass index (BMI),¹⁶ as these characteristics were reported to influence platelet function.

On **visit 1**, demographic, clinical, and medication use data were collected. Data on the following variables were obtained: 1) *diabetes mellitus* (defined as glycosylated hemoglobin level $\geq 6.3\%$, fasting blood glucose level ≥ 126 mg/dL, or use of hypoglycemic agents); 2) *hyperlipidemia* (use of anti-lipid medications, total cholesterol ≥ 240 mg/dL or serum low density lipoprotein (LDL) ≥ 160 mg/dL), *current smoking* (any cigarette smoking in the past 30 days); and 3) *hypertension* (an average of 4 office BP readings $\geq 140/90$ mm Hg and/or use of antihypertensive medication). *Dietary supplements* were discontinued for 2 weeks prior to the study.^{4, 17} Patients were also asked to not smoke¹⁸ or eat foods known to affect platelet function (*coffee, chocolate, grapes, and alcohol*) 48 hours prior to every sample collection.¹⁷ Diet and physical activity were assessed at every visit by the administration of the Modified 24-hour Dietary Recall and the Stanford 7-day Physical Activity Recall interviewer-administered questionnaires.^{16, 17} Venipuncture was also performed at each visit for complete blood count, WBPA measurements and adenosine triphosphate (ATP) secretions. Patients were dispensed chewable aspirin 81 mg/d. After 2 weeks, patients returned for **Visit 2**. WBPA and ATP secretion measurements were repeated. Two buccal swabs were collected for genotype analysis. Aspirin 81 mg/d and clopidogrel 75 mg/d were dispensed. Patients returned in 2 weeks for **Visit 3**. WBPA induced by ADP was measured to analyze clopidogrel effect. Patients were asked to take APA on the morning of venipuncture so that everyone received phlebotomy within 3–4 hours of pill intake. Pill counts were performed for adherence.

The *primary outcome measure* was the inhibition of platelet aggregation (IPA), defined as follows:^{5, 7} %IPA = (baseline aggregation) - (post-treatment aggregation)/(baseline aggregation). The *secondary outcome measure* was RPA, defined as the proportion with $<50\%$ inhibition of aggregation from baseline.^{5, 10} WBPA was measured by *ex vivo* whole blood impedance platelet aggregometry (Chrono-log Corporation, Havertown, PA 19083,

USA).¹⁹ ATP secretion was measured using the firefly luciferin-luciferase reaction (Chrono-log, Havertown, PA 19083, USA). Genotyping of all CYP2C19 alleles was performed from buccal swabs using real-time polymerase chain reaction (Harmonyx Diagnostics Inc. Cordova, TN, USA).¹² Major alleles, CYP2C19*2 and CYP2C19*17 genotypes were classified based on the standardized metabolic phenotypes.¹² For the purposes of testing 2-way interactions with CKD, intermediate (CYP2C19*1/*2 and CYP2C19*2/*17) and poor (CYP2C19*2/*2) metabolic groups were collapsed into one category as poor metabolic phenotype.¹²

For sample size calculations, we used published data on healthy volunteers as historical controls¹⁷ and unpublished pilot data from the Chronic Kidney Disease Antidepressant Sertraline Trial for CKD patients.²⁰ From log-transformed pilot data (non-Gaussian), a coefficient of variation of 0.84 (SD/Mean= 1.35/1.6 in log units) was calculated. With a sample of 30 patients (20 CKD and 10 non-CKD) and a two-sided α of 0.05, the study would have 84% power to detect at least a 4 Ω between-group difference in the geometric means of WBPA induced by 0.5 mM AA at baseline or on-treatment with APA. Student's *t*-test or Wilcoxon rank sum test was used to compare continuous and Fisher's Exact to compare categorical variables among CKD vs. non-CKD groups. Mixed model repeated measures (MMRM) analyses were used to compare WBPA induced by 0.5 mM AA, 2 μ g/mL collagen and ADP responses (change) to aspirin and dual therapy between groups.²¹ The model had group factor, treatment factor (repeated, pre- vs. post-), and interaction between group and treatment as covariates; the participant was modeled as a random effect. RPA was compared between-groups using the Fisher's Exact test. Proportions of metabolic phenotypes of CYP2C19 polymorphisms were analyzed between groups based on the presence of CKD and compared using Fisher's Exact test. ANOVA was used to test any 2-way interactions of poor (vs. normal) metabolic phenotype X CKD and rapid (vs. normal) metabolic phenotype X CKD on IPA after clopidogrel treatment. All available data were included in the analyses; the mixed model can accommodate cases with incomplete data. Analyses were performed with SAS version 9.3 (SAS Institute, NC).

RESULTS

Figure 1 shows the exclusionary cascade for derivation of the cohorts. Baseline characteristics, based on presence or absence of CKD, are listed in Table 1. A higher proportion of participants with CKD vs. no CKD reported use of beta-blockers and statins (Table 1). Those with CKD compared to no CKD had a lower mean hemoglobin concentration and higher glycosylated hemoglobin, serum uric acid, phosphorus, and intact parathyroid hormone levels (Table 1). There were no between-group differences in the baseline values for ATP secretions and WBPA except when induced by ADP (Table 2 and Figure 2).

Aspirin effect^{17, 21} (Table 2; Figure 3) was evaluated at Visit 2 by the change from baseline (Visit 1) in platelet aggregation induced by AA and collagen after 2 weeks of aspirin 81 mg/d. Both groups had near complete IPA to AA on aspirin (Table 3), group x time interaction $p=0.78$. Response to collagen was incomplete in both groups, group x time interaction $p=0.29$. Finally, proportion of patients with RPA on-aspirin was not different

between-groups (Table 4). Clopidogrel effect¹⁹ was evaluated at Visit 3 by ADP response after 2 weeks of aspirin/clopidogrel (Table 2; Figure 4). IPA in response to clopidogrel was significantly lower in CKD vs. non-CKD individuals (Table 3). Additionally, a higher proportion of CKD vs. non-CKD participants had RPA after clopidogrel treatment (Table 4). Group x time interaction was not significant ($p=0.46$ for 10 μ M ADP and $p=0.42$ for 20 μ M ADP). There were no differences in the metabolizer phenotypes between groups (Table 5). Figure 5 shows platelet-inhibitory responses to clopidogrel in the two groups based on the four metabolic phenotypes (CKD x metabolic phenotype interaction $p>0.1$). There were no hospitalizations or bleeding episodes requiring APA discontinuation or blood transfusion. Three CKD and 2 non-CKD participants experienced self-limited spontaneous bruises or nose bleeds on aspirin alone. Two CKD and 7 non-CKD patients experienced similar episodes on aspirin/clopidogrel.

DISCUSSION

Our study reports 3 key findings in CKD patients when compared to those without kidney disease: 1) similar platelet function profiles at baseline, except higher ADP-induced platelet aggregation in CKD; 2) similar aspirin effect; and, 3) poor response to the platelet-inhibitory effects of clopidogrel among CKD patients, independent of CYP2C19*2 and CYP2C19*17 polymorphism effects.

We report baseline similarity in platelet aggregation of non-dialysis patients with CKD stages 4–5 compared to non-CKD, except an abnormality in the ADP pathway. These findings are particularly important as scarce data exist on platelet aggregation and secretion profiles of non-dialysis CKD patients,^{4, 6} and there are no published controlled studies reporting baseline platelet aggregation profiles of patients with non-dialysis CKD *before* treatment with APA.² Early published case series used bleeding time (BT), capillary fragility time (CFT) or other methods to measure platelet dysfunction in patients with severe uremia,^{22–24} and are of limited clinical utility in modern-day practice given that it is uncommon to find such severe degree of untreated uremia. Studies in uremic dogs reported no between-group differences in platelet aggregation profiles when compared to controls.²⁵ Later uncontrolled reports from ESRD cohorts that used older methods of platelet function testing were conflicting, reporting either normal or impaired ADP-induced platelet aggregation.^{8, 9} These studies employed assays including optical aggregometry, multi-plate analyzers or platelet function analyzer (PFA)-100 which are sub-optimal for testing platelet function and the results may not be reproducible.^{19, 21, 26} The WBPA method is superior to these methods as it is more sensitive, evaluates platelets in a physiological milieu in the presence of red and white blood cells known to affect platelet function, and does not require centrifugation which can injure platelets.²⁶ As a result, knowledge gaps existed in our understanding of uremic platelet dysfunction from studies that were limited by lack of controls, or performed in uremic dogs²⁵ or ESRD.^{6, 22–24} Findings of our study address these gaps in knowledge by including a control group, studying CKD stages 4–5 patients where previous data on platelet aggregation was lacking and using a validated method of platelet aggregation measurement in whole blood.¹⁹

We report no differences in platelet response to 81 mg of aspirin between two groups. These are similar to findings in healthy volunteers where WBPA induced by AA and collagen were inhibited completely and incompletely, respectively, by 2 weeks of aspirin 81 mg/d.¹⁷ Although, we observed reduction in WBPA by collagen from 29 to 20 Ω in CKD and from 25 to 19 Ω in non-CKD individuals on-aspirin ($p=0.56$), a cross sectional study of aspirin-treated diabetic patients observed higher proportion of CKD vs. non-CKD patients had RPA measured by collagen (49% vs. 41%, $p=0.004$).⁴ This study did not define CKD based on guidelines and failed to assess before- and after- aspirin effects. Therefore, we addressed these limitations and found that AA and collagen pathways of platelet aggregation may be inhibited to a similar extent in patients with stages 4–5 CKD as in those without kidney disease.

We extend findings of previous studies by reporting poor response to clopidogrel in CKD stages 4–5 vs. non-CKD patients, independent of CYP2C19 polymorphism effects.¹¹ Previous cross-sectional studies reported presence of RPA in nearly 40–60% of patients with CKD on-clopidogrel.^{4, 6} However, these studies had various limitations: a) Angiolillo *et al.* did not measure before- and after-treatment effects on platelets and over-fitted the model;⁴ b) Woo *et al.* showed lower ADP-induced platelet aggregation in ESRD patients vs. controls but used the optical method of platelet assay;⁵ c) Htun *et al.* reported poor response to clopidogrel in an uncontrolled analysis of CKD and ESRD patients without measuring before- and after-treatment effects;⁶ and, d) Park *et al.* reported similar findings of high RPA on-clopidogrel in Korean men with CKD but failed to adjust for DM.⁷ Our study addresses these limitations and extends these findings by using a validated contemporary method to measure aggregation; including a control; measuring before- and after-treatment effects of clopidogrel on platelet aggregation; and analyzing CYP2C19 polymorphisms.

Although our study may be limited by residual confounding due to small sample size and was not randomized, the two groups were frequency-matched for characteristics previously reported to influence platelet function. Another limitation is that given hemostatic mechanisms are complex, platelet function measured *ex vivo* by WBPA may be different from platelet function *in vivo*, and we investigated only one pathway of hemostasis that would be potentially different among patients with and without CKD. In addition, platelet function assays may have suboptimal reproducibility.²⁷ As such, previously reported variability in antiplatelet effects of clopidogrel is partly explained by CYP2C19 polymorphisms.^{28,29} Therefore, we measured CYP2C19 polymorphisms that may affect clopidogrel-induced platelet inhibition.³⁰ Future studies are needed to confirm these findings, determine if such differences may explain the increased incidence of thrombotic events in APA-treated patients with vs. without CKD, and investigate whether RPA on-clopidogrel translates into a modifiable clinical risk.

Acknowledgments

We thank Robert F. Reilly (M.D.) for advice about study conception and design. We thank Steven Creacy, PhD, at Harmonyx Diagnostics for analyzing and interpreting genetic polymorphisms. We also thank Kyle West (M.S.), Mieshia Beamon (M.S.), and Staci Schwartz (B.A.) for assistance with recruitment and study visits.

Grant support: Supported by an American Heart Association grant (12CRP11830004) awarded to N.J.; VA MERIT grant (CX000217-01) and grant R01DK085512, National Institute of Diabetes and Digestive and Kidney

Diseases (NIDDK), National Institutes of Health (NIH) awarded to S.S.H; and by UTSW O'Brien Kidney Research Core Center (NIDDK, P30DK079328) and Center for Translational Medicine (NIH, UL1TR001105).

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Figure 1.
Exclusionary cascade for derivation of sample. CKD, Chronic Kidney Disease.

Figure 2.

A. Whole blood platelet aggregation (WBPA) induced by various agonists at baseline in chronic kidney disease (CKD) participants (closed bars) vs. non-CKD controls (open bars), expressed in ohms: 10 μ M adenosine diphosphate (ADP10), 20 μ M adenosine diphosphate (ADP20), 2 μ g/mL collagen (collA2), 0.25 mM arachidonic acid (AAA0.25), 0.5 mM arachidonic acid (AAA0.5) and 1 mg/mL ristocetin (R_1mg_mL). **B.** Adenosine triphosphate (ATP) secretion response to various agonists at baseline in the CKD vs. non-CKD groups, expressed in nanomoles (nmoles): ATP secretion to 0.25 mM arachidonic acid (AAS0_25), to 0.5 mM arachidonic acid (AAS0_5), to 2 μ g/mL collagen (CollS2), and to 1 unit/mL thrombin (ThrombinS). Results are presented as means $\pm$ standard deviation. Between-group significant differences are shown with asterisk (CKD vs. non-CKD), $p=0.02$ for ADP10 and $p=0.007$ for ADP20.

Figure 3.

Aspirin effect in the chronic kidney disease (CKD) and the non-CKD groups as measured by the change in whole blood platelet aggregation (WBPA) induced by **A**) 0.5 mM arachidonic acid (AAA0.5) and **B**) 2 µg/mL collagen (CollA2) after 2 weeks of 81 mg of aspirin daily. Results are presented as mean and standard deviation. CKD is represented with closed circles and non-CKD with open triangles. There was a significant decrease in WBPA in both groups with CollA2 ($p < 0.0001$) and with AAA0.5 ($p < 0.0001$). The response at 2 weeks was not statistically different between CKD and non-CKD groups: group x time interaction $p = 0.29$ for CollA2 and $p = 0.78$ for AAA0.5.

Figure 4.

Clopidogrel effect in the chronic kidney disease (CKD) (closed circles) vs. the non-CKD (open triangles) groups in whole blood platelet aggregation (WBPA) induced by **A.** 10 μ M adenosine diphosphate (ADP10) and **B.** 20 μ M adenosine diphosphate (ADP20) on 2 weeks of 81 mg of aspirin and 75 mg of clopidogrel daily. Results are presented as means $\pm$ standard deviation. Asterisk (*) shows that at Week 4 vs. Week 2, there was a significant decrease in WBPA in both groups induced by 10 μ M ADP ($p < 0.0001$) and 20 μ M ADP ($p < 0.0001$). The responses over time, *i.e.* at 2 weeks (aspirin 81 mg) and 4 weeks (aspirin 81 mg + clopidogrel 75 mg) of treatment, were not statistically different between CKD and non-CKD groups.

Figure 5.

Clopidogrel effect in the chronic kidney disease (CKD) (right-side) vs. the non-CKD (left-side) groups in whole blood platelet aggregation (WBPA) induced by 20 μ M adenosine diphosphate (ADP 20) based on metabolic phenotypes of CYP2C19 polymorphisms.

Table 1

Baseline Characteristics of the Cohort

Variable	Chronic Kidney Disease		
	No (n =16)	Yes (n =28)	p-value
Age (years)	49 ± 11	52 ± 10	0.48 *
BMI (kg/m ²)	29.8 ± 4.9	32.0 ± 5.5	0.19 *
Woman	8 (50%)	12 (43%)	0.65
Black	4 (25%)	15 (54%)	0.06
Diabetes mellitus	7 (44%)	13 (46%)	0.86
Proton pump inhibitor use	1 (6%)	1 (4%)	1.00
Beta blocker use	0	21 (75%)	<0.0001
Statin use	5 (31%)	19 (68%)	0.02
Angiotensin converting enzyme inhibitor or angiotensin receptor blocker use	5 (31%)	12 (43%)	0.45
Allopurinol use	0	6 (21%)	0.07
Baseline use of aspirin	4 (25%)	12 (43%)	0.24
eGFR (mL/min/1.73 m ²)	101 ± 15	17 ± 7	<0.0001 *
Urine albumin-to-creatinine ratio (mg/g)	5 (2.5–8.8)	1,282 (143–1,848)	<0.0001 **
Hemoglobin (g/dL)	14.4 ± 1.7	11.2 ± 1.7	<0.0001 *
Hematocrit (%)	42.6 ± 4.2	33.9 ± 5.1	<0.0001 *
White blood cell count (K per µL)	6 ± 2	8 ± 2	0.06 *
Platelet count (K per µL)	217 ± 55	232 ± 55	0.43 *
Serum total cholesterol (mg/dL)	180 ± 33	170 ± 42	0.46 *
Serum triglycerides (mg/dL)	112 (55–211)	124 (104–161)	0.44 **
Serum low density lipoprotein (mg/dL)	106 (70–124)	94 (61–118)	0.38 **
Serum total bilirubin (mg/dL)	0.6 (0.4–0.7)	0.3 (0.2–0.5)	0.02 **
Glycosylated hemoglobin (%)	6.4 ± 0.4	6.8 ± 0.4	<0.0001 *
Serum albumin (g/dL)	4.2 ± 0.4	4.0 ± 0.5	0.13 *
Serum uric acid (mg/dL)	5.1 ± 1.8	8.9 ± 2.0	<0.0001 *

Variable	Chronic Kidney Disease		
	No (n =16)	Yes (n =28)	p-value
Serum calcium (mg/dL)	9.3 ± 0.3	9.1 ± 0.8	0.31 *
Serum phosphorus (mg/dL)	3.3 ± 0.3	4.6 ± 1.1	<0.0001 **
Serum parathyroid hormone (pg/mL)	42 (34–46)	197 (143–358)	<0.0001 **
Serum 25-hydroxyvitamin D (ng/mL)	29 ± 15	28 ± 14	0.80 *

* Student's *t*-test used for comparison

** Wilcoxon rank sum test used for comparison

Table 2

Whole Blood Platelet Aggregation and Adenosine Triphosphate Secretion at Baseline, on Aspirin and on Aspirin/Clopidogrel

Profile	Chronic kidney disease		
	No	Yes	p-value
	Visit 1: Baseline		
Aggregation to 0.5 mM arachidonic acid (Ω)	18.0 (10.0, 20.0)	21.0 (10.5, 24.5)	0.27
Aggregation to 0.25 mM arachidonic acid (Ω)	17.0 (9.0, 21.0)	19.5 (12.0, 22.0)	0.43
Aggregation to 20 μM adenosine diphosphate (Ω)	9.0 (6.0, 12.0)	13.5 (9.5, 16.0)	0.007*
Aggregation to 10 μM adenosine diphosphate (Ω)	8.0 (5.0, 11.0)	12.5 (8.0, 14.5)	0.02*
Aggregation to 2 μg/mL collagen (Ω)	25.0 (20.0, 30.0)	28.5 (23.5, 31.5)	0.21
Aggregation to 1 mg/mL ristocetin (Ω)	14.0 (9.0, 22.0)	14.0 (9.5, 20.0)	0.92
Secretion to 0.5 mM arachidonic acid (nmoles)	0.81 (0.65, 0.98)	0.67 (0.52, 1.05)	0.79
Secretion to 0.25 mM arachidonic acid (nmoles)	0.92 (0.60, 1.33)	0.75 (0.5, 0.94)	0.24
Secretion to 2 μg/mL collagen (nmoles)	1.05 (0.79, 1.28)	0.92 (0.62, 1.32)	0.72
Secretion to 1 unit/mL thrombin (nmoles)	1.28 (0.82, 1.64)	1.32 (0.93, 1.84)	0.54
Visit 2: On Aspirin			
Aggregation to 0.5 mM arachidonic acid (Ω)	0	0	0.67
Aggregation to 0.25 mM arachidonic acid (Ω)	0	0	0.50
Aggregation to 20 μM adenosine diphosphate (Ω)	10.0 (8.0, 11.0)	11.0 (8.0, 16.0)	0.19
Aggregation to 10 μM adenosine diphosphate (Ω)	9.0 (7.0, 11.0)	10.5 (8.0, 14.0)	0.14
Aggregation to 2 μg/mL collagen (Ω)	19.0 (16.0, 22.0)	19.5 (16.0, 26.0)	0.72
Aggregation to 1 mg/mL ristocetin (Ω)	6.0 (4.0, 6.0)	7.5 (6.0, 12.0)	0.04
Secretion to 0.5 mM arachidonic acid (nmoles)	0	0 (0, 0.15)	0.03*
Secretion to 0.25 mM arachidonic acid (nmoles)	0 (0, 0.1)	0 (0, 0.11)	1.0
Secretion to 2 μg/mL collagen (nmoles)	0.48 (0.4, 0.61)	0.59 (0.4, 0.83)	0.20
Secretion to 1 unit/mL thrombin (nmoles)	0.82 (0.77, 1.55)	1.32 (1.09, 1.47)	0.21
Visit 3: On Aspirin/Clopidogrel			
Aggregation to 20 μM adenosine diphosphate (Ω)	3.0 (0, 4.0)	8.0 (1.0, 12.0)	0.04*

Profile	Chronic kidney disease		p-value
	No	Yes	
Aggregation to 10 μ M adenosine diphosphate (Ω)	1.0 (0, 3.0)	7.0 (1.0, 12.0)	0.02 *

* Denotes statistically significant p-values for Wilcoxon rank sum test comparing CKD yes vs. no

Table 3

Inhibition of Platelet Aggregation from Baseline in Response to Aspirin and Aspirin/Clopidogrel

Chronic kidney disease				
Inhibition of platelet aggregation measured by	Visits	No	Yes	p-value
Aspirin effect				
0.25 mM arachidonic acid (%)	Visit 2 vs. Visit 1	95 ± 17	95 ± 21	0.58
0.5 mM arachidonic acid (%)	Visit 2 vs. Visit 1	99 ± 3	97 ± 17	0.67
2 µg/mL collagen (%)	Visit 2 vs. Visit 1	28 (16, 37)	30 (7, 42)	0.56
Clopidogrel effect				
10 µM adenosine diphosphate (%)	Visit 3 vs. Visit 1	88 (65, 100)	50 (10, 85)	0.046
20 µM adenosine diphosphate (%)	Visit 3 vs. Visit 1	72 (59, 100)	38 (20, 88)	0.04

p-value for Wilcoxon rank sum test comparing CKD yes vs. no

Table 4

Residual Platelet Aggregation on Aspirin and on Aspirin/Clopidogrel

		Chronic kidney disease		
Residual platelet aggregation measured by	Visits	No	Yes	p-value
On aspirin				
0.25 mM arachidonic acid (%)	Visit 2 vs. Visit 1	7.7	4.2	1.0
0.5 mM arachidonic acid (%)	Visit 2 vs. Visit 1	0.0	3.9	1.0
2 µg/mL collagen (%)	Visit 2 vs. Visit 1	92.3	84.6	0.65
On aspirin/clopidogrel				
10 µM adenosine diphosphate (%)	Visit 3 vs. Visit 1	16.7	48.0	0.08
20 µM adenosine diphosphate (%)	Visit 3 vs. Visit 1	8.3	56.0	0.01

p-value for Fisher's Exact test comparing proportion of participants with CKD yes vs. no

Table 5
Frequencies for CYP2C19 According to Allele, Genotype, and Predicted Metabolic Phenotype

Allele	NCBI SNP ID	Genotype	Chronic kidney disease		Metabolic phenotype	Chronic kidney disease	
			Yes	No		Yes	No
			(n=26)	(n=13)		(n=26)	(n=13)
*17	rs12248560	*1/17	6 (23%)	2 (15%)	Rapid	7 (27%)	2 (15%)
		*17/*17	1 (4%)	0			
		*1/*1	9 (35%)	5 (38%)	Normal	9 (35%)	5 (38%)
*2	rs4244285	*2/*17	2 (8%)	3 (23%)	Intermediate	9 (35%)	6 (47%)
		*1/*2	7 (27%)	3 (23%)			
		*2/*2	1 (4%)	0	Poor	1 (3%)	0

p-value = 0.84 for Fisher's Exact test comparing proportions of metabolic phenotypes between CKD yes vs. no
CYP2C19, cytochrome P450 2C19; CYP2C19*1, wild type; NCBI, National Center for Biotechnology Information; SNP ID, single nucleotide polymorphism identification number