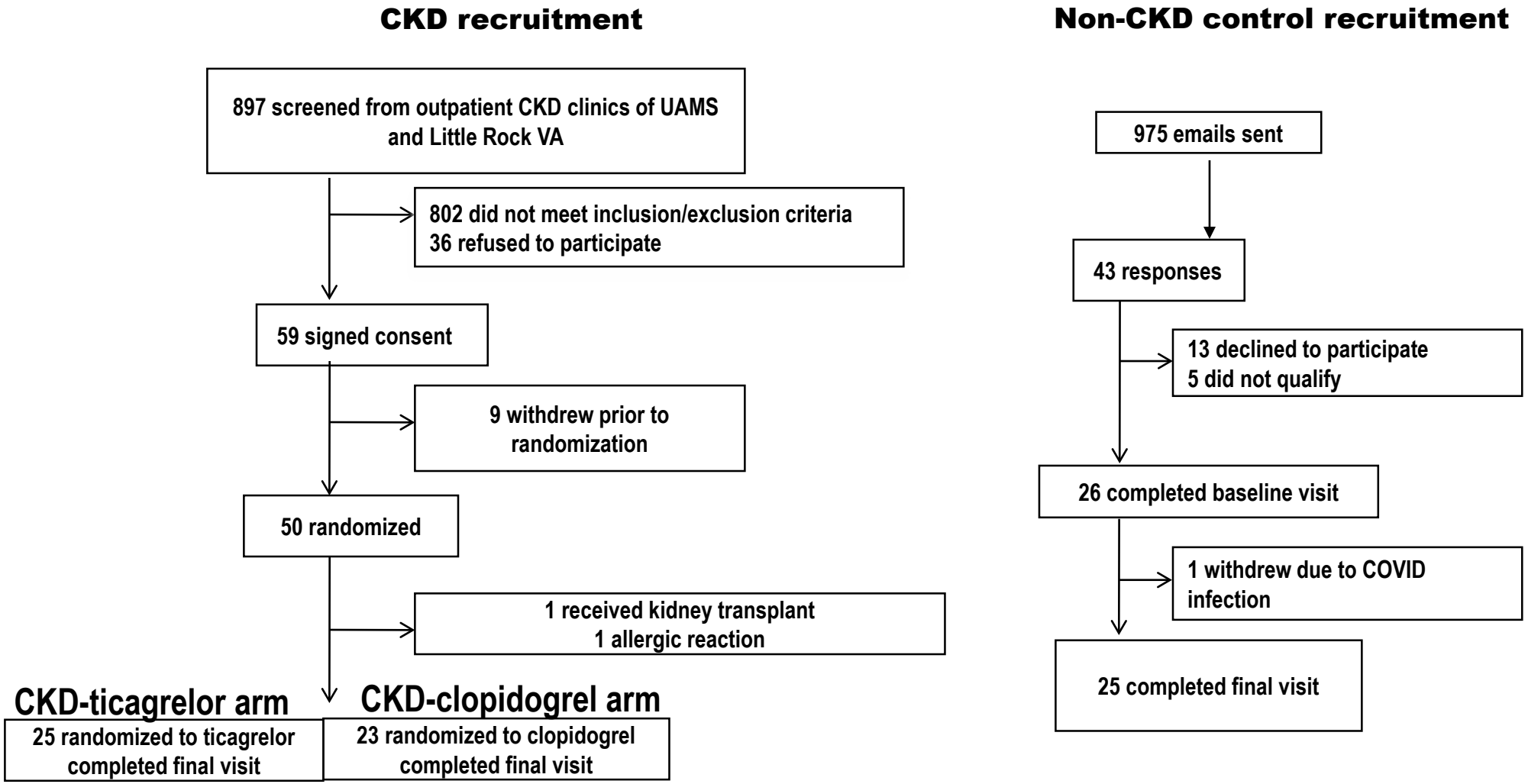
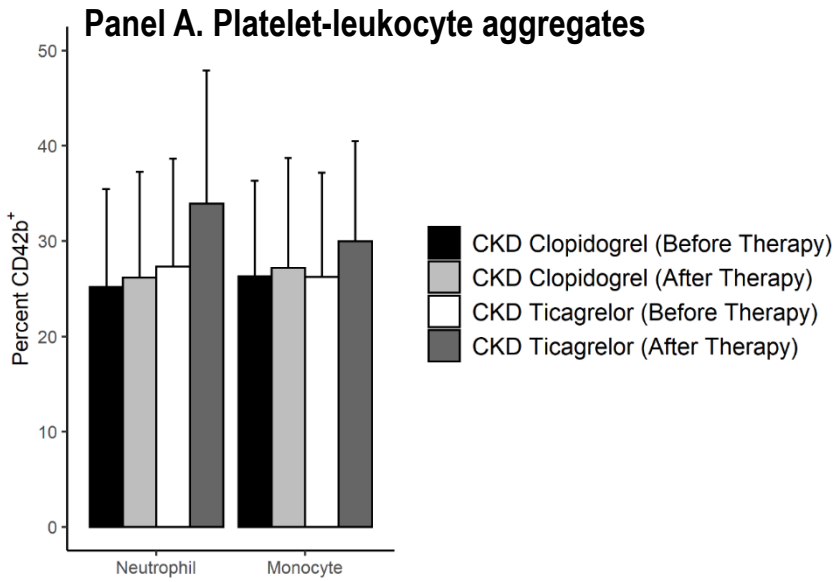
## SUPPLEMENTAL APPENDIX

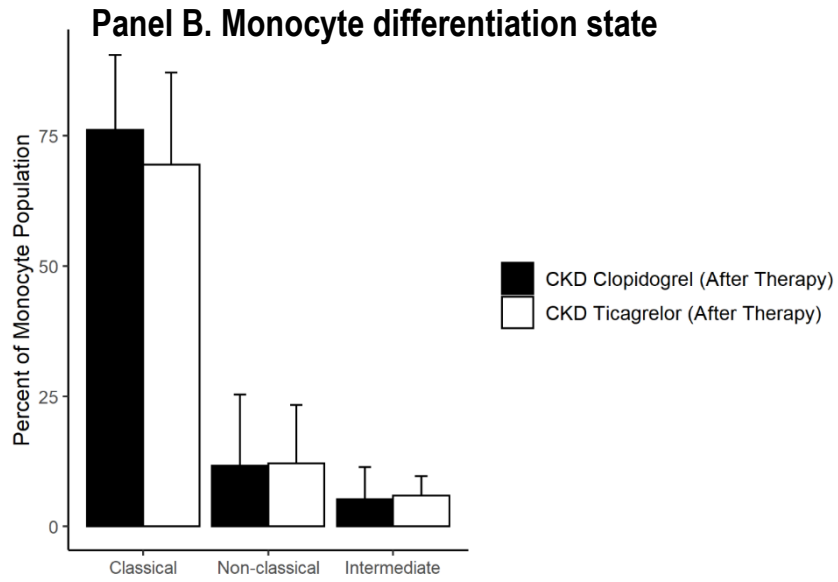
1. **Figure S1** shows the derivation of the study cohort
2. **Figure S2** shows platelet–leukocyte aggregates in a) patients with CKD randomized to clopidogrel or ticagrelor **(Panels A-B)** before and after therapy and b) patients with CKD and controls before and after ticagrelor treatment **(Panels C-D)**.
3. **Figure S3** shows plasma levels of IL-6 and IL-1RA in CKD patients and controls before and after treatment with ticagrelor.
4. **Table S1** shows protocol for flow cytometry
5. **Table S2** shows protocol for ticagrelor and drug measurement performed at the Metabolomics Lab, University of Missouri, Columbia.
6. **Table S3** shows baseline characteristics of the cohort
7. **Table S4** shows data on CKD and controls on aspirin and ticagrelor treatment including platelet aggregation to various agonists, expression of platelet surface receptors and circulating platelet-leukocyte aggregates.
8. **Table S5** shows data on CKD randomized to receive ticagrelor vs. clopidogrel treatments including platelet aggregation to various agonists, expression of platelet surface receptors and circulating platelet-leukocyte aggregates.
9. **Table S6 A.** shows adverse events of CKD during the 2-weeks of treatment with ticagrelor or clopidogrel. Everyone received concomitant aspirin 81 mg/d. **B**. shows adverse events of CKD and controls during the 2-weeks of treatment with ticagrelor and aspirin.

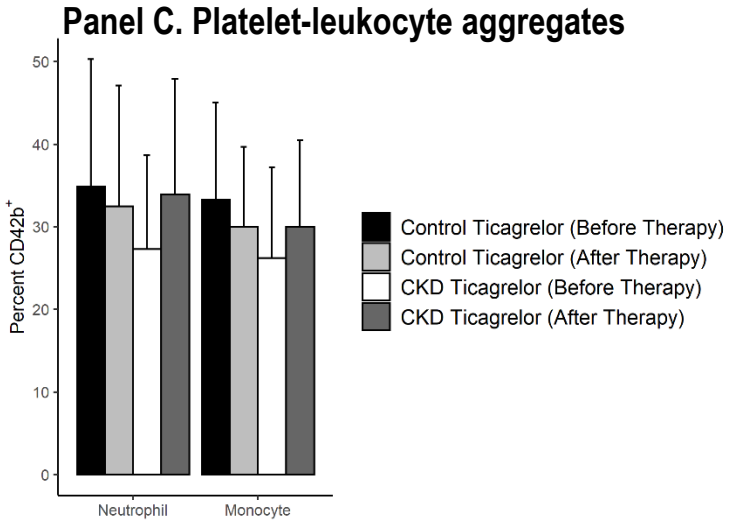
# Figure S1. Flow of the Study Cohort




clinicaltrials.gov # NCT03649711. Study enrollment period from 11.01.2018 through 08.19.2021


Panel A. Platelet-leukocyte aggregates
Percent CD42b+
50
40
30
20
10
0
Neutrophil
Monocyte
CKD Clopidogrel (Before Therapy)
CKD Clopidogrel (After Therapy)
CKD Ticagrelor (Before Therapy)
CKD Ticagrelor (After Therapy)


Panel B. Monocyte differentiation state
Percent of Monocyte Population
75
50
25
0
Classical
Non-classical
Intermediate
CKD Clopidogrel (After Therapy)
CKD Ticagrelor (After Therapy)


Panel C. Platelet-leukocyte aggregates
Percent CD42b+
50
40
30
20
10
0
Neutrophil
Monocyte
Control Ticagrelor (Before Therapy)
Control Ticagrelor (After Therapy)
CKD Ticagrelor (Before Therapy)
CKD Ticagrelor (After Therapy)

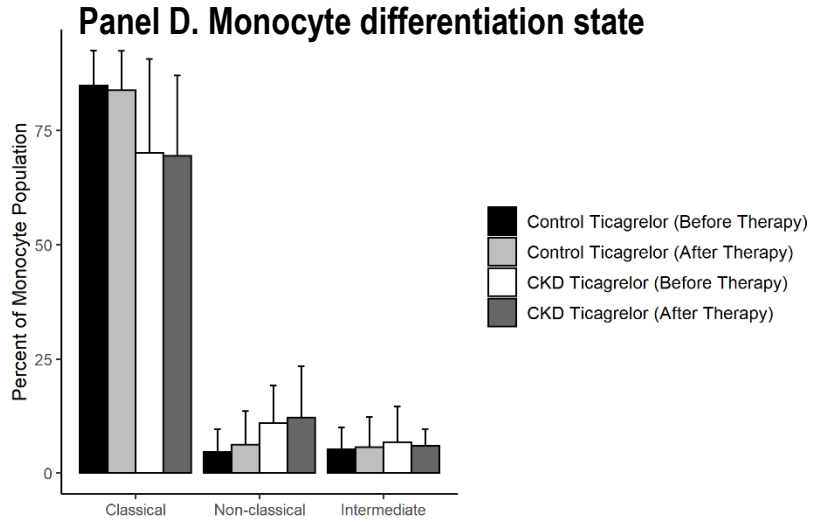

Panel D. Monocyte differentiation state
Percent of Monocyte Population
75
50
25
0
Classical
Non-classical
Intermediate
Control Ticagrelor (Before Therapy)
Control Ticagrelor (After Therapy)
CKD Ticagrelor (Before Therapy)
CKD Ticagrelor (After Therapy)

**Figure S2**

**Table S1.** Ticagrelor and Metabolite Analysis

Internal Standards and Calibration:
An internal standard solution was made containing ticagrelor-d7 and des-ticagrelor-d7 in methanol. A calibration stock solution was made containing ticagrelor and 4.50 ug/mL des-ticagrelor in methanol. The calibration stock solution was diluted 5x repeatedly with methanol to obtain 7 concentrations, ranging from 0.2–10,000 ng/mL. A 25-µL aliquot of the internal standard solution was added to 400 µL of each calibration solution.

Sample Preparation:
A 100-µL aliquot of each plasma sample was placed into an Eppendorf tube. A 25-µL aliquot of internal standard solution was added to each sample, along with 300 µL acetonitrile, and each sample was vortexed for 10 minutes. The samples were centrifuged at 12,500 *g* for 5 minutes, then the supernatant was transferred to an autosampler vial with insert and stored at -20°C until analysis by LCMS/MRM.

LCMS/MRM Analysis:
LCMS analyses were carried out with a Waters Xevo-TQ triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) coupled to an electrospray ionization source (ESI) in both positive and negative modes, depending on the compound, as shown in the supplementary data table below. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode. The fragmentation transitions for each compound, along with the cone voltages and collision energies, are listed in the supplementary table.

The separation was performed using a Waters Acquity UHPLC equipped with an Acquity column (BEH C28 150 x 2.1 mm ID, 1.7 um) with a flow rate of 0.375 mL/min and column temperature of 45°C. Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. The mobile phase gradient began with 99% A, decreasing to 50% A at 5 minutes, 10% A at 7 minutes, and 1% A at 7.5 minutes, returning to the initial condition of 99% A at 9 minutes and held at 99% A until 10 minutes. Sample injection volume was 2 µL.

Data were analyzed with MassLynx software version 4.1 (Waters Corporation). The calibration curves were calculated with least-square linear regression analysis of the peak area ratio of analytes to the internal standard versus the concentration of the standards.

| | Qual transition | Quant transition | Cone (V) | CE (eV) | RT (min) | ESI |
|---|---|---|---|---|---|---|
| Ticagrelor | 523→453 | 523→495 | 42 V | 26, 22 | 6.6-7.1 | + |
| Ticagrelor-d7 | 530→153 | 530→502 | 50 V | 40, 22 | 6.6-7.1 | + |
| Des-Ticagrelor | 479→145 | 479→461 | 56 V | 74, 24 | 6.6-7.2 | + |
| Des-Ticagrelor | 486→153 | 486→370 | 40 V | 32, 30 | 6.6-7.2 | + |

**Table S2.** Protocol for Whole-Blood Flow Cytometry Staining

Protocol #1. **Platelet–Leukocyte Interactions:**

1. Collect whole blood from patient in 4-ml BD vacutainer® EDTA tube using butterfly needle.
2. Label 5 ml round-bottom tube “Leukocyte” and place in foil-covered tube rack.
3. Pipette 5 µl of the following into the same 5-ml tube: anti-human CD42b, anti-human CD14, anti-human CD16, anti-human CD66b, and anti-human CD11b.
4. Pipette 100 µl whole blood into tube containing antibodies. Cover tube/rack with foil and shake for 20-30 min.
5. Pipette 1,000 µl BD FACSLyse buffer (BD Biosciences catalog # 349202) into tube and cover tube/rack with foil and shake for 20-30 min.
6. Centrifuge tube at 1,000 *g* for 5 min to pellet leukocytes. Remove supernatant.
7. Reconstitute pellet in 1000 µl PBS.
8. Store covered at 4°C in the UAMS Flow Cytometry Core until analysis on a BD LSRFortessa (capturing ~50,000 events in the $BV605^{+}/BV785^{-}$ population).

Protocol #2. **Platelet Receptors:**

1. Use same tube of whole blood collected from patient in Protocol #1.
2. Label 5-ml round-bottom tube “Platelet” and place in foil-covered tube rack.
3. Pipette 24 µl PBS into 5-ml tube.
4. Pipette 5 µl of the following into the same 5-ml tube: anti-human CD41b, anti-human CD42b, anti-human CD62P, and anti-human P2Y12.
5. Pipette 1 µl whole blood into tube containing antibodies. Cover tube/rack with foil and shake for 20-30 min.
6. Pipette 400 µl BD FACSLyse buffer (BD Biosciences catalog # 349202) into tube. Cover tube/rack with foil and shake for 20-30 min.
7. Store covered at 4°C in the UAMS Flow Cytometry Core until analysis on a BD LSRFortessa (capturing ~100,000 events from the platelet population).

Reagents:

**Antibodies:**

*BD Biosciences:*
a. Alexa Fluor 700 mouse anti-human CD11b (Clone ICRF44) (#557918)
b. PE mouse anti-human CD66b (Clone G10F5) (#561650)
c. BV605 mouse anti-human CD16 (Clone 3G8) (#563172)
d. BV786 mouse anti-human CD14 (Clone M5E2) (#563698)
e. FTIC mouse anti-human CD41b (Clone HIP2) (#555469)
f. BV711 mouse anti-human CD42b (Clone HIP1) (#740779)
g. BV421 mouse anti-human CD62P (Clone AK-4) (#564038)

*BioLegend:* PE anti-human P2RY12 (Clone S16001E) (#392104)

**Buffers:**

*BD Biosciences (FACSLyse Buffer):*
a. Lysing Solution 10X Concentrate (100ml) (#349202)

*PBS (Tablets)*
b. Phosphate Buffered Saline (100 tablets) (#P4417-100TAB)

Protocol #3. **Platelet adherence to leukocytes: Whole-blood Imaging Flow Cytometry Staining**

1. Use same tube of whole blood collected from patient in Protocol #1.
2. Label 1.5-ml round-bottom tube “Platelet” and place into foil-covered tube rack.
3. Pipette 25 µl PBS into 5-ml tube.
4. Pipette 5 µl of the following into the same 5-ml tube: anti-human CD42, anti-human CD14, anti-human CD16, and anti-human CD66b.
5. Pipette 75 µl whole blood into tube containing antibodies. Cover tube/rack with foil and shake for 20-30 min.
6. Pipette 1,000 µl BD FACSLyse buffer into tube. Cover tube/rack with foil and shake for 20-30 min.
7. Centrifuge tube at 1,000 *g* for 5 min to pellet leukocytes. Remove supernatant.
8. Reconstitute pellet in 1,000 µl PBS and centrifuge again at 1,000 *g* for 5 min. Remove supernatant.
9. Reconstitute pellet in 30 µl PBS.
10. Store covered at 4°C in the UAMS Flow Cytometry Core until analysis on an Amnis ImageStream (capturing ~2,500-5,000 events from the neutrophil–platelet group ($CD42^+$ then $CD14^-/CD16^+/CD66b^+$) and from the monocyte–platelet group ($CD42^+$ then $CD14^+/CD66b^-$).

Reagents:

**Antibodies:**

*BD Biosciences:*

a. PE mouse anti-human CD66b (Clone G10F5) (#561650)
b. BV605 mouse anti-human CD16 (Clone 3G8) (#563172)
c. BV786 mouse anti-human CD14 (Clone M5E2) (#563698)
d. BV711 mouse anti-human CD42b (Clone HIP1) (#740779)

**Buffers:**

*BD Biosciences (FACSLyse Buffer):*

c. Lysing Solution 10X Concentrate (100ml) (#349202)

*PBS (Tablets)*

d. Phosphate Buffered Saline (100 tablets) (#P4417-100TAB)

**Table S3. Baseline characteristics of the cohort**

| Variable | Non-CKD | CKD | | |
|---|---|---|---|---|
| | Controls (n=26) | All (n=48) | Ticagrelor (n=25) | Clopidogrel (n=23) |
| Age, years, mean (SD) | 48.0 (15.2)* | 53.7 (13.3) | 55.2 (11.5) | 52.2 (15.1) |
| Chronic kidney disease stage 5, n (%) | 0 (0) | 20 (41.7) | 10 (40.0) | 10 (43.5) |
| Body mass index, kg/m$^2$, mean (SD) | 31.2 (7.0)* | 33.5 (8.8) | 34.5 (8.6) | 32.5 (9.1) |
| Women, n (%) | 16 (61.5)* | 25 (52.1) | 13 (52.0) | 12 (52.2) |
| African American, n (%) | 3 (11.5) | 26 (54.2) | 13 (52.0) | 13 (56.5) |
| Diabetes mellitus, n (%) | 0 (0) | 31 (64.6) | 17 (68.0) | 14 (60.9) |
| Diuretics, n (%) | 1 (3.9) | 33 (68.8) | 16 (64.0) | 17 (73.9) |
| Other antihypertensive drug, n (%) | 4 (15.4) | 37 (77.1) | 20 (80.0) | 17 (73.9) |
| Proton pump inhibitor use, n (%) | 1 (3.9) | 9 (18.8) | 5 (20.0) | 4 (17.4) |
| Beta blocker use, n (%) | 1 (3.9) | 34 (70.8) | 18 (72.0) | 16 (69.6) |
| Statin use, n (%) | 4 (15.4) | 29 (60.4) | 15 (60.0) | 14 (60.9) |
| ACEI/ARB use, n (%) | 2 (7.7) | 21 (43.8) | 12 (48.0) | 9 (39.1) |
| Baseline use of aspirin, n (%) | 3 (11.5) | 17 (35.4) | 10 (40.0) | 7 (30.4) |
| Serum creatinine, in mg/dl, mean (SD) | 0.9 (0.1) | 4.2 (1.9) | 4.2 (1.9) | 4.3 (1.8) |
| eGFR, mL/min/1.73 m$^2$, mean (SD) | 89.6 (13.2) | 16.5 (6.0) | 16.7 (6.5) | 16.4 (5.7) |
| Urine albumin-to-creatinine ratio, mg/g, median (IQR) | 3.9 (2.1, 5.5) | 1,229.4 (638.0, 3,329.0) | 1,039.0 (715.6, 2,382.5) | 2,400.0 (519.7, 4,243.0) |
| Hemoglobin, g/dL, mean (SD) | 13.5 (1.3) | 11.1 (1.8) | 11.1 (1.8) | 11.1 (1.9) |
| White blood cell count, K per µL, mean (SD) | 6.2 (1.6) | 7.6 (2.4) | 7.7 (2.4) | 7.5 (2.4) |
| Platelet count, K per µL, mean (SD) | 266.5 (57.9) | 236.7 (61.5) | 243.0 (71.1) | 229.9 (49.7) |
| Serum total cholesterol, mg/dL, mean (SD) | 188.4 (39.6) | 175.5 (49.6) | 178.5 (47.9) | 172.1 (52.5) |
| Serum triglycerides, mg/dL, mean ±SD | 128.9 (91.9) | 159.0 (101.9) | 161.9 (95.3) | 155.4 (111.6) |
| Serum LDL, mg/dL, median (IQR) | 108.0 (86.0, 122.0) | 98.0 (74.0, 120.0) | 103.0 (86.0, 120.0) | 92.0 (63.5, 118.3) |
| Serum total bilirubin, mg/dL, mean (SD) | 0.6 (0.3) | 0.5 (0.2) | 0.5 (0.2) | 0.6 (0.2) |
| Hemoglobin A1c, %, mean (SD) | 5.3 (0.4) | 6.9 (1.6) | 6.8 (1.3) | 7.0 (1.9) |
| Serum albumin, g/dL, mean (SD) | 4.2 (0.3) | 3.5 (0.4) | 3.4 (0.4) | 3.5 (0.4) |
| Serum uric acid, mg/dL, mean (SD) | 5.1 (1.2) | 8.4 (2.1) | 8.5 (2.2) | 8.4 (1.9) |
| Serum calcium, mg/dL, mean (SD) | 9.5 (0.3) | 8.4 (1.6) | 8.5 (1.5) | 8.3 (1.6) |
| Serum phosphorus, mg/dL, mean (SD) | 3.7 (0.7) | 4.9 (1.7) | 4.8 (1.6) | 4.9 (1.9) |

Abbreviations. ACEI/ARB- angiotensin converting enzyme inhibitor or angiotensin receptor blocker, eGFR- estimated glomerular filtration rate based on CKD-EPI equation; LDL- low density lipoprotein. ***Represents no difference in the non-CKD control and CKD in the ticagrelor arm groups where all *P*-values >0.05.**

**Table S4**. Whole Blood Platelet Aggregation to Various Agonists, and the Fluorescence Cytometry of Platelet Surface Receptors and Platelet-Leukocyte Aggregates in Patients with stages 4-5 CKD and Controls with Normal Kidney Function on DAPT (aspirin 81 mg/d + ticagrelor 90 mg twice daily)

| Variable | CKD Ticagrelor (n=25) | Control Ticagrelor (n=26) | P-Value* | CKD Ticagrelor (n=25) | Control Ticagrelor (n=26) | P-Value* |
|---|---|---|---|---|---|---|
| | Post-treatment values on visit 3 | | | Mean changes on DAPT | | |
| **Whole blood platelet aggregation** | | | | | | |
| 20 μM ADP (in Ω) | 0 (0, 2) | 1 (0, 3) | 0.15 | -8 (-12, -6) | -6 (-10, -3) | 0.18 |
| 2 μg/mL collagen (in Ω) | 4 (1, 9) | 1 (0, 8) | 0.29 | -4 (-16, 1) | 0 (-5, 1) | 0.19 |
| 0.5 mM arachidonic acid (in Ω) | 0 (0, 0) | 0 (0, 0) | 0.65 | -6 (-9, 0) | -2 (-6, 0) | 0.39 |
| 1 mg/mL ristocetin (in Ω) | 4 (3, 7) | 3 (2, 8) | 0.47 | -8 (-11, -4) | -7 (-10, -4) | 0.64 |
| **Platelet surface receptor** | | | | | | |
| Glycoprotein Ib, MFI | 9,255 (7,405, 10,563) | 10,096 (8,635, 11,896) | 0.07 | 755 (-1,154, 1,388) | -606 (-1,760, 961) | 0.54 |
| Glycoprotein IIb/IIIa, MFI | 2,225 (1,020, 3,209) | 1,989 (1,473, 2,430) | 0.79 | 73 (-234, 611) | -269 (-910, 363) | 0.10 |
| P2Y12, MFI | 3,429 (2,232, 3,831) | 3,113 (2,647, 3,451) | 0.50 | -61 (-1,311, 433) | 168 (-144, 687) | 0.10 |
| P-selectin, MFI | 1,002 (758, 1,149) | 1,052 (810, 1,279) | 0.45 | -72 (-212, 279) | -61 (-433, 143) | 0.52 |
| **Platelet-leukocyte aggregates** | | | | | | |
| Platelet-neutrophil, % | 32 (29, 40) | 32 (25, 36) | 0.61 | 4 (-4, 12) | 0 (-4, 2) | 0.21 |
| Platelet-monocytes, % | 30 (24, 40) | 33 (21, 35) | 0.96 | 2 (-1, 7) | 0 (-3, 3) | 0.13 |

*P-value represent Wilcoxon rank sum test of comparison between groups

CKD. Chronic kidney disease with glomerular filtration rate <30 ml/min/1.73m$^2$, DAPT. Dual antiplatelet therapy with aspirin 81 mg/day plus ticagrelor 90 mg twice daily, MFI. Mean fluorescence intensity

**Table S5**. Post-treatment Values of Whole Blood Platelet Aggregation to Various Agonists, and the Fluorescence Cytometry of Platelet Surface Receptors and Platelet-Leukocyte Aggregates in Patients with stages 4-5 CKD in the Ticagrelor Arm (aspirin 81 mg/d + ticagrelor 90 mg twice daily) and the Clopidogrel Arm (aspirin 81 mg/d + clopidogrel 75 mg/d and a matching placebo at night)

| Variable | CKD Ticagrelor (n=25), median (IQR) | CKD Clopidogrel (n=23), median (IQR) | P-Value* |
|---|---|---|---|
| **Whole blood platelet aggregation** | | | |
| 2 μg/mL collagen (in Ω) | 4 (1, 9) | 6 (1, 11) | 0.73 |
| 0.5 mM arachidonic acid (in Ω) | 0 (0, 0) | 0 (0, 0) | 0.11 |
| 1 mg/mL ristocetin (in Ω) | 4 (3, 7) | 6 (4, 9) | 0.54 |
| **Platelet surface receptor** | | | |
| Glycoprotein Ib, MFI | 9,255 (7,406, 10,563) | 9,155 (8,517, 10,655) | 0.50 |
| Glycoprotein IIb/IIIa, MFI | 2,225 (1,021, 3,210) | 1,962 (1,675, 2,732) | 0.74 |
| P2Y12, MFI | 3,430 (2,233, 3,831) | 3,075 (2,603, 3,172) | 0.52 |
| P-selectin, MFI | 1,003 (759, 1,150) | 1,038 (849, 1,263) | 0.22 |
| **Platelet-leukocyte aggregates** | | | |
| Platelet-neutrophil, % | 32 (29, 40) | 28 (23, 32) | 0.05 |
| Platelet-monocytes, % | 30 (24, 40) | 30 (18, 37) | 0.32 |

*P-value represents ANCOVA of comparison between groups adjusted for presence of diabetes mellitus

**Table S6.** Adverse Events

**Table A. Adverse events in study participants with CKD randomized to each study drug**

| | Ticagrelor (N=25) | Clopidogrel (N=23) | P-value |
|---|---|---|---|
| Adverse events, n | 14 | 15 | 0.85 |
| Bruise, n | 12 | 9 | 0.74 |
| Dyspnea, n | 5 | 1 | 0.19 |
| Fatigue, n | 2 | 4 | 0.42 |
| Dyspepsia, n | 0 | 1 | 0.49 |

Adverse events reported during the 2 weeks of treatment

**Table B. Adverse events in study participants with CKD and controls treated with ticagrelor**

| | CKD (N=25) | Controls (N=26) | P-value |
|---|---|---|---|
| Adverse events, n | 14 | 13 | 0.87 |
| Bruise, n | 12 | 14 | 0.89 |
| Dyspnea, n | 5 | 1 | 0.10 |
| Fatigue, n | 2 | 0 | 0.23 |
| Dyspepsia, n | 0 | 2 | 0.49 |

Adverse events reported during the 2 weeks of treatment