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CLINICAL RESEARCH

Ticagrelor Reduces Thromboinflammatory Markers in Patients With Pneumonia

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CME/MOC Objective for This Article: Upon completion of this activity, the learner should be able to: 1) identify the potential role for treatment with the P2Y12 inhibitor ticagrelor in settings outside of acute coronary syndrome; 2) examine the effect that ticagrelor has on systemic biomarkers of inflammation; and 3) discuss the mechanism of platelet inhibition with ticagrelor and the role of treatment in acute coronary syndrome

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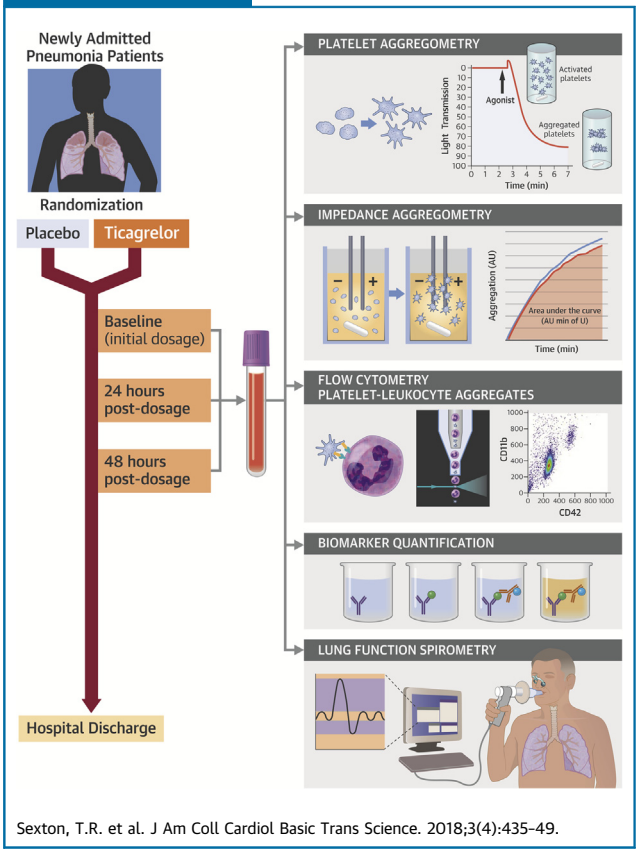
VISUAL ABSTRACT



Sexton, T.R. et al. J Am Coll Cardiol Basic Trans Science. 2018;3(4):435–49.

HIGHLIGHTS

- **As expected, ticagrelor reduced ex-vivo ADP-induced aggregation in patients with pneumonia compared with placebo.**
- **Ticagrelor reduced platelet-leukocyte interactions as well as plasma interleukin-6 within 24 h in patients with pneumonia compared with placebo.**
- **Ticagrelor acutely altered NETosis biomarkers, whereas placebo had no effect.**
- **Ticagrelor improved lung function and reduced need for supplemental oxygen in patients with pneumonia compared with placebo.**

All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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SUMMARY

Despite treatment advances for sepsis and pneumonia, significant improvements in outcome have not been realized. Antiplatelet therapy may improve outcome in pneumonia and sepsis. In this study, the authors show that ticagrelor reduced leukocytes with attached platelets as well as the inflammatory biomarker interleukin (IL)-6. Pneumonia patients receiving ticagrelor required less supplemental oxygen and lung function tests trended toward improvement. Disruption of the $P2Y_{12}$ receptor in a murine model protected against inflammatory response, lung permeability, and mortality. Results indicate a mechanistic link between platelets, leukocytes, and lung injury in settings of pneumonia and sepsis, and suggest possible therapeutic approaches to reduce complications.(Targeting Platelet-Leukocyte Aggregates in Pneumonia With Ticagrelor [XANTHIPPE]; NCT01883869) (J Am Coll Cardiol Basic Trans Science 2018;3:435–49) Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABBREVIATIONS AND ACRONYMS

ADP = adenosine diphosphate

CAP = community-acquired pneumonia

CI = confidence interval

COPD = chronic obstructive pulmonary disease

dsDNA = doubled-stranded DNA

HAP = hospital-acquired pneumonia

ELISA = enzyme-linked immunosorbent assay

FEV-1 = forced expiratory volume in 1 s

IL = interleukin

IQR = interquartile range

K_{fc} = capillary filtration coefficient

LPS = lipopolysaccharide

LTA = light transmission aggregometry

MPO = myeloperoxidase

MVV = maximum ventilation velocity

NE = neutrophil elastase

NET = neutrophil extracellular trap

OR = odds ratio

PRP = platelet-rich plasma

TNF = tumor necrosis factor

TRAP = thrombin receptor activating peptide

WT = wild-type

Sepsis is a complex syndrome of dysregulated host responses to infection that results in organ damage and carries a substantial risk of mortality. Pneumonia is a leading cause of sepsis and is the most common fatal infection acquired in hospitals. Despite advances in treatments, such as antibiotic therapy and intensive care, significant improvement in mortality rates remains elusive (1). Additionally, there is increasing awareness of the burden of cardiovascular complications in patients hospitalized with pneumonia (2–9). Novel treatment strategies in this high-risk patient population are needed.

In addition to an integral role in hemostasis, platelets contribute to inflammatory and immune responses. Platelets alter properties of endothelial cells and leukocytes, release soluble pro- and anti-inflammatory mediators, internalize microorganisms, and bind and sequester pathogens. Platelet activation and sequestration in pulmonary tissue is a key feature in inflammatory or infectious states such as sepsis and acute respiratory distress syndrome (10–12). Recent evidence suggests that antiplatelet therapy may improve outcomes in patients hospitalized with pneumonia. A retrospective, cohort study to evaluate the effects of antiplatelet therapy on the incidence and severity of community-acquired pneumonia (CAP) reported trends toward reduced instances of mechanical ventilation, mortality, and the composite endpoint of death in CAP inpatients (13). Similarly, an observational study of 224 hospitalized CAP patients showed lower use of intensive care unit care in patients receiving antiplatelet agents for at least 6 months compared with unmatched controls (14). A study of 615 patients consecutively admitted to medical and surgical intensive care units found that use of antiplatelet agents was associated with reductions in mortality and attenuation in thrombocytopenia (15). More recently, a post hoc analysis on the PLATO (Platelet Inhibition and Patient Outcomes) trial, in which 18,624 patients presenting with acute coronary syndrome were randomized to receive standard of care with the adenosine diphosphate receptor $P2Y_{12}$ antagonist clopidogrel or ticagrelor, which provides stronger and more consistent $P2Y_{12}$ antagonism than clopidogrel, revealed that ticagrelor was associated with lower mortality in patients with subsequent pulmonary events or sepsis (16). In addition, ticagrelor was able to decrease the absolute risk of ischemic events compared with clopidogrel in patients enrolled in the PLATO trial that had a history of chronic obstructive pulmonary disease (COPD) (17).

Taken together, these observations suggest that antiplatelet therapy may improve outcomes in ambulatory patients with pneumonia, and potentially in sepsis. The mechanism for a beneficial effect could be multifactorial. Platelet activation is associated with worse cardiovascular outcomes in patients with pneumonia (2). Platelet activation results in adhesion, the expression of cell surface receptors, and the release of small molecules that can promote thrombosis and amplify the immune response. Specifically, platelet activation results in expression of P-selectin, increases platelet-leukocyte aggregates, and triggers the formation of neutrophil extracellular DNA nets. Platelet-leukocyte aggregates predict mortality in severe sepsis (18), and DNA nets associate with severity of sepsis and septic organ dysfunction (19). In animals, platelets and neutrophils accumulate in lungs in models of sepsis, during both bacterial and viral infection (20). Furthermore,

TABLE 1 Inclusion and Exclusion Criteria

Inclusion criteria
Men or nonpregnant women 18 yrs of age or older
Subjects must be willing and able to give informed consent
Diagnosis of CAP or HAP within 48 h of diagnosis or presentation to hospital
Subjects must have new radiographic finding(s) consistent with pneumonia and at least 2 of the following signs: • Cough • Fever • Hypothermia • Purulent sputum production or respiratory secretion • Total WBC count >10,000/mm^3, or >15% band forms, regardless of total peripheral white count; or leukopenia with total WBC <4,500/mm^3 • Auscultatory findings on pulmonary examination of rales and/or evidence of pulmonary consolidation (dullness on percussion, bronchial breath sounds, or egophony) • Hypoxemia • Increase in dyspnea and/or tachypnea (>20 breaths/min)
Exclusion criteria
Contraindication to ticagrelor (hypersensitivity of reaction to ticagrelor or another $P2Y_{12}$ antagonist)
Active or suspected major bleeding history
Platelet count <100,000/mm^3 or International normalized ratio >1.5
Surgery within 30 days or anticipated major surgery
Oral anticoagulant that cannot be stopped
Inability or unwillingness of treating physician to reduce dose of aspirin to 100 mg
Fibrinolytic therapy in the last 24 h
Increased risk of bradycardic events—2nd or 3rd degree heart block, bradycardia induced syncope—unless pacemaker in place
Underlying immunodeficiency (HIV, neutropenia, receiving immunomodulating reagents, active hematologic malignancy, functional or anatomic asplenia, and hypogammaglobunemia)
Concomitant therapy with CYP3A inducer; rifampin/rifampicin, phenytoin, carbamazepine
Pregnancy or lactation
Active treatment for cancer
Acute, decompensated congestive heart failure
Participation in another investigational drug or device study in the last 30 days
Inability to administer enteric medication

CAP = community-acquired pneumonia; HAP = hospital-acquired pneumonia; WBC = white blood count.

TABLE 2 Baseline Characteristics of XANTHIPPE Subjects

	Placebo	Ticagrelor	p Value
Demographics			
Men	14 (47)	13 (43)	1.000
Women	16 (53)	17 (57)	1.000
Age, yrs	55.1 ± 16.0	56.6 ± 15.9	0.705
Caucasian	28 (93)	29 (97)	1.000
African American	2 (7)	1 (3)	1.000
Medical history			
Hypertension	15 (50)	23 (77)	0.060
History of smoking	21 (70)	23 (77)	0.771
Diabetes	9 (30)	8 (27)	1.000
COPD	17 (57)	16 (53)	1.000
Medication on admission			
Aspirin	7 (23)	8 (27)	1.000
$P2Y_{12}$ inhibitor	4 (13)	7 (23)	0.506
Antibiotics on enrollment			
Levofloxacin	8 (27)	14 (47)	0.180
Azithromycin	7 (23)	2 (7)	0.146
Ceftriaxone	5 (17)	5 (17)	1.000
Vancomycin	8 (27)	10 (33)	0.779
Levaquin	6 (20)	4 (13)	0.731
Cefepime	3 (10)	4 (13)	1.000
Penicillin	1 (3)	0 (0)	1.000
Zosyn	3 (10)	1 (3)	0.612
Doxycycline	1 (3)	2 (7)	1.000
Peracillin/tazobactam	2 (7)	1 (3)	1.000
Clindamycin	1 (3)	0 (0)	1.000
Metronidazole	1 (3)	3 (10)	0.612
Acyclovir	0 (0)	1 (3)	1.000
Aztreonam	0 (0)	1 (3)	1.000

Values are n (%) or mean ± SD. p Values for the qualitative variables were calculated using Fisher's exact test. p Values for quantitative variables were calculated with a 2-sample Student's *t*-test.
COPD = chronic obstructive pulmonary disease.

antiplatelet therapy decreases platelet-leukocyte aggregates and reduces lung injury.

To explore the possibility that platelets underpin adverse events in pneumonia and sepsis and that potent antiplatelet therapy may attenuate the end-organ or cellular and tissue complications of a dysregulated immune response, we evaluated the effect of the direct-acting $P2Y_{12}$ antagonist ticagrelor on biomarkers of cardiovascular outcomes in a double-blinded, placebo-controlled randomized trial in patients diagnosed with pneumonia. We then performed mechanistic studies of ticagrelor on endotoxemia-associated mortality in mice. Our findings support a role for platelets as a link between adverse outcomes in pneumonia and sepsis, and provide mechanistic insight into potential beneficial actions of potent antiplatelet therapy in pneumonia.

METHODS AND MATERIALS

PATIENTS AND STUDY DESIGN. XANTHIPPE (Examining the effect of Ticagrelor on Platelet Activation, Platelet-Leukocyte Aggregates, and Acute Lung Injury in Pneumonia is an investigator-designed and initiated trial) (NCT01883869) was approved by the institutional review board at the University of Kentucky. All subjects provided written informed consent before participation. The XANTHIPPE trial enrolled patients admitted to the University of Kentucky hospitals with a diagnosis of pneumonia between March 26, 2014, and June 10, 2016. Patients were enrolled within 48 h of diagnosis of either CAP or hospital-acquired pneumonia (HAP), which was defined as pneumonia occurring in a patient with any hospital admission in the previous 3 months. Complete inclusion and exclusion criteria can be found in Table 1. Demographics of all enrolled patients are presented in Table 2. Patients that were taking a $P2Y_{12}$

antagonist upon enrollment were not included on the analysis of flow cytometry, platelet function, biomarker analysis, and spirometry.

The XANTHIPPE trial was a double-blind study in which subjects were randomized to placebo or ticagrelor (180-mg loading dose followed by 90 mg twice a day) for up to 7 days or until discharge from the hospital. Randomization and drug distribution were performed by the Investigational Drug Service at the University of Kentucky. Blood samples were collected at baseline just before administration of the loading dose of study medication and at approximately 24 and 48 h following the initial study dose. An additional blood sample was taken on day of discharge or day 7, whichever came first. Study follow-up occurred daily while admitted to the hospital and at 30 days after enrollment.

The primary efficacy endpoint of XANTHIPPE was the change in platelet-leukocyte aggregates (defined as the percentage of leukocytes with attached platelets) from baseline to 24 h following the initial dose of study medication. Secondary endpoints included change in platelet function, change in biomarkers associated with inflammation and thrombosis, and lung function as determined by bedside spirometry. Rates of serious adverse events and/or rehospitalization within 30 days of enrollment were determined by patient follow-up, by clinic or phone call, as well as by review of electronic medical records.

FLOW CYTOMETRY. Platelet-leukocyte aggregates were determined by flow cytometry as previously described (21). Briefly, fresh whole blood collected in hirudin blood tubes (Diapharma, West Chester, Ohio) and incubated with the leukocyte-specific allophycocyanin (APC)-conjugated anti-CD11b/Mac-1 (BD Biosciences, San Jose, California) and platelet-specific fluorescein isothiocyanate (FITC)-conjugated anti-CD42b (BD Biosciences) for 15 min at room temperature while protected from light. Samples were fixed and red blood cells were lysed using FACS Lysing Solution (BD Biosciences). Flow cytometry was performed at the core facility at the University of Kentucky. Leukocytes (50,000/gate) were identified by the CD11b-positive signal compared with the isotype control. Platelet-leukocyte aggregates were identified by the subset of CD11b-positive cells that were also CD42b positive as compared with the isotype control. FlowJo software version 10 (TreeStar, Ashland, Oregon) was used to analyze samples. Patients that were taking a $P2Y_{12}$ antagonist upon enrollment were excluded from analysis.

PLATELET AGGREGATION AND PLASMA BIOMARKERS. Ex vivo platelet aggregation using platelet-rich plasma (PRP) and whole blood was performed as previously described (22). Aggregation with PRP prepared from citrated blood was determined using a light transmission aggregometer (Chrono-log Corp., Havertown, Pennsylvania). PRP was incubated at 37°C for 2 min before treatment with thrombin receptor activating peptide (TRAP) or adenosine diphosphate (ADP) to final concentrations of 15 μmol/l and 5 μmol/l, respectively. Maximal aggregation and slope within the first 5 min were calculated using the AGGROLINK software (Chrono-log Corp.). For multiplate impedance analysis, blood was collected in hirudin tubes and incubated at 37°C for 3 min before the addition of agonist. The area under the curve after 6 min of agonist was recorded with the Multiplate system (Roche, Basel, Switzerland). Patients that were taking a $P2Y_{12}$ antagonist upon enrollment were excluded from platelet aggregation analysis.

Plasma for biomarker analysis was collected from citrate-theophylline-adenosine-dipyridamole (CTAD)-anticoagulated blood that was supplemented with ethylenediaminetetraacetic acid (EDTA) (final concentration of 10 mmol/l) before centrifugation at 3,000 g for 10 min. Plasma was aliquoted, flash frozen, and stored at −80°C. Tumor necrosis factor (TNF)-α, IL-6, IL-1β, IL-10, and sCD40L analytes were measured with a MAGPIX multiplex reader using a Milliplex MAP Kit (Millipore, Burlington, Massachusetts). Angiopoietin-1, angiopoietin-2, ELA-2, ENA-78, NAP-2, and platelet factor (PF)-4 analytes were measured using DuoSet enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minnesota). Patients taking a $P2Y_{12}$ antagonist upon enrollment were excluded from biomarker analysis.

Neutrophil extracellular traps (NET) were measured by ELISA using anti-myeloperoxidase (MPO) (part 842842 from R&D Systems) for capture and biotinylated anti-neutrophil elastase (NE) (Ab79962 from

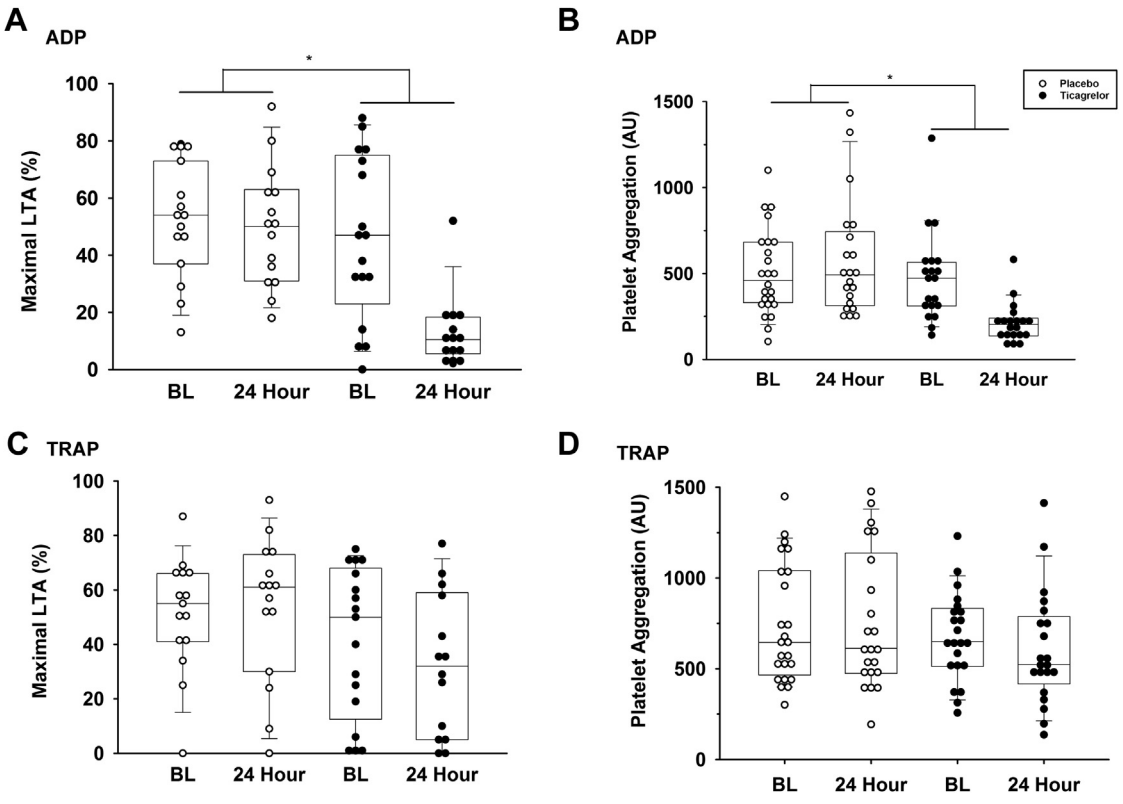
TABLE 3 Clinical Characteristics of XANTHIPPE Patients

	Placebo	Ticagrelor	p Value
Pneumonia			
CAP	21 (70)	17 (57)	0.422
HAP	9 (30)	13 (43)	0.422
Blood counts			
Baseline platelet count	248 ± 74	283 ± 85	0.094
24-h platelet count	263 ± 94	291 ± 97	0.324
Baseline WBC	12.04 ± 5.18	12.13 ± 5.83	0.951
24-h WBC	10.72 ± 5.10	12.91 ± 6.02	0.183

Values are n (%) or mean ± SD. p Values for the qualitative variables were calculated using Fisher's exact test. p Values for quantitative variables were calculated with a 2-sample Student's *t*-test.

Abbreviations as in Table 1.

FIGURE 1 Ticagrelor Reduces Ex Vivo ADP-Induced Platelet Aggregation in Pneumonia Patients



Maximum platelet aggregation was measured by light transmission aggregometry in PRP in response to 5 μmol/l ADP **(A)** and 15 μmol/l TRAP **(C)**. Area under the curve values in the Multiplate assay are shown with ADP **(B)** or TRAP **(D)** as an agonist. Patients randomized to placebo are represented by open circles and patients randomized to ticagrelor are represented by **filled circles**. A significant difference between groups on the change from baseline to 24 h is indicated by an **asterisk**. ADP = adenosine diphosphate; AU = arbitrary units; BL = baseline; LTA = light transmission aggregometry; TRAP = thrombin receptor activating peptide

Abcam, Cambridge, Massachusetts) for detection. MPO and NE are in complex with chromatin in NETs. Samples were calibrated to a serial dilution of serum and assigned arbitrary units based on detected signal. Quant-iT Picogreen kits (Thermo Fisher Scientific, Waltham, Massachusetts) were used to measure doubled-stranded DNA (dsDNA) in plasma.

SPIROMETRY. Spirometry was performed following American Thoracic Society/European Respiratory Society guidelines (23) at the patient bedside using a CareFusion MicroLoop spirometer (Vyaire Medical, Mettawa, Illinois). Relaxed spirometry, forced spirometry, and maximum ventilation velocity (MVV) were performed at baseline (before drug randomization), 24 h after dosage, and day of discharge on subjects that were able and willing to perform.

MOUSE STUDY. Mice deficient in $P2Y_{12}$ were generated as described previously (24). Littermate wild-type (WT) mice from heterozygous breeding were used as controls. The mice were bred and maintained in the University of Kentucky Animal Care Facility following institutional and National Institutes of Health guidelines after approval by the Animal Care Committee.

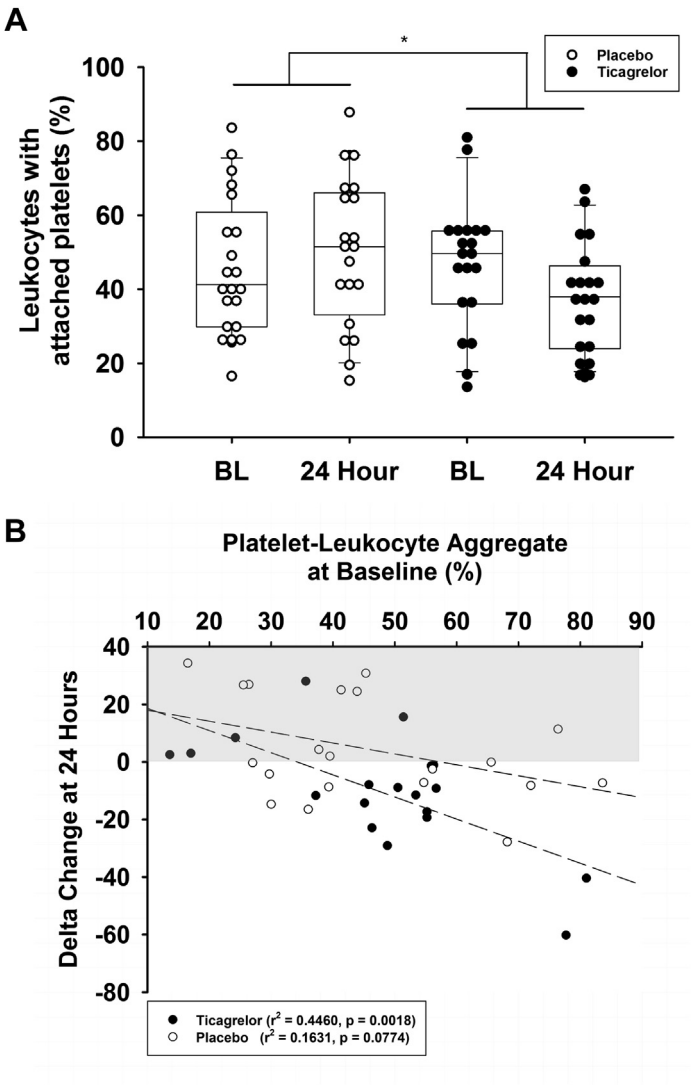
WT and *P2ry12*$^{-/-}$ male mice (8 to 10 weeks of age) were fed placebo, clopidogrel (25 mg/kg body weight per 24 h), or ticagrelor (10, 25, 50 mg/kg body weight, per 12 h) by oral gavage. Two h later, the animals were injected intraperitoneally with lipopolysaccharide (LPS) (0111:B4, 10 mg/kg in 200 μl, Sigma Aldrich, St. Louis, Missouri). Mice were assessed every 12 h for 5 days. Blood was collected at the indicated time points after LPS injection, and plasma was prepared by

centrifugation. TNF-α and IL-6 were measured by ELISA assay (eBioscience, San Diego, California).

DETERMINATION OF PULMONARY MICROVASCULAR PERMEABILITY. Lungs were isolated 6 h after injection of LPS and microvessel capillary filtration coefficient (K_{fc}) was measured as described previously (23). In brief, after 20-min equilibration perfusion to establish an isogravimetric condition, the outflow pressure was rapidly elevated by 10 cm of H_2O for 20 min. In response to the pressure increase, lung preparations gained weight due to net fluid accumulation. Lungs were dissected free of nonpulmonary tissue, and lung dry weight was determined. K_{fc} was calculated from the slope of the weight change normalized to the pressure change and lung dry weight.

STATISTICAL ANALYSES. Baseline and clinical characteristics in Tables 2 and 3 were compared between the 2 groups using Student's *t*-test and Fisher's exact test. For the repeated-measures data in the platelet aggregation, platelet-leukocyte aggregate assays, and for non-IL biomarkers (sCD40L, TNF-α, ENA-78, NAP-2, and PF-4), we fitted a linear mixed-effects model to compare groups at day 1, at day 2, and on change from day 1 to day 2. Logarithmic transformation was applied to some non-normally distributed data. For the IL-6, IL-10, and IL-1β biomarkers, we compared groups at day 1, at day 2, and on change from day 1 to day 2 using Mann-Whitney rank sum tests. Linear regression was used to determine the relationship between baseline platelet-leukocyte aggregates and change at 24 h. For comparison of baseline biomarkers of treatment groups to healthy controls, a Mann-Whitney rank sum test was used. The MPO-NE assay measurements were non-normally distributed with some negative values so a logarithmic transformation was inappropriate. To contrast MPO-NE assay measurements between the ticagrelor group and the placebo group, we used non-parametric rank sum tests with a Bonferroni correction for multiple comparisons. The Picogreen assay measures were analyzed using the parametric mixed-modelling approach with unstructured covariance and a Tukey correction for multiple comparison. To assess the effects of ticagrelor on lung function, for each patient, we first computed differences between baseline and 24-h lung function measurements and then contrasted mean lung function differences between the ticagrelor group and the placebo group by a 2-sample Student's *t*-test with unequal variances. To evaluate the effects of $P2Y_{12}$ antagonists across doses as well as changes over time, appropriate contrasts were considered. For multiple group comparison with a continuous response variable, we performed a 1-way analysis of variance. Relationships between mortality and $P2Y_{12}$ antagonists were analyzed using the log-rank test. To explore the role of $P2Y_{12}$ receptor in mice treated with either a placebo or with the $P2Y_{12}$ antagonists (ticagrelor or clopidogrel), Cox proportional hazards models were employed. To examine whether $P2Y_{12}$ antagonism (ticagrelor or clopidogrel) affects pulmonary microvascular permeability, K_{fc} were

FIGURE 2 Ticagrelor Reduces Platelet-Leukocyte Aggregates



The percentage of leukocytes with attached platelets for each subject is plotted at baseline (BL) and at 24 h in patients given placebo **(A, open circles)** and ticagrelor **(A, filled circles)**. A significant difference between groups on the change from baseline to 24 h is indicated by an **asterisk**. Delta change of platelet-leukocyte aggregates at 24 h (compared with baseline) is represented for patients randomized to placebo **(B, open circles and top dashed line)** and ticagrelor **(B, filled circles and bottom dashed line)**.

TABLE 4 Biomarkers

		Ticagrelor		Placebo	
	Healthy Plasma	Day 1	Day 2	Day 1	Day 2
NAP-2	295 (228–333)	291 (156–533)	355 (213–676)	148 (95–404)	186 (115–337)
ENA-78	52.55 (40.42–64.38)	48.9 (32.8–87.5)	50.0 (39.8–102.5)	39.4 (31.0–76.2)	46.2 (35.7–73.6)
PF-4	273 (224–325)	225 (119–339)	232 (136–419)	248 (121–360)	291 (179–362)
IL-10	3.08 (1.81–4.17)	3.76 (0.00–16.38)	4.40 (1.70–16.35)	4.67 (1.39–20.59)	3.15 (0.00–35.28)
IL-6	0.82 (0.77–2.03)	19.25 (3.20–42.52)*	3.20 (1.80–14.19)*	8.53 (0.99–17.74)*	9.93 (2.00–37.33)†
TNF-α	6.86 (4.66–8.08)	8.02 (4.19–18.69)	9.36 (5.76–23.02)	9.98 (5.62–16.66)	8.76 (6.40–21.44)
IL-1β	0.64 (0.42–0.86)	0.60 (0.04–2.30)	1.00 (0.28–2.44)	0.62 (0.00–2.93)	0.62 (0.00–7.54)
sCD40L	57.2 (35.03–77.61)	111.6 (50.0–255.4)	140.2 (81.0–239.6)	95.5 (53.5–211.5)	142.3 (66.4–217.5)

Values are median (interquartile range) in pg/ml. *Day 1 value is significantly different from healthy plasma (Mann-Whitney rank sum test). †Significant difference between the 2 treatment groups in the change from baseline to 24 h (linear mixed modeling or Mann-Whitney rank sum test).

contrasted using 1-way analysis of variance with a Tukey adjustment for multiple comparison. Statistical analysis was executed on SAS versions 9.3 and 9.4 (SAS Institute, Cary, North Carolina), SigmaPlot version 13.0 (Systat Software, San Jose, California) and R version 3.2.3 (R Project for Statistical Computing, Vienna, Austria). Graphs in figures were generated in SigmaPlot version 13.0.

RESULTS

TICAGRELOR MODULATES INFLAMMATORY RESPONSE IN PATIENTS HOSPITALIZED WITH PNEUMONIA. To evaluate the effect of the direct acting $P2Y_{12}$ antagonist ticagrelor in pneumonia, we randomized patients hospitalized within 48 h of diagnosis to receive placebo or ticagrelor for up to 7 days. Table 2 lists demographic and baseline characteristics of patients enrolled in the XANTHIPPE trial. The average age was 55.1 ± 16.0 years in the placebo group (53% female) and 56.6 ± 15.9 years in the ticagrelor group (57% female). There were no significant differences in history of hypertension, smoking history, diabetes, and COPD between the 2 groups. There was no significant difference between the 2 groups with regard to aspirin use before enrollment. There were no significant differences in antibiotic treatment at the time of enrollment between the 2 groups. Twenty-one subjects were diagnosed with CAP in the placebo arm (70%) and 17 subjects in the ticagrelor arm (57%) (Table 3). At 30 days, there were a total of 7 rehospitalizations (4 in the placebo group and 3 in the ticagrelor group) and 3 deaths (3 in the placebo group and 0 in the ticagrelor group). No major bleeding occurred in any of the patients.

No significant differences in platelet or white blood cell counts were observed in the 2 groups at baseline or at 24 h after intervention (Table 3). At baseline, maximum ADP- and TRAP-induced platelet aggregation were similar in the 2 groups (Figure 1). At 24 h, patients randomized to ticagrelor had lower ADP-induced aggregation as measured by light transmission aggregometry [LTA] (Figure 1A) and multiple electrode impedance assay (Figure 1B). No significant differences were observed between the groups at 24 h in aggregation induced by the PAR1 agonist TRAP by LTA (Figure 1C) or multiple electrode impedance assay (Figure 1D). Consistent with the effects of ticagrelor, the change in ADP-induced aggregation from baseline to 24 h was significantly different between subjects assigned to placebo and ticagrelor ($p = 0.0065$ [LTA] and $p < 0.0001$ [impedance assay]). No significant difference was noted for TRAP-induced aggregation ($p = 0.1283$ [LTA] and $p = 0.2937$ [impedance assay]).

Local inflammatory events mediated by leukocytes and platelets have been proposed to underlie acute lung injury, and circulating platelet-leukocyte heterotypic cell aggregates increase in animal models of lung injury (25). Therefore, we determined whether ticagrelor altered platelet-leukocyte aggregates in patients with pneumonia. No difference in platelet-leukocyte aggregates in placebo and ticagrelor groups was present at baseline (Figure 2A). Between baseline and 24 h after drug administration, a significant difference in the change in heterotypic cell aggregates was observed in the placebo and ticagrelor groups ($p = 0.0244$). The percentage of leukocytes with attached platelets declined from a median of 49.65% (interquartile range [IQR]: 36.00% to 55.73%) at baseline to 37.90% (IQR: 23.93% to 46.35%) at 24 h after ticagrelor; whereas in subjects randomized to placebo, the median percentage of leukocytes with attached platelets was 41.30% (IQR: 31.35% to 60.85%) at baseline and increased to 52.20% (IQR: 40.40% to 66.30%) at 24 h. Interestingly, the magnitude of the response to ticagrelor depended on the

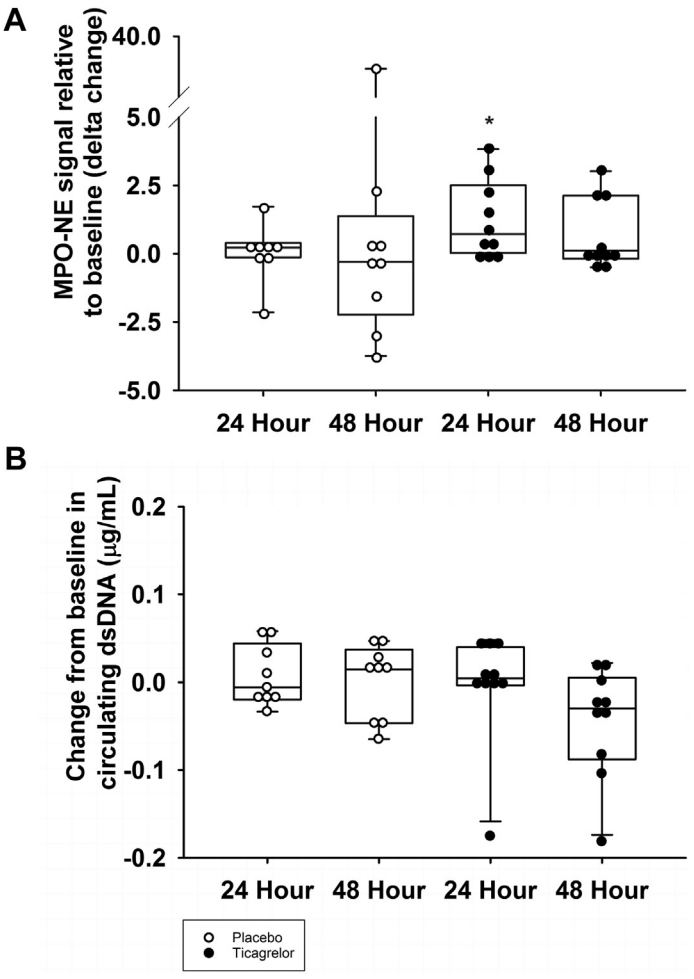
percentage of leukocytes with attached platelets at baseline, with larger responses to ticagrelor (Figure 2B) observed in individuals with more platelet-leukocyte aggregates at baseline.

The effects of ticagrelor on other markers of platelet activation and systemic plasma biomarkers of inflammation were examined next. sCD40 ligand is released from the platelet membrane during activation. Baseline plasma sCD40L levels in both the placebo and ticagrelor groups were similar to levels in healthy controls and increased at 24 h irrespective of randomization arm (Table 4). Of a series of inflammatory biomarkers, including IL-6, TNF-α, IL-1β, IL-10, NAP-2, and ENA-78, only IL-6 was above normal levels at baseline in the 2 groups. From baseline to 24 h, IL-6 levels changed significantly differently in the placebo and ticagrelor groups ($p = 0.0226$). In subjects randomized to ticagrelor, median plasma IL-6 levels declined by 83% from 19.25 pg/ml at baseline to 3.20 pg/ml at 24 h. No decrease was observed in the placebo group, which had plasma IL-6 levels of 8.53 pg/ml at baseline and 9.93 pg/ml at 24 h after randomization.

Platelets may also contribute to the formation of NETs, protein-DNA complexes released from neutrophils that contain MPO and NE in complex with chromatin. NETs facilitate pathogen clearance; however, their presence has also been associated with sepsis and sepsis organ dysfunction. We therefore measured the levels of MPO-NE complexes as a marker of NETosis in XANTHIPPE patients up to 48 h after placebo or ticagrelor. Somewhat surprisingly, relative to the baseline values, a significant increase in MPO-NE complexes was observed at 24 h ($p = 0.0410$) in the ticagrelor group. No significant change occurred in the placebo group (Figure 3A). Circulating ds-DNA quantified by the PicoGreen assay (Thermo Fisher Scientific), did not significantly change at 24 h after treatment in either group (Figure 3B), although a near-significant reduction in dsDNA occurred in the ticagrelor group at 48 h after therapy ($p = 0.0511$).

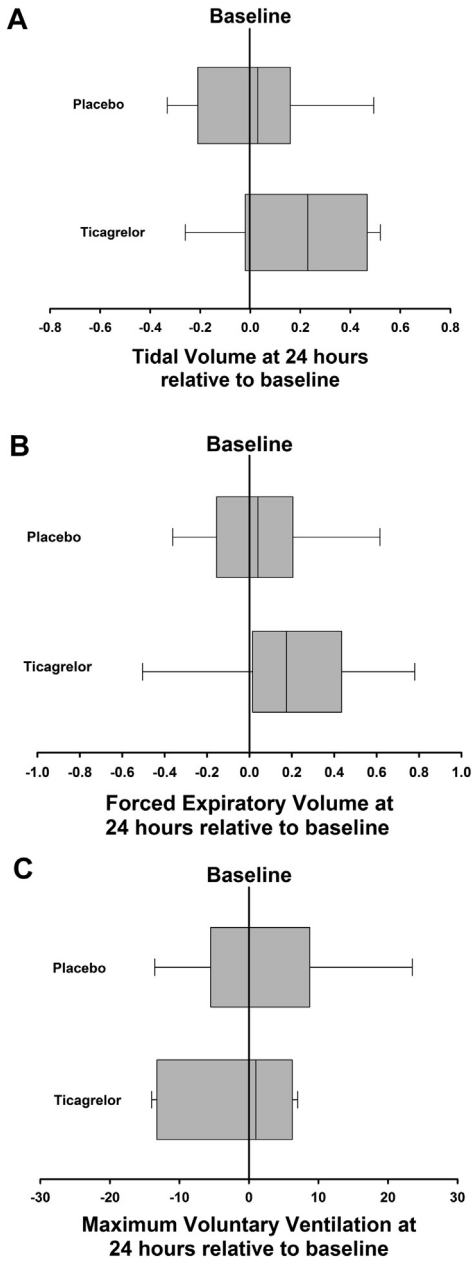
The effects of ticagrelor on lung function and supplemental oxygen requirements were assessed, respectively, by bedside spirometry on patients willing and able to perform the test and by patient records. In patients not receiving a $P2Y_{12}$ antagonist at baseline, 10 of the patients randomized to ticagrelor and 13 randomized to placebo were able to perform spirometry tests at both baseline and 24 h. In patients receiving ticagrelor, the mean tidal volume increased by 0.205 l from baseline to 24 h, whereas the change in the placebo group was only 0.008 l (Figure 4A). The ticagrelor group also displayed a mean increase of 0.185 l in forced expiratory volume in 1 s (FEV-1) at 24 h, as compared with a mean 0.057-l change in placebo patients (Figure 4B). Finally, MVV decreased on an average by 1.51 l/min at 24 h in ticagrelor patients, whereas MVV in the placebo group increased by 2.31 l/min (Figure 4C). However, none of the differences between ticagrelor-treated patients and the placebo group were statistically significant (change in tidal volume p value = 0.187, change in FEV-1 $p = 0.387$, change in MVV $p = 0.447$). Ticagrelor patients did, however, have a significant reduction away from supplemental oxygen (odds ratio [OR]: 1.08, 95% confidence interval [CI]: 1.01 to

FIGURE 3 Ticagrelor and NETosis Biomarkers in Pneumonia Patients



The delta change at 24 and 48 h after dosing for the MPO-NE NETosis assay **(A)** and circulating dsDNA **(B)** were measured in placebo patients **(open circles)** and ticagrelor patients **(filled circles)** that had samples through 48 h after dosing. Significant changes are indicated by an **asterisk**. dsDNA = doubled-stranded DNA; MPO = myeloperoxidase; NE = neutrophil elastase.

FIGURE 4 Ticagrelor and Lung Function Within 24 h in Pneumonia Patients



Lung function was tested in subjects willing and able to undergo spirometry testing. Data were collected at baseline and at 24 h. Changes at 24 h from baseline (midline in all 3 graphs) are shown for tidal volume **(A)**, FEV-1 **(B)**, and MVV **(C)**. Units reported are liters for tidal volume and FEV-1 **(A, B)** and liters per minute for MVV **(C)**. Data from patients randomized to placebo are shown in the **open boxes**, whereas patients randomized to ticagrelor are represented in the **shaded boxes**. Each **box** represents the interquartile range (25% to 75%), whereas the **whiskers** represent the full range of the data. The **vertical line in each interquartile range box** represents the median value (note that the median for the placebo group in **C** overlaps with the midline). FEV-1 = forced expiratory volume in 1 s; MVV = maximum ventilation velocity.

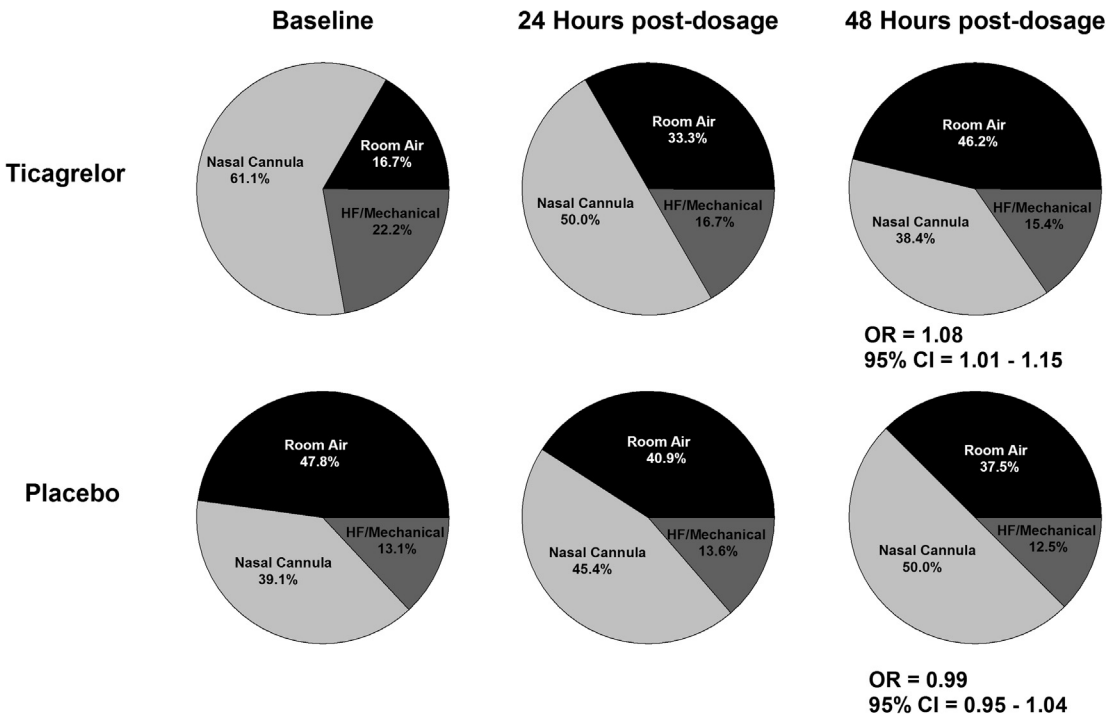
1.15) compared with no change in placebo patients (OR: 0.99; 95% CI: 0.95 to 1.04) (Figure 5).

$P2Y_{12}$ RECEPTOR FUNCTION MODULATES RESPONSE IN ACUTE INFLAMMATORY PRECLINICAL MODELS. Our results indicate that ticagrelor has effects on systemic biomarkers of inflammation in patients with pneumonia. To explore the role of $P2Y_{12}$ receptor function in an acute inflammatory model, mice were treated with placebo, ticagrelor, or clopidogrel before intraperitoneal LPS (10 mg/kg). Within hours of LPS administration, plasma inflammatory and anti-inflammatory cytokine levels increased. At 1 h in WT mice, plasma TNF-α levels were approximately 20-fold higher than baseline (Figure 6A). Ticagrelor treatment at all doses reduced TNF-α at 1 h, but this decrease was statistically significant only for 25 mg/kg dose ($p < 0.01$). Over longer times (2 to 8 h), ticagrelor had no significant effect on reducing TNF-α levels (Figure 6A). Ticagrelor elicited an increase in IL-10 levels after LPS. At higher doses (25 and 50 mg/kg), but not the lower dose (10 mg/kg), ticagrelor-treated mice had increased IL-10 at all time points compared with controls ($p < 0.05$ for all treatment doses and times, except ticagrelor dose 50 mg/kg at 4 h; $p = 0.06$) (Figure 6B). Clopidogrel had no significant effect on TNF-α, but IL-10 levels were elevated at 2 h and 8 h ($p < 0.05$ for both time points).

In comparison to WT controls, mice lacking the $P2Y_{12}$ receptor (*P2ry12*$^{-/-}$) were protected from death from endotoxemia ($p < 0.001$) (Figure 6C). Ticagrelor, but not clopidogrel, conferred protection in WT animals ($p < 0.05$) (Figure 6D) without an additive effect in the *P2ry12*$^{-/-}$ background.

Endotoxemia causes an inflammatory reaction, associated with disruption in endothelial barrier function, enhanced permeability, and the development of tissue edema. In the lung, this can manifest as acute lung injury. To examine whether $P2Y_{12}$ antagonism affects pulmonary microvascular permeability, K_{fc} was measured in isolated perfused lung preparations. Lungs from mice pre-treated with ticagrelor at higher doses (25 and 50 mg/kg) displayed lower K_{fc} after LPS treatment ($p < 0.05$) (Figure 6E), suggesting that ticagrelor reduced LPS-induced pulmonary capillary permeability. Conversely, ticagrelor at a lower dose (10 mg/kg) and clopidogrel did not yield a statistically significant reduction in K_{fc} levels. Taken together, our findings in a preclinical model indicate that ticagrelor can attenuate inflammation and may protect against inflammation-associated acute lung permeability, which could have a benefit in patients with pneumonia.

FIGURE 5 Pneumonia Patients Taking Ticagrelor Became Less Reliant on Supplemental Oxygen



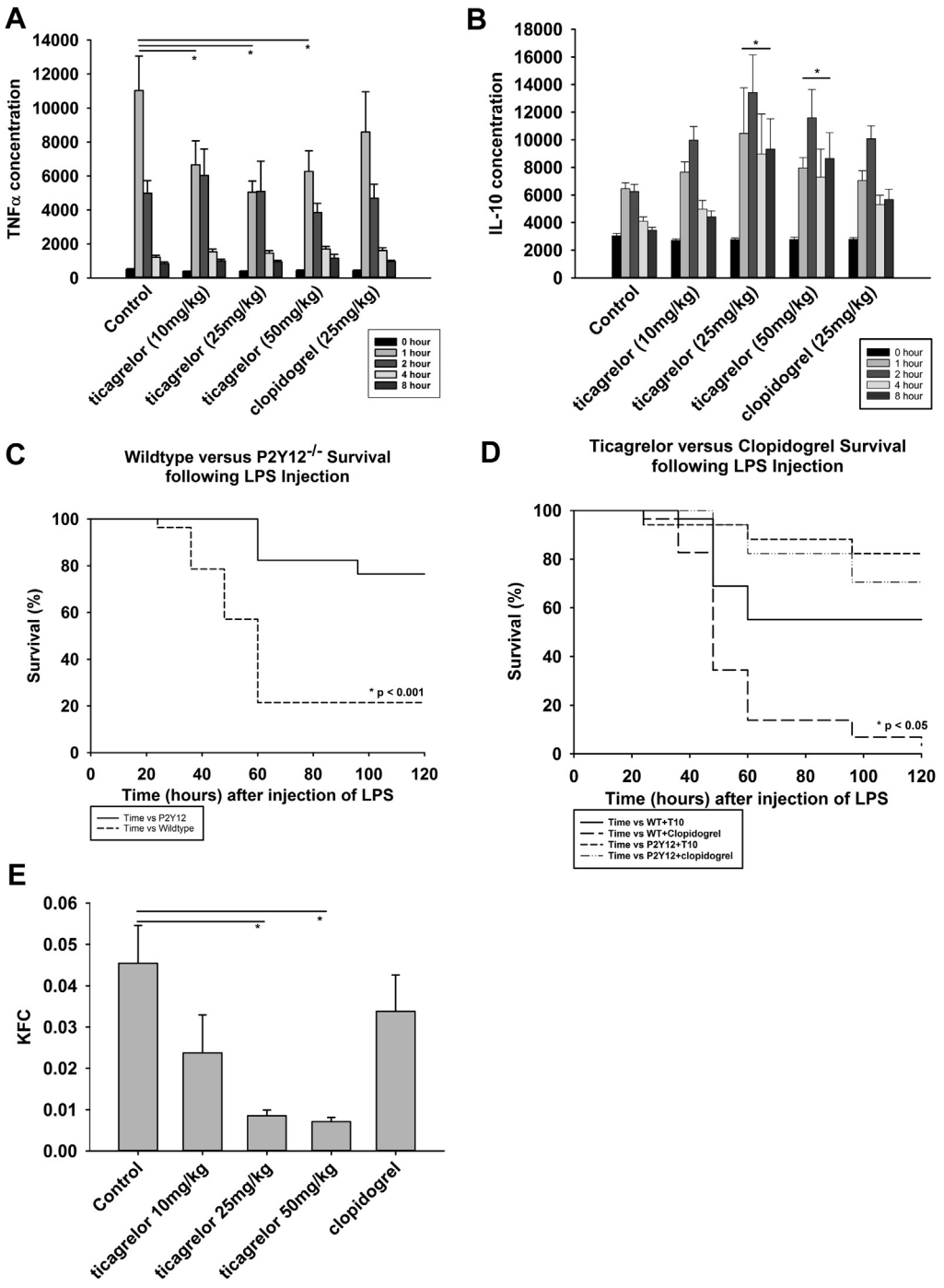
Supplemental oxygen requirements were recorded from patient records. Data were collected at the time closest to when the patient received the study medication and 24 and 48 h following study dosage. Supplemental oxygen was recorded as none (room air), nasal cannula, and high-flow (HF) devices and mechanical ventilation. Patients randomized to ticagrelor demonstrated a progression to room air and away from supplemental oxygen (odds ratio [OR]: 1.08; 95% confidence interval [CI]: 1.01 to 1.15). Patients randomized to placebo did not change over 48 h after receiving the study dose (OR: 0.99; 95% CI: 0.95 to 1.04).

DISCUSSION

Pneumonia is one the most common causes of death from infectious diseases worldwide. Left untreated, it can lead to serious complications such as acute lung injury and sepsis. Sepsis, a complex and poorly understood condition, carries a high mortality rate, and current treatments are generally supportive. Here, we identify the platelet $P2Y_{12}$ receptor as a potential target to prevent the complications of sepsis and for reducing lung injury, and suggest that the $P2Y_{12}$ antagonist ticagrelor may be of clinical benefit in the treatment of pneumonia. Taken together, these findings support a novel mechanism to prevent life-threatening complications of pneumonia and may explain the mortality benefit observed in patients randomized to ticagrelor in the PLATO trial (26).

Ticagrelor, but not clopidogrel, attenuated the inflammatory response to LPS in mice by blunting the increase in TNF-α and elevating IL-10 levels. The elevation of IL-10 exclusively occurred at 25 and 50 mg/kg doses of ticagrelor and not at the lower 10 mg/kg dose indicating a threshold concentration of ticagrelor must be achieved in order to elicit an anti-inflammatory effect. Furthermore, there seems to be a ceiling to IL-10 increase because we did not observe higher levels in the 50 mg/kg dose compared with the 25 mg/kg dose. Moreover, animals pretreated with ticagrelor or lacking $P2Y_{12}$ receptors were more likely to survive endotoxemia. Inflammation and infections have been associated with platelet activation and platelet-leukocyte heterotypic aggregate formation. Ticagrelor administration to patients with pneumonia reduced platelet-leukocyte aggregates in circulation and lowered IL-6 levels, consistent with an anti-inflammatory effect. Given that platelet-leukocyte aggregates serve as a sensitive in vivo marker of platelet activation, and increase from a mean of approximately 19% to 34% in patients presenting with acute myocardial

FIGURE 6 Ticagrelor Reduces Inflammation, Increases Lung Function, and Protects Against Mortality in a Murine Sepsis Model



The concentrations of TNF-α **(A)** and IL-10 **(B)** at 0 h up to 8 h post-LPS injection are graphed for control mice and mice treated with ticagrelor or clopidogrel (**A and B**, reported as mean with bar indicating SE). Significant difference over the time course compared with the control is indicated by an **asterisk in A and B**. Survival curves of wild-type (WT) mice and *P2y12*$^{-/-}$ mice injected with LPS are plotted in **C**, whereas **D** shows survival curves of WT and *P2y12*$^{-/-}$ mice treated with clopidogrel or ticagrelor (T10) before LPS injection. Significance between mutant and WT **(A)** and $P2Y_{12}$ antagonist treated and untreated **(D)** is indicated by an **asterisk**. Capillary filtration coefficients (K_{FC}) are graphed for control mice and mice treated with ticagrelor or clopidogrel (**E**, reported as mean with bar indicating SE). Significant differences between treatment group and control are indicated by an **asterisk**. IL = interleukin; LPS = lipopolysaccharide; TNF = tumor necrosis factor.

infarction, the differences observed with ticagrelor likely reflect a meaningful effect of platelet activation in pneumonia. These anti-inflammatory effects of $P2Y_{12}$ antagonism are consistent with those observed in a rat model of LPS-induced sepsis in which pre-treatment with clopidogrel reduced IL-6 and TNF-α (27), as well as a mouse model of sepsis and acute lung injury in which clopidogrel-treated and $P2Y_{12}$-null mice were resistant to sepsis-induced lung injury (28). These anti-inflammatory effects of $P2Y_{12}$ are also consistent with retrospective clinical studies in which anti-platelet drugs had a favorable effect on patients with organ dysfunction (such as sepsis) and CAP (14,15,29). However, in all of those studies, there was no benefit of clopidogrel on mortality. Here, we demonstrate, not only an anti-inflammatory effect of ticagrelor apparently equal to or greater than that of clopidogrel, but also improvement on survival in LPS-treated mice. As a potent, direct-acting $P2Y_{12}$ antagonist, ticagrelor may be particularly well suited to reduce adverse events mediated by platelets in the setting of inflammation. In the setting of acute coronary syndrome, patients taking clopidogrel have high on-treatment platelet reactivity that can be overcome with ticagrelor (30,31). Although not yet reported, a similar phenomenon may occur in other inflammatory settings such as pneumonia and sepsis. Additionally, by inhibiting the adenosine transport ENT1, ticagrelor may have adenosine-mediated effects that could promote vasodilation, platelet inhibition, and modulate inflammation (32). Although work in our lab has traditionally focused on anti-inflammatory effects by reducing platelet-leukocyte interactions, there is some evidence from translational work that these platelet-leukocyte interactions can be beneficial. In a study by Tunjungputri et al. (33), pro- and anti-inflammatory effects of ticagrelor were observed in blood exposed to Pam3CSK4 (TLR2-mediated) and LPS (TLR4-mediated), respectively. Interestingly, in a study investigating the effectiveness of the $P2Y_{12}$ antagonist prasugrel in reducing coagulation and inflammation in a human LPS model, prasugrel had no effect on platelet-leukocyte aggregation (34). Thus, it is possible that ticagrelor has unique effects on platelet-leukocyte interactions.

During sepsis, endothelial dysfunction leads to systemic capillary leak and multiple-organ failure. The pulmonary microvessels are especially prone to disruptions in integrity, and they may also be affected in pneumonia-type infections. Sequestration of platelets with leukocytes in pulmonary tissue is a key feature in inflammatory or infectious states (35). Activated platelets alone or neutrophil-platelet aggregates may occlude small pulmonary vessels to perpetuate local inflammation or line the endothelium to contribute to leakage of plasma and cellular components across a normally impermeable barrier into the alveolar and interstitial spaces of the lung. Through these and other mechanisms, platelet-leukocyte interactions can disrupt lung endothelial barrier function in sepsis and acute respiratory distress syndrome (11,36). Our results suggest that potent $P2Y_{12}$ antagonism with ticagrelor can interrupt key molecular signaling pathways responsible for lung permeability.

In this work, we have shown both murine and clinical measures that suggest improved lung function during sepsis and pneumonia following treatment with ticagrelor. Furthermore, we observed a baseline-dependent effect of ticagrelor on circulating platelet-leukocyte aggregates following ticagrelor treatment in pneumonia patients, although this observation could be due, in part, to regression to the mean. Patients with higher baseline platelet-leukocyte aggregates also had greater reductions in platelet-leukocyte aggregates following ticagrelor treatment. These reductions in platelet-leukocyte aggregates could possibly explain the improvements observed in lung function in both our animal and human models.

STUDY LIMITATIONS. The XANTHIPPE study was a prospective study designed to target pneumonia patients. Due to a combination of pneumonia patients having the capacity to consent as well as exclusion criteria, the recruited population was less sick than we had anticipated. We also acknowledge that cecal ligation and puncture model of sepsis may carry more clinical relevance than the endotoxin model that was employed; however, the inflammatory response to sepsis and the anti-inflammatory effects of ticagrelor are likely similar between the 2 models.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Ticagrelor is commonly used in patients with acute coronary syndrome. This work indicates a potential use for ticagrelor to target inflammation and improve lung function in the setting of pneumonia. The findings suggest the possibility that ticagrelor could prevent complications of sepsis, although larger-scale clinical studies are required.

TRANSLATIONAL OUTLOOK: Over the last decade, it has become clear that platelets play vital roles that extend well beyond hemostasis. In this work, the authors demonstrate that targeting the platelet $P2Y_{12}$ receptor reduces platelet–leukocyte interactions, alters inflammatory biomarkers, and can improve lung function in the context of pneumonia and in experimental models of sepsis. Preclinical models should provide additional mechanistic insight into the benefits of antiplatelet therapy beyond preventing the complications of atherosclerotic disease.

REFERENCES

1. Restrepo MI, Faverio P, Anzueto A. Long-term prognosis in community-acquired pneumonia. Curr Opin Infect Dis 2013;26:151–8.

2. Cangemi R, Casciaro M, Rossi E, et al. Platelet activation is associated with myocardial infarction in patients with pneumonia. J Am Coll Cardiol 2014;64:1917–25.

3. Chen LF, Chen HP, Huang YS, Huang KY, Chou P, Lee CC. Pneumococcal pneumonia and the risk of stroke: a population-based follow-up study. PLoS One 2012;7:e51452.

4. Chiang CH, Huang CC, Chan WL, et al. Association between Mycoplasma pneumonia and increased risk of ischemic stroke: a nationwide study. Stroke 2011;42:2940–3.

5. Mandal P, Chalmers JD, Choudhury G, Akram AR, Hill AT. Vascular complications are associated with poor outcome in community-acquired pneumonia. QJM 2011;104:489–95.

6. Ribeiro DD, Lijfering WM, Van Hylckama Vlieg A, Rosendaal FR, Cannegieter SC. Pneumonia and risk of venous thrombosis: results from the MEGA study. J Thromb Haemost 2012;10:1179–82.

7. Ruane L, Buckley T, Hoo SYS, et al. Triggering of acute myocardial infarction by respiratory infection. Intern Med J 2017;47:522–9.

8. Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet 2006;367:1075–9.

9. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 2004;351:2611–8.

10. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest 2012;122:2661–71.

11. Grommes J, Alard JE, Drechsler M, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. Am J Respir Crit Care Med 2012;185:628–36.

12. Weyrich AS, Zimmerman GA. Platelets in lung biology. Annu Rev Physiol 2013;75:569–91.

13. Gross AK, Dunn SP, Feola DJ, et al. Clopidogrel treatment and the incidence and severity of community acquired pneumonia in a cohort study and meta-analysis of antiplatelet therapy in pneumonia and critical illness. J Thromb Thrombolysis 2013;35:147–54.

14. Winning J, Reichel J, Eisenhut Y, et al. Antiplatelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets 2009;20:50–7.

15. Winning J, Neumann J, Kohl M, et al. Antiplatelet drugs and outcome in mixed admissions to an intensive care unit. Crit Care Med 2010;38:32–7.

16. Storey RF, James SK, Siegbahn A, et al. Lower mortality following pulmonary adverse events and sepsis with ticagrelor compared to clopidogrel in the PLATO study. Platelets 2014;25:517–25.

17. Andell P, James SK, Cannon CP, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes and chronic obstructive pulmonary disease: an analysis from the Platelet Inhibition and Patient Outcomes (PLATO) trial. J Am Heart Assoc 2015;4:e002490.

18. Rondina MT, Carlisle M, Fraughton T, et al. Platelet-monocyte aggregate formation and mortality risk in older patients with severe sepsis and septic shock. J Gerontol A Biol Sci Med Sci 2015;70:225–31.

19. Czaikoski PG, Mota JM, Nascimento DC, et al. Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. PLoS One 2016;11:e0148142.

20. Lowe KL, Finney BA, Deppermann C, et al. Podoplanin and CLEC-2 drive cerebrovascular patterning and integrity during development. Blood 2015;125:3769–77.

21. Sexton TR, Wallace EL, Macaulay TE, et al. The effect of rosuvastatin on thromboinflammation in the setting of acute coronary syndrome. J Thromb Thrombolysis 2015;39:186–95.

22. Sexton TR, Wallace EL, Chen A, et al. Thromboinflammatory response and predictors of outcomes in patients undergoing transcatheter aortic valve replacement. J Thromb Thrombolysis 2016;41:384–93.

23. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26:319–38.

24. Foster CJ, Prosser DM, Agans JM, et al. Molecular identification and characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. J Clin Invest 2001;107:1591–8.

25. Zarbock A, Ley K. The role of platelets in acute lung injury (ALI). Front Biosci (Landmark Ed) 2009;14:150–8.

26. Cannon CP, Harrington RA, James S, et al. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. Lancet 2010;375:283–93.

27. Seidel M, Winning J, Claus RA, Bauer M, Losche W. Beneficial effect of clopidogrel in a mouse model of polymicrobial sepsis. J Thromb Haemost 2009;7:1030–2.

28. Liverani E, Rico MC, Tsygankov AY, Kilpatrick LE, Kunapuli SP. P2Y12 receptor modulates sepsis-induced inflammation. Arterioscler Thromb Vasc Biol 2016;36:961–71.

29. Winning J, Claus RA, Pletz MW, Bauer M, Losche W. Adenosine diphosphate receptor antagonist clopidogrel sulfate attenuates LPS-induced systemic inflammation in a rat model. Shock 2011;36:317; author reply 318.

30. Alexopoulos D, Galati A, Xanthopoulou I, et al. Ticagrelor versus prasugrel in acute coronary syndrome patients with high on-clopidogrel

platelet reactivity following percutaneous coronary intervention: a pharmacodynamic study. J Am Coll Cardiol 2012;60:193–9.

31. Li P, Yang Y, Chen T, et al. Ticagrelor overcomes high platelet reactivity in patients with acute myocardial infarction or coronary artery instent restenosis: a randomized controlled trial. Sci Rep 2015;5:13789.

32. Cattaneo M, Schulz R, Nylander S. Adenosine-mediated effects of ticagrelor: evidence and potential clinical relevance. J Am Coll Cardiol 2014;63:2503–9.

33. Tunjungputri RN, Van Der Ven AJ, Schonsberg A, et al. Reduced platelet hyperreactivity and platelet-monocyte aggregation in HIV-infected individuals receiving a raltegravir-based regimen. AIDS 2014;28:2091–6.

34. Schoergenhofer C, Schwameis M, Hobl EL, et al. Potent irreversible P2Y12 inhibition does not reduce LPS-induced coagulation activation in a randomized, double-blind, placebo-controlled trial. Clin Sci (Lond) 2016;130:433–40.

35. Akinosoglou K, Alexopoulos D. Use of antiplatelet agents in sepsis: a glimpse into the future. Thromb Res 2014;133:131–8.

36. Kuebler WM. Selectins revisited: the emerging role of platelets in inflammatory lung disease. J Clin Invest 2006;116:3106–8.

KEY WORDS inflammation, leukocytes, platelets, pneumonia, sepsis

APPENDIX For supplemental figures, please see the online version of this paper.

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