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# Ticagrelor Inhibits Platelet Aggregation and Reduces Inflammatory Burden More than Clopidogrel in Patients with Stages 4 or 5 Chronic Kidney Disease

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## Abstract

**Background:** No study has compared pharmacologic properties of ticagrelor and clopidogrel in non-dialysis patients with stage 4 – 5 chronic kidney disease (CKD).

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AUTHOR CONTRIBUTIONS

JW, JMA, JLM, SSH, and NJ designed the study; AC and NJ carried out experiments; JD and MAP analyzed the data; NA assisted with data entry and performing literature searches; YR performed correlation analysis of cytokines data; NJ drafted and revised the manuscript; and SS provided critical expertise in reviewing and providing meaningful insights for the manuscript. All authors approved the final version of the manuscript.



PRIOR PRESENTATION

Some of these results were submitted in an abstract for the American Society of Nephrology Kidney Week and the American Heart Association Scientific Sessions to be held in Fall 2022.

Declaration of interests

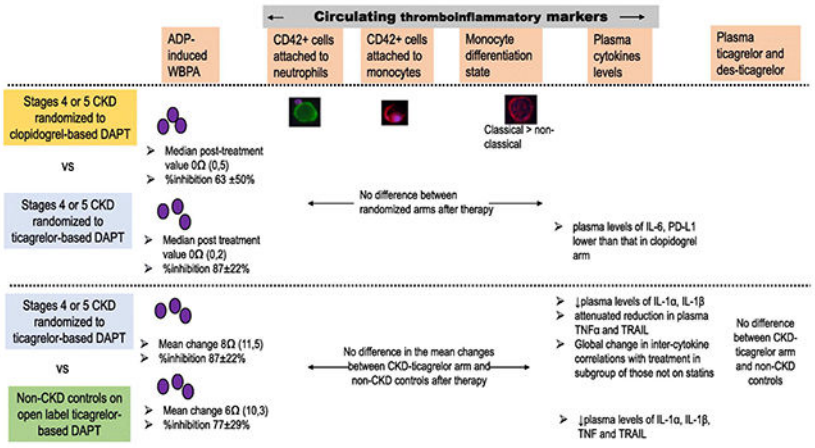
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Methods:** We conducted a double-blind RCT to compare effects of ticagrelor and clopidogrel in 48 CKD, with the primary outcome of ADP-induced platelet aggregation (WBPA) after 2 weeks of DAPT. In a parallel arm, we compared effects of 2 weeks of ticagrelor plus aspirin on mean changes in WBPA and markers of thromboinflammation among non-CKD controls (n=26) with that of CKD in the ticagrelor-arm.

**Results:** Average age of CKD was 53.7 years, with 62% women, 54% African American, and 42% with stage 5 CKD. Ticagrelor generated statistically lower WBPA values post treatment [median 0 Ω (IQR 0, 2)] vs. clopidogrel [median 0Ω (IQR 0, 5)] ($P$=0.002); percent inhibition of WBPA was greater (87 ± 22% vs. 63 ± 50%; $P$=0.04; and plasma IL-6 levels were much lower (8.42 ±1.73 pg/ml vs. 18.48 ±26.56 pg/ml; $P$=0.04); No differences in mean changes in WBPA between CKD-ticagrelor and control groups were observed. Ticagrelor- DAPT reduced levels of IL-1α and IL-1β in CKD-ticagrelor and control groups, attenuated lowering of TNFα and TRAIL levels in CKD-ticagrelor (vs controls), and had global changes in correlation between various cytokines in a subgroup of CKD-ticagrelor subjects not on statins (n=10). Peak/trough levels of ticagrelor/metabolite were not different between CKD-ticagrelor and control groups.

**Conclusions:** We report significant differences in platelet aggregation and anti-inflammatory properties between ticagrelor- and clopidogrel-based DAPT in non-dialysis people with stage 4-5 CKD. These notable inflammatory responses suggest ticagrelor-based DAPT might lower inflammatory burden of asymptomatic patients with stage 4 or 5 CKD. (clinicaltrials.gov # NCT03649711)

## Graphical Abstract




### Keywords

platelets; chronic kidney disease; ticagrelor; clopidogrel; inflammation

## INTRODUCTION

Ticagrelor has the most desirable pharmacological properties among the oral P2Y12 inhibitors.[1] The Platelet Inhibition and Patient Outcomes (PLATO) trial reported that in the subgroup with glomerular filtration rate (GFR) <30 ml/min/1.73 $m^2$ (n=214), use

of ticagrelor was associated with a 23% reduction in mortality and ischemic events.[1] Ticagrelor use was also associated with reduced sepsis-related death in 2 observational studies.[2,3] However, there were only 15 individuals with GFR of ≤15 ml/min/1.73 $m^2$ (not on dialysis) included in the PLATO trial.[4] Recent post-marketing observational studies failed to demonstrate benefits of ticagrelor over clopidogrel in reducing thrombotic events among CKD patients with GFR ≤15 ml/min/1.73 $m^2$.[5–9] To date, there is a lack of mechanistic data to dissect the antiplatelet effects of ticagrelor vs. clopidogrel among patients with stages 4-5 CKD (GFR <30 ml/min/1.73 $m^2$). Studies exploring the antiplatelet effects of ticagrelor in CKD have been limited by the lack of a control arm,[10] high dropout rates,[11] or failure to enroll participants with stages 4-5 CKD.[12–17]

The pharmacological characteristics of ticagrelor and clopidogrel may differ between individuals without kidney disease or with stages 1-3 CKD (GFR >60 ml/min/1.73 $m^2$) and patients with more severe stages 4-5 CKD (<30 ml/min/1.73 $m^2$).[18–20] Alterations in the hemostatic pathway in patients with stages 4-5 CKD make it difficult to extrapolate mechanistic data to this population.[18–20] First, as CKD progresses, the ability of platelet surface receptors to undergo conformational changes upon activation may be impaired.[21] Ticagrelor binds reversibly to a site on the platelet P2Y12 receptor distant from the ADP-binding site and blocks ADP binding to the receptor via allosteric modulation.[22] Binding of ticagrelor and consequent allosteric modulation of the receptor may be altered in the CKD milieu.[23] Second, ADP-induced platelet aggregation may be higher in stages 4-5 CKD and may not be inhibited completely by a P2Y12 antagonists.[19] Finally, elevated levels of circulating cytokines in CKD patients may alter drug absorption (peak drug levels) or metabolism (trough drug levels) based on severity of CKD.[24] To date, no randomized controlled trial (RCT) has been conducted to compare the antiplatelet effects of ticagrelor and clopidogrel in people with stages 4-5 CKD, and no controlled studies have compared the pharmacologic properties of ticagrelor between people with stages 4-5 CKD or without CKD. Therefore, we performed a mechanistic, double-blind RCT to investigate the efficacy of ticagrelor and clopidogrel in inhibiting platelet aggregation in patients with stages 4-5 CKD. We also investigated the effect of ticagrelor on mean changes in platelet aggregation in patients with stages 4-5 CKD compared to the frequency-matched non-CKD controls. Finally, we explored mechanisms underlying the effects of ticagrelor in patients with stages 4-5 CKD by measuring changes in circulating platelet–leukocyte aggregates, differentiation state of monocytes, cytokine levels, and drug/metabolite levels.

## METHODS AND MEASUREMENTS

### Study design

The Chronic Kidney Disease (CKD-Platelet) study is a prospective double-blind, parallel-design, RCT (clinicaltrials.gov # NCT03649711) comparing the effects of ticagrelor and clopidogrel on platelet activation, aggregation, and inflammation in 48 CKD outpatients who were asymptomatic for cardiovascular diseases (i.e., not having experienced thrombotic events). Institutional Review Board approvals were obtained and participants gave written informed consent prior to participation. CKD subjects were randomized in a double-blind manner to receive ticagrelor 90 mg orally twice daily or clopidogrel 75 mg orally in the

morning and a matching placebo at night for two weeks. All participants also received aspirin 81 mg/day for two weeks. In a parallel open-label, fixed-dose, controlled study, 26 non-CKD controls were recruited and matched with the CKD participants for sex, body mass index within 5 kg/$m^2$ and decade of age. Visits for controls were similar to CKD participants except for randomization and blinding. Non-CKD controls were dispensed aspirin (81 mg/day) and open-label ticagrelor (90 mg twice daily) for 2 weeks.

### Sample size and power calculations

For power calculations, measurements of platelet aggregation in CKD patients at baseline and after treatment with clopidogrel, values from previously published work were used (CKD at baseline: 11.32 Ω, SD=5.14 and CKD after clopidogrel treatment: 6.88Ω, SD= 5.95Ω).[19,25] Because phase I and II studies of ticagrelor in non-CKD patients reported a 75% decrease in ADP-induced platelet aggregation,[26,27] the mean post-ticagrelor treatment platelet aggregation value was calculated to be 2.83 Ω (75% drop from 11.32Ω) for CKD, implying a mean difference of 4.05 Ω (6.88–2.83) in post-treatment platelet aggregation values between ticagrelor and clopidogrel groups. Additional statistics, such as the R-squared value of baseline platelet aggregation accounting for the adjustment for presence of diabetes mellitus ($R^2$=0.25) and the common within-group SD of 5.56, were taken from previously published work.[19,25] On the basis of these assumptions, a total sample of 48 CKD participants (24 per arm) provides 81.40% power to detect a mean difference of 4.05 Ω with the use of an ANCOVA F test at a significance level of 0.05 (NCSS Pass 20).

### Inclusion and Exclusion criteria

Inclusion criteria for the non-CKD controls were GFR ≥60 ml/min/1.73 $m^2$, urine albumin-to-creatinine ratio (UACR) <30 mg/g, and no known kidney disease. Inclusion criteria for CKD patients were GFR <30 ml/min/1.73 $m^2$ for at least 3 months (calculated with the CKD-EPI equation).[28] Exclusion criteria for both groups were lack of health care power of attorney to sign informed consent, unwillingness or inability to participate, pregnancy, acute kidney injury, kidney transplant or any other solid organ transplant recipient, end-stage kidney disease treated with maintenance dialysis (peritoneal or hemodialysis), nephrotic syndrome (defined as nephrotic-range proteinuria, hypoalbuminemia, hyperlipidemia, and generalized edema), recent hospitalization or surgery <3 months, acute coronary or cerebrovascular event in the preceding 12 months, blood dyscrasias, active bleeding, bleeding diathesis, gastrointestinal bleeding in the last 6 months, concomitant use of antiplatelet agents other than aspirin or antithrombotic agents, treatment within 30 days with a glycoprotein IIb/IIIa antagonist, hematocrit <25%, white blood cell count >20,000/μl, platelet count <50,000/μl, or any active malignancy or liver disease.

### Recruitment

We recruited asymptomatic outpatients with CKD from renal clinics at the University of Arkansas for Medical Sciences ([UAMS] protocol # 227997) and the Central Arkansas Veterans Affairs Hospital ([VA] protocol # 1241997), Little Rock, Arkansas, from November 1, 2018, through August 19, 2021. Controls were recruited using the data from the UAMS Translational Research Institute Research Participant Registry. People who signed up to be part of the Arkansas Research volunteer database were contacted via emails.

Those who responded to the emails were pre-screened by telephone using an IRB-approved transcript, and subsequently scheduled for informed consent and study visits.

## Study visits and measurements

**Screening visit:** Patients with CKD were prescreened prior to their routine visits to renal clinics, and invited to participate and sign informed consent at a study screening visit when demographic and clinical variables were collected, along with information on concomitant medications and comorbidities. Routine blood laboratory data were collected to ensure individuals met inclusion/exclusion criteria. In women of childbearing potential, urine pregnancy test was also performed. Those on aspirin for primary prevention of cardiovascular disease, or those taking nonsteroidal anti-inflammatory drugs were asked to discontinue use for 2 weeks prior to baseline visit. During the COVID-19 pandemic, this visit was amended to a tele-visit for controls while CKD patients underwent the screening visit during their regular renal clinic appointments. Blood work and urine tests for this visit were deferred to the baseline visit during the pandemic to minimize contact time with the study participants. Recruitment at the VA site was stopped during the pandemic.

**Baseline visit:** Medication lists were reviewed, and study staff confirmed that participants were not consuming non-steroidal anti-inflammatory drugs. Blood was collected for complete blood count (CBC) and whole-blood platelet aggregation induced with various agonists (0.5 mM arachidonic acid, 2 μg/mL collagen, 20 μM ADP, or 1 mg/mL ristocetin) and measured using a Chrono-log aggregometer (Chrono-log Corporation Model 500, Havertown, PA 19083, USA). Expression of platelet surface receptors (P-selectin, P2Y12, Glycoprotein IIb/IIIa, and Glycoprotein Ib) and platelet–leukocyte aggregates were measured with a BD LSRFortessa flow cytometer (see detailed protocol in Supplementary Table 1). Thromboinflammatory markers including differentiation state of monocytes, and platelet–leukocyte aggregates were quantified with FlowJo software (TreeStar) by counting platelets ($CD42b^+$) adherent to neutrophils ($CD14^-/CD16^+/CD66b^+$) and monocytes ($CD14^+/CD66b$) in whole blood. Levels of multiple cytokines were determined with the Bio-techne Human XL Cytokine Luminex® Performance Assay 45-Plex Fixed Panel (catalog # LKTM014).

**Randomization of CKD participants:** CKD participants were randomized in a double-blind manner at the baseline visit to receive either 2 weeks of ticagrelor (90 mg twice daily) or clopidogrel (75 mg/day in the morning, plus a matching placebo once daily at night). Stratified randomization was used to ensure balance between the 2 treatments in the strata defined by diabetic status and was performed by a research pharmacist. To minimize imbalances in treatment allocation and to maximize power, a computerized random number generator was used to create a blocked randomization list separately for diabetic and non-diabetic strata. Block size and variable for each stratum were determined by the statistician and revealed to the research pharmacist but not to the research personnel. All study pills were placed in larger identical capsules to conceal allocation. The matching placebo was compacted by the research pharmacist to conceal the frequency of dosing for clopidogrel. All CKD patients received concomitant therapy with aspirin (81 mg/day). Non-CKD controls were dispensed dual antiplatelet therapy (DAPT) consisting of aspirin

(81 mg/day) and open-label ticagrelor (90 mg twice daily) for 2 weeks. All participants received phone calls mid-treatment (one week on study drugs) to confirm adherence and record any side effects. We also continued to confirm that the participants were not taking non-steroidal anti-inflammatory drugs or other supplements during the phone call.

**Final visit (2 weeks after randomization):** Physical exam was performed at this visit including vitals, height and weight. Pill count was performed to confirm adherence. Adverse events were noted. All blood tests performed at the baseline visit were repeated for each study participant. In addition, samples were collected for trough levels of ticagrelor and its metabolite, des ticagrelor (AR-C124910XX), 12 hours after the last dose of the study drug.[29] Subsequently, all participants were administered the study drug and blood was re-drawn for peak drug/metabolite levels 3 hours later. During the COVID-19 pandemic, all participants were screened for COVID symptoms before coming to study visits. Once the study completed enrollment, and unblinding occurred, frozen samples were measured for peak and trough blood levels of ticagrelor and its metabolite using multiple reaction monitoring liquid chromatography mass spectrometry (LCMS) in a subgroup of CKD participants randomized to the ticagrelor arm, and all non-CKD controls (see detailed protocol in Supplementary Table 2).[29]

## Outcome measure

The primary outcome measure in CKD subjects randomized to ticagrelor or clopidogrel was post-treatment platelet aggregation value induced by 20 μM ADP (expressed in ohms, Ω) in participants with stages 4-5 CKD administered ticagrelor or clopidogrel. The secondary outcome measure was percent inhibition of ADP-induced platelet aggregation (IPA), defined as [(baseline aggregation value–post-treatment aggregation value) / (baseline aggregation value)].

To compare ticagrelor treatment effect between CKD-ticagrelor and non-CKD control subjects, the secondary outcome variable was the difference in the mean change in ADP-induced platelet aggregation between treatment and baseline. Markers of thromboinflammation, adverse events, and levels of the drug/metabolite and cytokines were also included as secondary outcomes.

## Statistical analysis

Summary statistics were used to describe the distribution of the data. Continuous variables were reported using mean and standard deviation when they were normally distributed, and, using median and interquartile range when they had skewed distributions. Categorical variables were reported using frequency counts and percentages. Post-treatment ADP-induced platelet aggregation value in ohms (Ω) was the primary outcome variable to compare the effect of drugs (ticagrelor vs. clopidogrel) in participants with CKD. An analysis of covariance (ANCOVA) model was used to compare the treatment effects where the primary outcome variable was modeled as the dependent variable, and baseline measurement of platelet aggregation, diabetic status (1: diabetics; 0: non-diabetics), and a binary treatment variable (1: ticagrelor arm; 0: clopidogrel arm) were included as independent variables. In cases of missing data, we performed complete case analysis. The

secondary outcome variables were compared using ANCOVA for comparing differences between CKD in the two randomized arms. Univariate analysis was done using Wilcoxon rank sum test adjusted for multiple comparisons (Bonferonni method) for comparing differences between CKD-ticagrelor arm, and the non-CKD controls. Paired t-test or Wilcoxon signed rank test was used to compare the before and after treatment differences within CKD-ticagrelor, and non-CKD control groups. Analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC) and statistical significance was set at 5%.

Data analysis for the cytokine measurements were also performed using the R language to create better visualization of baseline levels, and also of the global changes occurring on therapy.[30] The measurements from a set of cytokines were analyzed using the Gene Sets Net Correlations Analysis (GSNCA) method as implemented in the Bioconductor package GSAR.[31,32] This nonparametric statistical method tests the hypothesis that the inter-cytokine correlations for the set of cytokines are significantly different before and after therapy, and estimates significance (p-value) using sample permutation approach. Although this method was originally proposed for the analysis of a set of gene expression profiles, it is equally applicable to any set of measurements regardless of their dynamic ranges (i.e., normalization is not required). This method helps us visualize complex changes occurring in the inflammatory pathways. This method assigns each cytokine a weight factor that is proportional to its correlations with the rest of the cytokines. The weight factors are centered around 1, and cytokines that change in similar patterns across subjects show values larger than 1, while cytokines that change in random patterns show values smaller than 1. The weight factors reveal hub cytokines that may directly or indirectly influence other cytokines. Visual network representations of the change in inter-cytokine correlations before and after therapy for specific groups of subjects were generated using package GSAR. The network is formed by the union of the first and second order minimum spanning trees (MSTs). Influential cytokines tend to occupy a central position in the network with dense links to other cytokines, while cytokines with low correlations with other members in the set tend to occupy a peripheral position in the network with sparse links to other cytokines.

## RESULTS

In this mechanistic, double-blind RCT, we investigated the efficacy of ticagrelor and clopidogrel in inhibiting ADP-induced platelet aggregation in CKD patients. Supplementary Figure S1 illustrates how the cohort was derived. None of the participants were on dialysis, and nearly half of the CKD participants had stage 5 CKD, with mean GFR in the ticagrelor arm of 16.7 ml/min/1.73 $m^2$ (SD=6.5) and mean GFR in the clopidogrel arm of 16.4 ml/min/1.73 $m^2$ (SD=5.7). Baseline characteristics were comparable between the CKD participants randomized to each drug arm (Table S3). The overall CKD sample consisted of 62% women and 54% African American individuals. Individuals in the non-CKD control arm were recruited with the goal to frequency-match for decade of age, sex and BMI within 5 $kg/m^2$ with the CKD participants in the ticagrelor arm. Table S3 illustrates that these three characteristics were balanced between the CKD-ticagrelor arm, and the non-CKD controls.

### Aspirin effect

All participants (CKD and controls) responded to aspirin 81 mg/day, as demonstrated by inhibition of arachidonic acid-induced platelet aggregation (Figure 1, **Panel A**).

### P2Y12 inhibition

The primary outcome variable of post-treatment ADP-induced platelet aggregation was lower in CKD subjects randomized to receive ticagrelor treatment [median 0 Ω (IQR 0, 2)] vs. clopidogrel treatment [median 0Ω (IQR 0, 5)] and remained statistically significant after adjusting for the presence of diabetes mellitus and baseline values of ADP-induced platelet aggregation, ANCOVA $P$=0.002 represents significant vertical distance in Figure 1, **Panel B**. Ticagrelor resulted in a greater percent inhibition of ADP-induced platelet aggregation ([IPA]= 87%, SD=22) than did clopidogrel (IPA=63%, SD=50; $P$=0.04). Ticagrelor was particularly superior to clopidogrel in inhibiting ADP-induced platelet aggregation in CKD subgroup with GFR <16 ml/min/1.73m$^2$ (Figure 2 Panels A-B) but not in those with GFR ≥16 ml/min/1.73m$^2$. When comparing the performance of ticagrelor between CKD-ticagrelor and non-CKD groups, there were no differences in the mean change in ADP-induced platelet aggregation between groups on ticagrelor therapy (Figure 1, **Panel C**) or, in their percent inhibition of ADP-induced platelet aggregation, IPA in CKD 87% (SD= 22) vs. IPA in controls 77% (SD= 29), $P$=0.14.

### Other platelet markers

The post-treatment values of platelet aggregation induced by collagen or ristocetin as well as platelet surface expression of GPIb, GPIIb/IIa, P2Y12, and P-selectin were not different between CKD-ticagrelor arm, and the non-CKD controls on ticagrelor-based DAPT (Supplementary Table 4). Similarly, there were no differences in the mean changes in these values on-treatment between CKD-ticagrelor arm, and non-CKD controls on ticagrelor-based DAPT (Supplementary Table 4).

### Thrombo-inflammatory markers

Post-treatment, plasma IL-6 levels were lower in the CKD group randomized to the ticagrelor arm compared to the clopidogrel arm, $P$=0.04 (Figure 3, **Panel A**). Similarly, PD-L1 was also lower in the CKD-ticagrelor arm. Plasma levels of several other cytokine post treatment were not different between the two randomized arms of CKD people (Figure 3, **Panels B-E**). Two weeks of ticagrelor-based DAPT resulted in significant lowering of IL-1α and IL-1β levels in both the CKD-ticagrelor, and the non-CKD control groups, all $P$<0.05 (Figure 4, **Panels A-B**). However, ticagrelor-based DAPT decreased levels of TNFα and TRAIL in the non-CKD control group but not in the CKD-ticagrelor group (Figure 4, **Panels C-D**). Mean change in TRAIL levels after treatment was 13.53 (1, 31.18) pg/mL in the non-CKD control arm vs. 3.08 (−18.03, 21.62) pg/mL in the CKD-ticagrelor arm, $P$=0.09. Finally, there were no differences in levels of IL-6 or IL-1RA before or after ticagrelor treatment in the CKD-ticagrelor or non-CKD control groups (Supplementary Figure S3).

The percent neutrophils and monocytes with attached platelets (Supplementary Figure S2 **Panel A** and Supplementary Table 5) and the monocyte differentiation state (Supplementary Figure S2 **Panel B**) was not different in CKD patients randomized to ticagrelor or clopidogrel. After two weeks of aspirin and ticagrelor, no difference was observed between the CKD-ticagrelor arm and the non-CKD control arm in the percent of neutrophils (CKD= median 32% [IQR 29%, 40%] and controls= median 32% [IQR 25%, 36%]) and monocytes (CKD= median 30% [IQR 24%, 40%] and non-CKD controls= median 33% [IQR 21%, 35%]) with attached platelets (all $P>0.05$; Supplementary Figure S2 **Panel C**, and Supplementary Table 4). Similarly, there were no differences in the differentiation state of monocytes on DAPT between CKD receiving ticagrelor, and non-CKD controls; all $P>0.05$ (Supplementary Figure S2 **Panel D**, and Supplementary Table 4).

The GSNCA test indicated a significant global change in inter-cytokine correlations with therapy in a subgroup of CKD-ticagrelor individuals who reported not to be on any statin therapy (n=10), $P<0.05$, Figure 5. Platelet-derived growth factor (PDGF) played a central role in tightly correlating with other cytokines before therapy in the CKD-ticagrelor arm (considered a hub), and its weight factor reduced after therapy, Figure 5. IL-1β was also tightly correlated various other cytokines, Figure 5.

### Drug levels

There were no differences in the trough or peak levels of ticagrelor or des ticagrelor of CKD-ticagrelor patients and the non-CKD controls (Figure 6, **Panels A and B**).

### Adverse events

In the CKD participants, 1 individual treated with clopidogrel developed an allergic rash that was attributed to clopidogrel, and 1 received kidney transplantation and withdrew from the study three days after randomization. Overall, in the CKD cohort, adverse events were minor with two weeks of DAPT (14 in the ticagrelor arm and 15 in the clopidogrel arm, $P=0.85$) and included bruising, dyspnea, dyspepsia, and fatigue (Supplementary Table 6A) but did not involve discontinuation of the study drug. These episodes resolved spontaneously. There were no differences in adverse events between CKD-ticagrelor and non-CKD control groups during the 2 weeks of DAPT (14 in the CKD arm and 13 in the non-CKD control arm, $P=0.87$), although there were numerically more patients experiencing dyspnea with ticagrelor (Supplementary Table 6B). One serious adverse event (event rate of 2%) occurred in a CKD participant related to hospital admission for symptomatic anemia requiring a blood transfusion.

## DISCUSSION

In the current double-blind, mechanistic RCT, we demonstrated that ticagrelor reduced ADP-induced platelet aggregation more than clopidogrel among asymptomatic patients with stages 4-5 CKD even after adjusting for the presence of diabetes mellitus and baseline values of WBPA. Our findings provide mechanistic evidence for superior efficacy of ticagrelor over clopidogrel in this patient population, as seen in the subgroup analysis of the PLATO trial.[1] In addition, ticagrelor-based DAPT reduced plasma IL-6 and PD-L1

levels much more than clopidogrel-based DAPT, and had other notable anti-inflammatory effects in asymptomatic patients with stages 4-5 CKD, such as a decrease in plasma IL-1α and IL–1β levels. This modulatory effect on inflammation in stages 4 or 5 CKD was heterogeneous and complex as evidenced by a significant global change in the shape of the correlation matrix of multiple inflammatory cytokines in CKD-ticagrelor individuals who were not on any statin therapy, and by an attenuated reduction in TNFα and TRAIL levels in CKD-ticagrelor arm vs. non-CKD controls. The sum of these results provides a potential mechanism how ticagrelor use may be associated with lower rates of lethal sepsis in CKD population as reported in recent observational studies.[2,3] Our findings also suggest that ticagrelor-based DAPT might lower inflammatory burden observed in asymptomatic stages 4 or 5 CKD patients which has been recently linked to recurrent cardiovascular events in the CANTOS trial.

Our data demonstrate that ticagrelor is better than clopidogrel in achieving inhibition of platelet aggregation in patients with stages 4-5 CKD. Approximately two-thirds of ticagrelor is active after absorption.[33] The remainder is metabolized by CYP3A4/5 to an active metabolite, des-ticagrelor (AR-C124910XX).[33] Ticagrelor and des-ticagrelor reversibly block the binding of ADP to the P2Y12 receptor. On the other hand, clopidogrel is a pro-drug—85% is hydrolyzed to an inactive metabolite in the blood after absorption, and the remaining 15% is metabolized to 2-oxo-clopidogrel and subsequently to the active metabolite (R-130964), primarily by CYP2C19, before irreversibly blocking the binding of ADP to the platelet P2Y12 receptor.[34] In the non-CKD population, this pharmacokinetic difference translates into greater antiplatelet effects for ticagrelor than clopidogrel, with reduced inter- and intra-individual variabilities.[35–37] Patients with stages 4-5 CKD, a population with the most severe form of kidney disease not on dialysis, have a 4-fold higher risk of thrombotic cardiovascular events despite treatment with clopidogrel compared to the general population.[38] In the subgroup of PLATO trial participants with GFR <30 ml/min/1.73 $m^2$, use of ticagrelor over clopidogrel was associated with a 23% reduction in mortality and ischemic events.[1] This double-blind randomized mechanistic RCT addresses limitations of previous studies[10–17] by enrolling stages 4-5 CKD individuals with mean GFR of 16 ml/min/1.73$m^2$ not on dialysis, including a control arm, minimizing drop-out rates, double-blinding and randomization of treatment allocation. Furthermore, the CKD patient population was diverse, including over 50% women and African Americans, attesting to effects of ticagrelor across sex and race.

In addition to better platelet inhibition, ticagrelor vs clopidogrel also generates better anti-inflammatory response in asymptomatic patients with stages 4-5 CKD. However, this response is complex, as indicated by decreases in pro-inflammatory plasma IL-1α and IL-1β in all CKD individuals, and a significant global change in the shape of correlation matrix of multiple cytokines in CKD individuals who were not on any statin therapy. Circulating platelets regulate inflammation as well as thrombosis.[39,40] Studies performed in the last decade highlighted the role of these anucleated cells in modulating inflammation through their interaction with inflammatory cells in the circulation.[41] Studies in animals demonstrated that platelet-depleting antibodies decreased the levels of pro-inflammatory cytokines in the plasma, with cytokine levels being restored by platelet transfusion.[42] *Ex vivo* studies of human blood samples from healthy volunteers demonstrated that platelet

inhibitors decreased plasma levels of cytokines.[43] CKD is a proinflammatory state marked by increased levels of inflammatory markers in the plasma (e.g., IL-1α, TNF-α, and IL-6) that worsens with worsening CKD severity.[24] Use of the more potent platelet inhibitors prasugrel and ticagrelor (vs. clopidogrel) has been associated with reductions in the rate of death from sepsis in recent observational studies of CKD patients.[3] In this clinical trial, we observed that ticagrelor-based DAPT decreased levels of pro-inflammatory cytokines in the plasma of individuals with stages 4-5 CKD not on dialysis regardless of statin use, and a global change in inter-correlations of cytokines among subgroup of individuals not on statin co-therapy. Statin therapy reduces inflammation and may be able to confound some of the inflammatory responses of DAPT in CKD individuals.[44] In summary, ticagrelor-based DAPT might be able to reduce inflammatory burden in select, if not all, asymptomatic stages 4 or 5 CKD individuals- a problem that remains complex in nature with no readily available clinical intervention for it.

In addition to the global changes in the correlation matrix of cytokines on ticagrelor-based DAPT in some CKD individuals, this treatment generated much less effect on plasma levels of TNFα and TRAIL in the CKD-ticagrelor group than in the non-CKD controls, and no effects on various other thromboinflammatory markers. The reason for the differential and heterogeneous effect is not known. We did note a correlation between plasma TNFα levels and mean platelet volume ($r$=0.30; $P$=0.01), which may indicate that younger, larger platelets have a ticagrelor-independent influence on TNFα. Shorter platelet half-life in circulation, platelet sequestration in the microvasculature, or inflammation-induced megakaryopoiesis could influence platelet production in patients with CKD. In mouse models of sepsis, platelets are sequestered in the microcirculation of the lungs and liver following interactions with circulating leukocytes—a phenomenon that culminates in microvascular occlusion and tissue damage, as well as a decrease in the mean platelet count and an increase in the mean platelet volume in the circulation.[45] A similar platelet sequestering could be at play in patients with CKD and result in high platelet turnover.[46,47] It is also possible that the inflammatory state of CKD is driving production of platelets by megakaryocytes. While an association between baseline plasma levels of proinflammatory cytokines with worse cardiorenal outcomes was previously reported,[24,48] the clinical relevance of phenotyping patients with CKD based on the degree of decrease in levels of proinflammatory cytokines with antiplatelet therapeutics needs to be further investigated.

Our study has several limitations. First, although adequately powered to address the primary outcome, the sample size was not powered for secondary outcomes. The sample size was comparable to that of similar published[43] and ongoing[49] mechanistic studies in this patient population that tends to present recruitment challenges and historically has been excluded from larger RCT. Second, use of concomitant medications known to affect chronic inflammation, such as statins, could have confounded the effects noted in the study.[44] Third, the specific P2Y12 inhibitors used could have exerted differential anti-inflammatory effects as reported in experimental studies of healthy human volunteers.[43] Such intraclass differences between the P2Y12 inhibitors in CKD patients remain unclear. Fourth, aspirin alone could have contributed to the various characteristics of thromboinflammatory markers observed,[50] as the effects of aspirin in CKD patients remain poorly defined. Fifth,

inflammatory dysregulation in stages 4 or 5 CKD is complex with large inter-individual variabilities.[24] Larger studies are required to adequately address this limitation. Finally, ticagrelor and clopidogrel are approved for use in the setting of acute myocardial infarction. Clopidogrel is also approved for use in chronic cardiovascular diseases. Our study participants were asymptomatic for cardiovascular diseases and would be considered off-label use of the drugs. Despite this limitation, our results are valid as CKD is commonly considered a coronary artery disease (CAD) equivalent regardless of the presence of CAD symptoms.[51]

In summary, this study demonstrates significant differences in platelet inhibition and inflammatory properties between ticagrelor and clopidogrel in asymptomatic non-dialysis people with stage 4-5 CKD, as well as additional inflammatory responses in CKD patients receiving ticagrelor-based DAPT. Our findings provide evidence for superior efficacy of ticagrelor over clopidogrel in non-dialysis people with stage 4-5 CKD, and also suggest DAPT with ticagrelor and aspirin might lower inflammatory burden observed in asymptomatic stages 4 or 5 CKD patients. Larger mechanistic clinical trials should investigate whether inflammatory responses of ticagrelor-based DAPT improve clinical outcomes in asymptomatic people with stages 4 or 5 CKD, and help us individualize treatment strategies for this understudied patient population in order to maximize therapeutic benefits.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (NJ) upon reasonable request.

## ABBREVIATION LIST

| | |
|---|---|
| **ANCOVA** | analysis of covariance |
| **CAD** | coronary artery disease |
| **CKD** | chronic kidney disease |
| **DAPT** | dual antiplatelet therapy |
| **GFR** | glomerular filtration rate |
| **GSNCA** | gene sets net correlations analysis |
| **LCMS** | liquid chromatography mass spectrometry |
| **PLATO** | Platelet Inhibition and Patient Outcomes |
| **RCT** | randomized controlled trial |
| **SD** | standard deviation |
| **UACR** | urine albumin-to-creatinine ratio |

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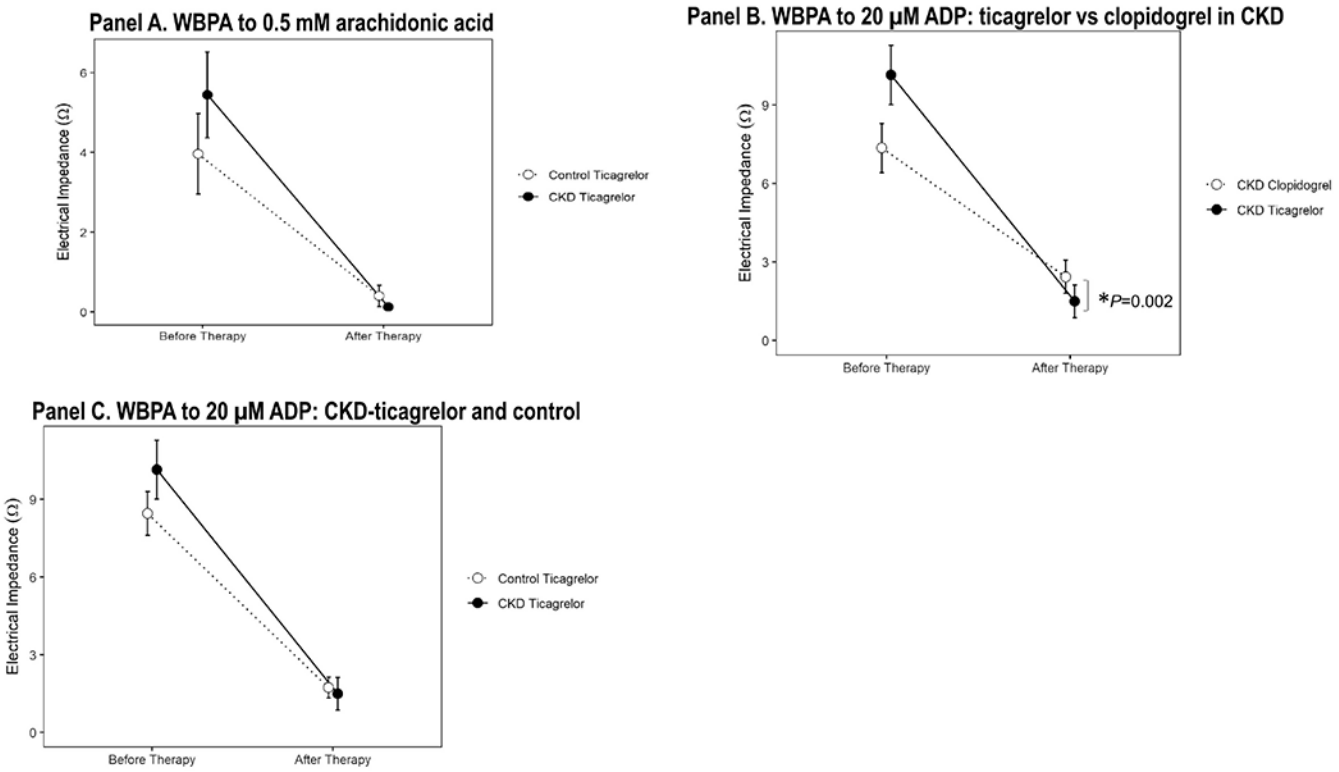


**Figure 1.**

**A.** Whole-blood platelet aggregation (WBPA) induced with 0.5 mM arachidonic acid in samples from patients with stages 4-5 CKD (closed circle) and non-CKD controls (open circle) at baseline and after 2 weeks of treatment with aspirin 81 mg/d and ticagrelor 90 mg twice daily. **B.** WBPA induced with 20 µM ADP in samples from participants as described in *ticagrelor vs. clopidogrel, ANCOVA *P*=0.002 represents significant vertical distance after adjusting for diabetes and baseline ADP values. **C.** WBPA induced with 20 µM ADP in samples from patients with stages 4-5 CKD randomized to the ticagrelor arm (closed circle) and non-CKD controls (open circle) at baseline and after 2 weeks of treatment with aspirin 81 mg/d and ticagrelor 90 mg twice daily. Data are presented as the mean ± standard deviation.

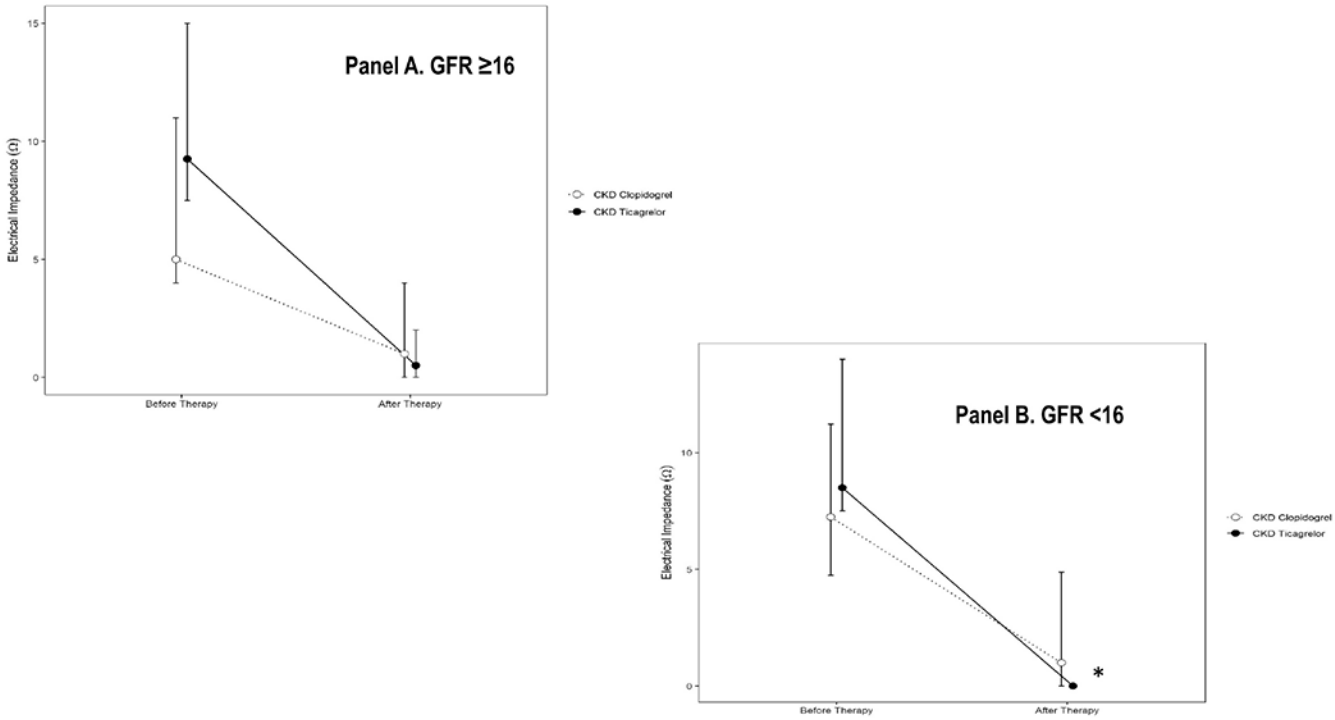


**Figure 2.**

Whole-blood platelet aggregation (WBPA) induced with 20 μM ADP in subgroup of CKD participants with glomerular filtration rate (GFR) **A.** ≥16 ml/min/1.73m$^2$ and **B.** *<16 ml/min/1.73m$^2$ randomized to receive ticagrelor (closed circle) vs. clopidogrel (open circle). Data are presented as the mean ± standard deviation.

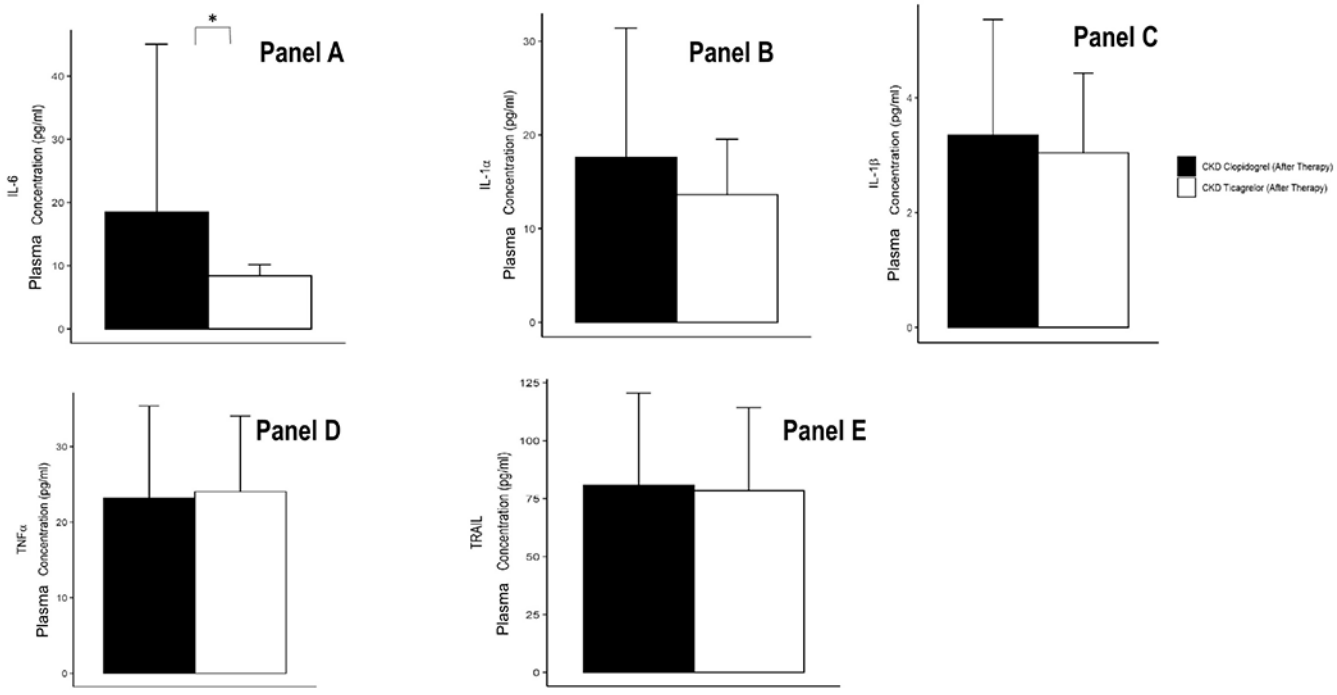


**Figure 3.**

Levels of **A.** IL-6, **B.** IL-1α, **C.** IL-1β, **D.** TNF-α, and **E.** TRAIL in plasma from patients with stages 4-5 CKD randomized to the ticagrelor arm, and clopidogrel arm after therapy with aspirin 81 mg/d and ticagrelor 90 mg twice daily. Cytokine concentrations measured with Luminex® assays and expressed in pg/ml. Data are presented as the mean ± standard deviation. *P* value calculated with a non-parametric test after adjusting for multiple comparisons with the Bonferroni method (**P*<0.05).

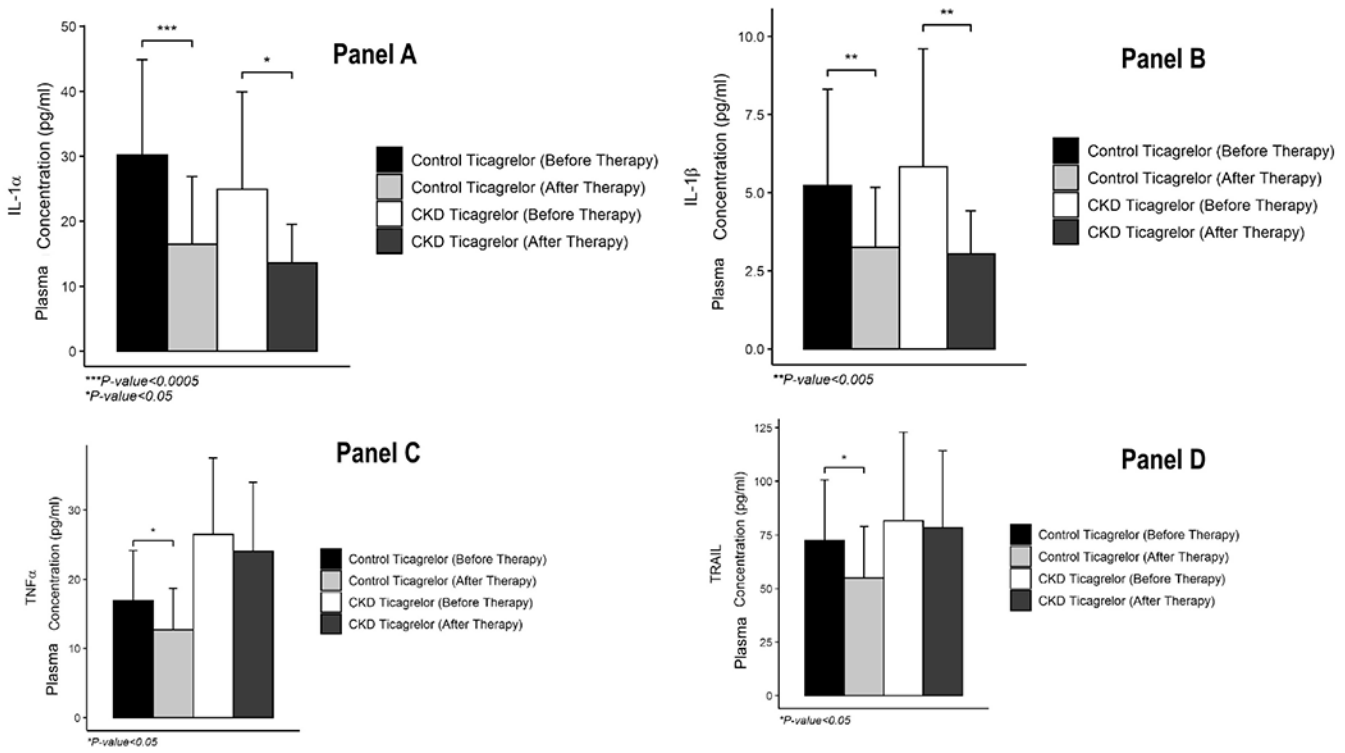


**Figure 4.**

Levels of **A.** IL-1α, **B.** IL-1β, **C.** TNF-α, and **D.** TRAIL in plasma from patients with stages 4-5 CKD randomized to the ticagrelor arm, and non-CKD controls (normal kidney function without proteinuria) before and after therapy with aspirin 81 mg/d and ticagrelor 90 mg twice daily. Cytokine concentrations measured with Luminex® assays and expressed in pg/ml. Data are presented as the mean ± standard deviation. *P* value calculated with a paired t-test or paired rank sum test. There were no between-group differences in the 4 analytes before or after therapy after adjusting for multiple comparisons with the Bonferroni method (all $P>0.05$).

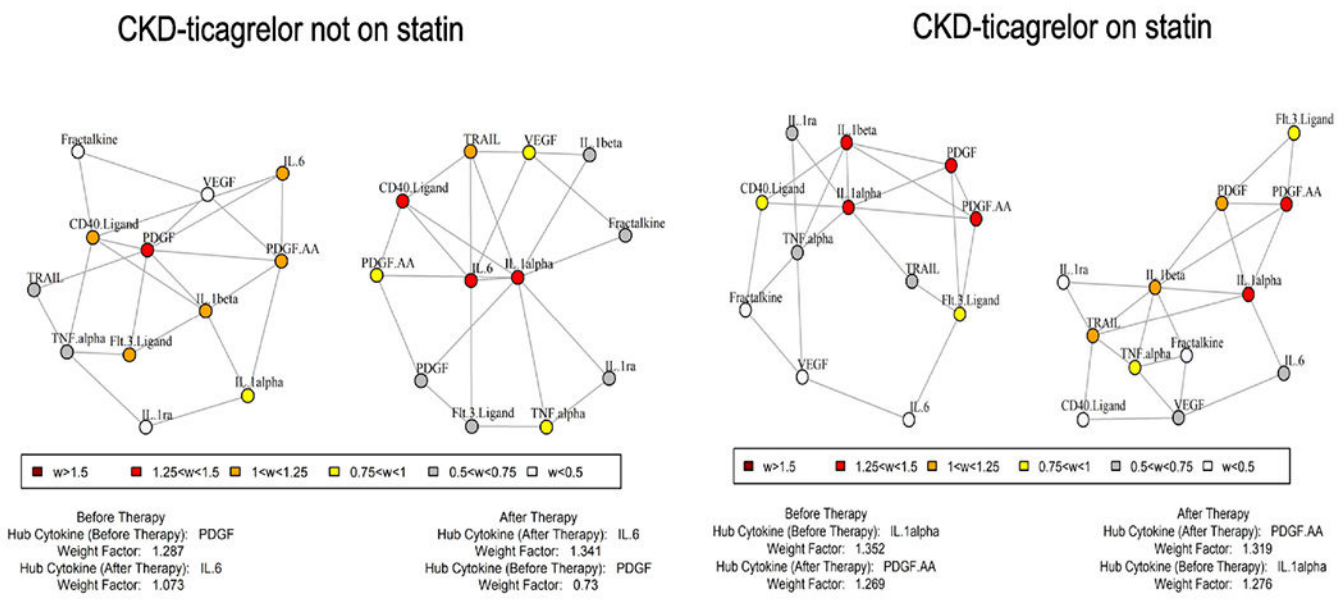


**Figure 5.**

Gene Sets Net Correlations Analysis (GSNCA) representing CKD-ticagrelor arm not on statin co-therapy and, on statin co-therapy. There was a global change in correlation matrix of CKD-ticagrelor arm not on statin ($P<0.05$). PDGF and IL-1β were tightly correlated with several other cytokines.

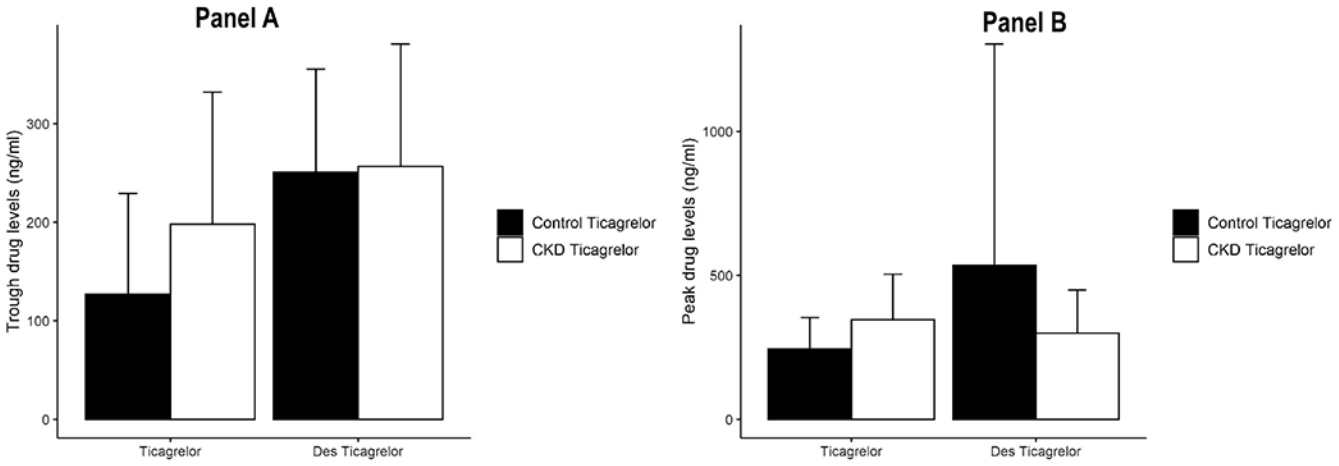


**Figure 6.**

**A.** Trough (measured at the last visit, 12 h after the final evening dose of ticagrelor) and **B.** peak (measured 3 h after administration of ticagrelor at the last visit) plasma levels of ticagrelor or des-ticagrelor (AR-C124910XX) in CKD-ticagrelor arm, and non-CKD controls before and after therapy with aspirin 81 mg/d and ticagrelor 90 mg twice daily. Ticagrelor and des-ticagrelor concentrations measured with mass spectrometry and expressed in ng/ml. Data are presented as the mean ± standard deviation. Within-group differences in the before- and after-therapy levels were compared with a paired t-test; all $P>0.05$. There were no between-group differences in the peak and trough levels of ticagrelor or des-ticagrelor after adjusting for multiple comparisons with the Bonferroni method (all $P>0.05$).