Reversal of the antiaggregant effect of ticagrelor in combination with acetylsalicylic acid, using normal platelets

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This thesis is presented in partial fulfilment of the requirements for the degree of Master of Clinical Research of The University of Western Australia

School of Medicine
2019
Thesis declaration

I, Paul Kruger, certify that:

This thesis has been substantially accomplished during enrolment in the degree of Master of Clinical Research at The University of Western Australia.

This thesis does not contain material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

No part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

The research involving human data reported in this thesis was assessed and approved by The University of Western Australia Human Research Ethics Committee. Approval RA/4/1/9101 (26-Apr-2017).

Written informed consent has been received from subjects and archived for the research involving subject data reported in this thesis.

The following approvals were obtained prior to commencing the relevant work described in this thesis:

1. Population Health Research Institute Authorisation to Conduct Clinical Research (08-Nov-2016).


The work described in this thesis was funded by:


No third-party editorial assistance was provided in preparation of this thesis.

This thesis contains published work, some of which has been co-authored.

Signature: 

Date: 28-May-2019
Abstract

Background
Ticagrelor is an antiplatelet agent which is administered, in combination with acetylsalicylic acid, for prevention of thrombosis in patients with acute coronary syndrome (ACS) or after percutaneous coronary artery stent implantation. However, ticagrelor also increases the risk of bleeding, which may occur spontaneously or during emergency surgery. The management of bleeding in patients who have taken ticagrelor is difficult because there is not yet an antidote and its antiplatelet effects persist for up to five days after the last dose. Transfusion of platelets has been studied to potentially prevent or treat bleeding in patients who have taken ticagrelor. The rationale for using donor platelets is that they are fully functional. A potential problem with the use of donor platelets is that they may be affected by residual ticagrelor in the circulation. Prior publications have generally shown that donor platelets have minimal effect on platelet aggregation if given within 12 hours of the last dose of ticagrelor. Two publications report on ticagrelor reversal over a longer time frame, up to 48 hours and 96 hours after the last dose; one study has shown that donor platelets produce partial reversal of the effect of ticagrelor, however the other study conflicts because it shows that donor platelets have no effect on ticagrelor reversal. Prior studies have not determined the optimal dose and time-point at which to transfuse platelets to reverse ticagrelor. A valid method to study ticagrelor reversal is mixing platelets from a person treated with ticagrelor and an untreated person in vitro, and measuring the platelet function of the mixture by adenosine diphosphate (ADP) mediated aggregation.

Aims
To determine the minimum quantity of donor platelets required for major (>90%) reversal of ticagrelor, and the minimum quantity of platelets required for 50% reversal of ticagrelor at time-points up to 96 hours after the last dose.
**Hypothesis**

Donor platelets given within 24 hours of ticagrelor will not reverse the *in vitro* effect of ticagrelor, but when given in a large quantity (>24 hours after ticagrelor will reverse the *in vitro* effect of ticagrelor.

**Method**

Subjects who had taken maintenance dose ticagrelor and ASA had blood collected immediately prior to the last dose, and at 2, 10, 24, 48, 72, 96 hours after the last dose. An untreated person provided donor platelets. Platelet rich plasma (PRP) from the subject and donor were mixed in nine different proportions. Platelet aggregation in PRP after stimulation by ADP was measured using light transmission aggregometry and the maximum extent of aggregation was analysed.

**Results**

Ten subjects were recruited, mean age 30.1 ± 6.7 years, 60% female, mean weight 70.0 ±12.9 kg. Prior to treatment with ticagrelor and ASA, all subjects had a normal platelet count and normal mean ADP-aggregation of 76.4 ± 6.3%. After five days of ticagrelor, the mean ADP-aggregation decreased to a nadir of 34.6 ± 11.1% at two hours post the last dose. ADP-aggregation spontaneously increased until 72 hours when the mean ADP-aggregation was 71.8 ± 5.5% and not significantly different from pretreatment. Between 2 and 48 hours, addition of donor platelets increased the ADP-aggregation in a stepwise fashion. The increase in ADP-aggregation was proportional to the quantity of donor platelets. Major (>90% reversal) at 2 hours after ticagrelor was not achieved, but at 10 hours was achieved in the subject 10%/donor 90% mix, at 24 hours in the subject 40%/donor 60% mix, at 48 hours in the subject 80%/donor 20% mix, and at 72 and 96 hours was achieved spontaneously. 50% reversal at 2 hours was achieved in the subject 30%/donor 70% mix, at 10 hours in the subject 40%/subject 60% mix, at 24 hours in the subject 80%/donor 20% mix, and at 48, 72, and 96 hours was achieved spontaneously. Donor platelets had no effect on ADP-aggregation at 72 or 96 hours. Inhibition of donor platelet function occurred when platelet poor plasma from the subject was added to donor platelets between 2 and 24 hours.
Conclusion

This study indicates that the spontaneous offset of the effect of ticagrelor is complete by 72 hours. Adding donor platelets reverses the effect of ticagrelor when measured by ADP-aggregation. Partial ticagrelor reversal can be achieved with platelet transfusion in the first 24 hours after dosing, but major (>90%) reversal is not feasible during this time. Major reversal of ticagrelor is feasible at 24 and 48 hours if a sufficient number of platelets are transfused. Platelet transfusion is irrelevant from 72 hours because ADP-aggregation returns to the pretreatment level after the spontaneous offset of ticagrelor. The results of this study allow clinicians to use their judgement to optimise the quantity and timing of platelet transfusion, which in turn may reduce blood product wastage and improve patient outcomes.
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Statement of Candidate Contribution

This thesis is my own composition, all sources have been acknowledged and my contribution is identified. For any work in the thesis that has been published with other co-authors, I have the permission of the co-authors to include this work in my thesis.

I was involved in the conception and design, and was responsible for obtaining approval from regulatory agencies, collection of blood specimens from subjects when possible, performing aggregation testing, analysing and interpreting data, drafting the thesis and critical revision of the thesis. This thesis is my own composition, all sources have been acknowledged and my contribution is clearly identified in the thesis.

I have completed this thesis during the course of enrolment in the Master of Clinical Research Degree at The University of Western Australia and it has not previously been accepted for the award of any other degree or diploma at this or another institution.
Scholarly Outcomes of Thesis

Publications


Presentations


Paul C Kruger, Jack Hirsh, Vinai C Bhagirath, Jeffrey S Ginsberg, John W Eikelboom, Noel C Chan. Platelet dysfunction and transfusion management. Haematology Society of Australia and New Zealand (Western Australia Branch) 2018 Annual Scientific Meeting, Perth, April 2018.


Paul C Kruger, Jack Hirsh, Vinai C Bhagirath, Ke Xu, Brian Dale, Tim AC de Vries, Jeffrey S Ginsberg, John W Eikelboom, Noel C Chan. In vitro reversal of the anti-aggregant effect of ticagrelor using untreated platelets. Blood 2018: The combined Annual Scientific Meeting of the Haematology Society of Australia and New Zealand, Australian and New Zealand Society of
Authorship declaration: Co-authored publications

This thesis contains work that has been published.

Details of the work:


Location in thesis:

1.4.2 Evidence that donor platelets reverse oral antiplatelet agents
1.6.2 Evidence for ticagrelor reversal

Student contribution to work:

First author on paper. Major contribution to work including literature review, writing first draft of manuscript, and revising the manuscript after critical review from co-authors.

Co-author signatures and dates:

- Noel Chan
  - June 25, 2019
Details of the work:


Location in thesis:

1.4 Treatment of bleeding associated with antiplatelet therapy  
1.6.1 Pharmacological properties of ticagrelor that affect reversal  
1.7.2 Validity of *in vitro* antiplatelet agent reversal studies  
2.2 Aims  
2.3 Outcomes  
3.7 Sample size calculation  
3.9 Statistical analysis  
4.1 Characteristics of study population  
4.4 Primary and secondary outcomes  
5.1 Key findings  
5.4 Clinical importance of this study  
5.6 Potential limitations

Student contribution to work:

First author on paper. Major contribution including to the work including designing the study in consultation with the group, applying for and obtaining approvals from regulatory authorities, applying for and obtaining funding, performing laboratory testing, data collection, statistical analysis, writing first draft of the manuscript, revising manuscript in light of critical revisions from co-authors, submitting the manuscript for publication, revising the manuscript in light of comments from anonymous reviewers.
Co-author signatures and dates:

Jack Hirsh  
Vinai Bhagirath  
Ke Xu  
Brian Dale  
Tim de Vries  
Jeff Ginsberg  
Noel Chan  

Student signature:  
Date: 22-Jun-2019  

I, Professor John Eikelboom certify that the student statements regarding their contribution to each of the works listed above are correct.

Coordinating supervisor signature:  
Date:  

Jun 24, 2019
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Abbreviations

All abbreviations are defined within the text as they arise.

AA          Arachidonic acid
AC          Adenylyl cyclase
ACS         Acute coronary syndrome
ADP         Adenosine diphosphate
AM          Active metabolite
ARU         Aspirin response unit
ASA         Acetylsalicylic acid
ATP         Adenosine triphosphate
Beta-HCG    Beta human chorionic gonadotrophin
BD          Becton, Dickinson and Company
CABG        Coronary artery bypass graft
CAD         Coronary artery disease
cAMP        Cyclic adenosine monophosphate
CI          Confidence interval
COX-1       Cyclooxygenase-1
CV          Cardiovascular
CYP         Cytochrome P450
DAPT        Dual antiplatelet therapy
EDTA        K$_2$ ethylenediaminetetraacetic acid
FDA         Food and Drug Administration
HR          Hazard ratio
ICH         Intracranial haemorrhage
IHD         Ischaemic heart disease
IQR         Interquartile range
LTA         Light transmission aggregometry
MI          Myocardial infarction
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>n</td>
<td>Number</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PPP</td>
<td>Platelet poor plasma</td>
</tr>
<tr>
<td>PRP</td>
<td>Platelet rich plasma</td>
</tr>
<tr>
<td>PRU</td>
<td>P2Y₁₂ reaction units</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>TXA</td>
<td>Thromboxane</td>
</tr>
<tr>
<td>tₘₐₓ</td>
<td>Time to maximum concentration</td>
</tr>
<tr>
<td>t₀.₅</td>
<td>Time to 50% concentration (half-life)</td>
</tr>
<tr>
<td>VASP</td>
<td>Vasodilator stimulated phosphoprotein</td>
</tr>
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<td>WB</td>
<td>Whole blood</td>
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Introduction

1.1 Ischaemic heart disease and the role of platelets

Ischaemic heart disease (IHD) accounted for over eight million deaths in 2015 and has been a leading cause of death globally for at least the last 15 years.\(^1\) IHD encompasses a clinical spectrum ranging from asymptomatic coronary artery atherosclerosis to acute coronary syndrome (ACS) which presents as unstable angina or acute myocardial infarction (MI). ACS is associated with significant mortality as about 33\% of patients with ST elevated myocardial infarction die within 24 hours, and about 15\% of patients with unstable angina or non-ST elevated myocardial infarction die within 30 days.\(^2\)

Activated platelets play a key role in the pathogenesis of IHD.\(^3\) During atherogenesis in the coronary arteries, platelets contribute to plaque progression by releasing platelet derived growth factor and chemokines. During ACS, rupture of an unstable atherosclerotic plaque leads to the release of tissue and exposes the blood to subendothelial proteins, resulting in coagulation and platelet activation which leads to intra-coronary artery thrombus formation. In patients who undergo percutaneous coronary intervention (PCI) and stent insertion, the foreign surface of the stent activates platelets which may lead to stent thrombosis. Antiplatelet therapy is highly recommended to decrease the risk of stent thrombosis. The potential consequences of platelet activation include abrupt arterial closure, stent thrombosis and restenosis.\(^4\)-\(^7\)

1.2 Antiplatelet therapy in coronary artery disease

Antiplatelet therapy prevents platelet activation and has led to substantial improvements in the clinical outcomes of patients with IHD.\(^8\) Acetylsalicylic acid (ASA), clopidogrel, prasugrel and ticagrelor are the oral antiplatelets in routine clinical use and the pathways targeted by each agent are shown in Figure 1.
Figure 1 Pathways targeted by oral antiplatelet agents

AA denotes arachidonic acid; AC, adenylate cyclase; ADP, adenosine diphosphate; AM, active metabolite; ASA, acetylsalicylic acid; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; CYP, cytochrome P450; P, phosphate; PG, prostaglandin; PKA, protein kinase A; TXA₂, thromboxane A₂; VASP, vasodilator
ASA has been the foundation of antiplatelet therapy in patients with coronary artery disease (CAD) for decades. ASA irreversibly acetylates and inactivates platelet cyclooxygenase-1 (COX-1), thereby preventing formation of thromboxane A\(_2\) and inhibiting platelet aggregation.\(^9\) In a collaborative meta-analysis, ASA was shown to reduce the composite of major coronary events, stroke, and vascular mortality in patients with cardiovascular (CV) disease by 19% during long-term treatment (hazard ratio, [HR] 0.81; 95% confidence interval [CI], 0.75 to 0.87; p<0.00001).\(^10\) The use of an inhibitor of the adenosine diphosphate (ADP)-platelet P2Y\(_{12}\) pathway in combination with ASA, thereby targeting two different platelet activation pathways, leads to greater antithrombotic benefit than either agent used alone.\(^11,12\) Ticagrelor, clopidogrel, and prasugrel are oral platelet P2Y\(_{12}\) receptor antagonists that antagonise ADP-mediated activation of glycoprotein IIb/IIIa receptors on the platelet surface, and prevent platelet aggregation, and have been used in combination with ASA.\(^13\) Ticlopidine is an earlier ADP antagonist that has been used to reduce the risk of stent thrombosis and other vascular events, but it is rarely used now because of the potential for bone marrow suppression and has been replaced by newer agents.\(^14\)

In the acute setting, intensified antiplatelet therapy is superior to ASA alone for reduction of thrombotic risk. In the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, dual antiplatelet therapy (DAPT) with clopidogrel and ASA as compared with ASA alone in patients with a recent ACS reduced the composite outcome, CV death, MI or stroke, by 20% (relative risk [RR], 0.80; 95% CI, 0.72 to 0.90; p<0.001).\(^15\) Subsequent to the development of clopidogrel, the second generation thienopyridines prasugrel and ticagrelor have become available. In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI 38) trial, prasugrel was compared to clopidogrel in patients with moderate to high risk of ACS with scheduled PCI and showed a 27% reduction in death from CV causes, nonfatal MI, or nonfatal stroke (HR, 0.81; 95% CI, 0.73 to 0.90; p<0.001).\(^16\) Ticagrelor was approved by the United States Food and
Drug Administration (FDA) in July 2011 for reduction of thrombotic risk following ACS.\textsuperscript{17} This original indication which was based on the Platelet Inhibition and Patient Outcomes (PLATO) study showed that ticagrelor when compared to clopidogrel in patients with ACS was associated with a 16\% reduction in the composite of death from vascular causes, MI or stroke (HR, 0.84; 95\% CI, 0.77 to 0.92; p<0.001). Furthermore, the ticagrelor arm had significantly lower rates of MI alone (5.8\% in ticagrelor versus 6.9\% in clopidogrel; p=0.005) and death from any cause (4.5\% versus 5.9\%; p<0.001). Ticagrelor is the first oral antiplatelet agent to show a significant reduction in death from vascular causes (4.0\% versus 5.1\%; p=0.001).\textsuperscript{18} Ticagrelor and prasugrel were compared head-to-head in the Primary Angioplasty in patients with myocardial infarction transferred from General community hospitals to angioplasty Units of tertiary cardiology centers with or without Emergency thrombolysis (PRAGUE-18) Study, which showed that the primary end point of death, reinfarction, urgent target vessel revascularisation, stroke or serious bleeding requiring transfusion or prolonging hospitalisation did not differ between treatment groups (4.0\% and 4.1\%; odds ratio [OR], 0.98; 95\% CI, 0.55 to 1.73; p=0.939) in patients with acute MI treated with primary PCI.\textsuperscript{19}

Long term antiplatelet treatment has also been studied. In the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial, long-term treatment with ASA and clopidogrel compared with ASA alone in a subgroup of patients with symptomatic stable CV disease, reduced CV death, stroke or MI by 12\% (RR, 0.88; 95\% CI, 0.77 to 0.998; p=0.046).\textsuperscript{20} The Prevention of Cardiovascular Events in Patients with Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin-Thrombolysis in Myocardial Infarction 54 (PEGASUS-TIMI 54) trial showed that the combination of ticagrelor 90 mg twice daily and ASA compared with ASA alone in patients with a history of MI 1-3 years earlier, reduced the risk of CV death, stroke or MI by 15\% (HR, 0.85; 95\% CI, 0.75 to 0.96; p=0.008). Furthermore, the combination of ticagrelor 60 mg twice daily and ASA reduced the risk of CV death, stroke or MI by 16\% (HR, 0.84; 95\% CI, 0.74 to 0.95; p=0.004).\textsuperscript{21} In the TRIOLOGY ACS Study, long term treatment with prasugrel compared to clopidogrel did not show a difference in the primary end point of death from CV causes, MI, or stroke (13.9\% versus 16.0\%; HR, 0.91; 95\% CI 0.79 to 1.05; p=0.21) among patients with unstable angina or
MI who did not undergo revascularization. The potential reason for the lack of difference between groups is that patients who have medical therapy alone likely have a lower thrombotic risk than patients who have a transient increase in platelet reactivity during a revascularization procedure, and are therefore less likely to benefit from more potent antiplatelet therapy. The FDA expanded the indication for ticagrelor in 2015 so that patients with a history of MI could continue ticagrelor beyond 12 months at a lower dose of 60 mg twice daily. This expanded indication was based on the PEGASUS-TIMI 54 study. In Australia, ticagrelor is prescribed to patients with ACS (MI or unstable angina), in combination with ASA. As a result of the increased efficacy of ticagrelor, the uptake of ticagrelor has increased in recent years with a corresponding reduction in clopidogrel and prasugrel use.

1.3 Bleeding associated with antiplatelet therapy

Antiplatelet agents increase the risk of bleeding. Data on the risk of bleeding during ticagrelor therapy compared to clopidogrel or placebo is available from the PLATO trial and PEGASUS-TIMI 54 trial, respectively, and are summarised in Table 1. Antiplatelet agents increase the risk of bleeding because they inhibit platelet aggregation. In a meta-analysis of randomised trials, ASA, compared to placebo, increased the risk of major gastrointestinal and other extracranial bleeds by more than two-fold (RR, 2.69; 95% CI, 1.25 to 5.76; p=0.01) in patients taking ASA for secondary prevention of vascular disease. Clopidogrel, compared to ASA, is associated with a similar rate of any bleeding disorder (9.27% versus 9.28%; p=not significant), intracranial haemorrhage (ICH) (0.35% versus 0.49%; p=not significant), but with a lower rate of gastrointestinal haemorrhage (1.99% versus 2.66%; p<0.05) in patients with atherosclerotic vascular disease. Prasugrel and ticagrelor cause greater inhibition of platelet function than clopidogrel and are associated with higher rates of bleeding. Prasugrel, compared to clopidogrel, is associated with an increased risk of major bleeding (2.4% versus 1.8%; HR, 1.32; 95% CI, 1.03 to 1.68; p=0.03), life-threatening bleeding (1.4% versus 0.9%; HR, 1.52; 95% CI, 1.08 to 2.13; p=0.01) and fatal bleeding (0.4% versus 0.1%; HR, 4.19; 95% CI, 1.58 to 11.1; p=0.002) in patients with ACS. Ticagrelor, compared to clopidogrel, has a similar rate of major bleeding (11.6% versus 11.2%; p=0.43) but is associated with a higher rate of major bleeding not related to coronary artery bypass graft (4.5% versus 3.8%; p=0.03), higher risk of fatal
intracranial bleeding (0.1% versus 0.01%; p=0.02) and lower risk of fatal bleeding of other types (0.1% versus 0.3%; p=0.03) in patients with ACS.\textsuperscript{18} Consequences of bleeding include morbidity and mortality which is directly attributable to the acute bleeding episode, increased risk of recurrent ischaemic events, and increased drug discontinuation.\textsuperscript{26}
**Table 1 Bleeding risk associated with ticagrelor**

<table>
<thead>
<tr>
<th></th>
<th>Aspirin&lt;sup&gt;10&lt;/sup&gt;</th>
<th>Clopidogrel</th>
<th>Prasugrel</th>
<th>Ticagrelor&lt;sup&gt;18&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Major bleeding</td>
<td>1%</td>
<td>1.3%</td>
<td>0.45%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Life-threatening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bleeding</td>
<td>0.002%</td>
<td>0.35%</td>
<td>0.3%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Fatal bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracranial</td>
<td>0.002%</td>
<td>0.35%</td>
<td>0.3%</td>
<td></td>
</tr>
<tr>
<td>bleeding</td>
<td></td>
<td></td>
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<tr>
<td>Non-CABG major</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bleeding</td>
<td>4.5%</td>
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</tr>
</tbody>
</table>

Data are presented as absolute risk.

Aspirin denotes aspirin meta-analysis; CABG, coronary artery bypass graft; CAPRIE, Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events; PLATO, Platelet Inhibition and Patient Outcomes; TRITON-TIMI 38, Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction.
1.4 Treatment of bleeding associated with antiplatelet therapy

The treatment of patients who take DAPT and present with life threatening bleeding or require emergency surgery is challenging. The antiplatelet effect of oral antiplatelet agents persists for days after the last dose, and complete offset takes about three days for ASA, about five days for ticagrelor, and ten days for clopidogrel and prasugrel.\textsuperscript{27-29} Waiting three or more days for spontaneous offset of antiplatelet agents is not a feasible option when patients develop a clinically significant bleed. None of these antiplatelet agents have antidotes that are available currently. A phase 1 trial has shown that intravenous recombinant human IgG1 monoclonal antibody antigen-binding fragment PB2452 binds specifically to ticagrelor and AR-C124910XX and neutralises their platelet inhibitory properties in healthy volunteers, as measured by light transmission aggregometry (LTA), P2Y\textsubscript{12} and platelet reactivity test.\textsuperscript{30} PB2452 is not expected to reverse the effect of clopidogrel or prasugrel because these agents circulate in free form for only a short time before binding irreversibly to the platelet. Preliminary studies of another antidote to ticagrelor, MEDI2452, and sorbent haemadsorption have been performed.\textsuperscript{31-33} However, none of these reversal strategies are available for clinical use.

Platelet transfusions have the potential to reverse the anti-aggregant effects of antiplatelet agents by providing platelets with functional COX-1 which aids reversal of ASA, and/or functional P2Y\textsubscript{12} receptors which aid reversal of clopidogrel, prasugrel and ticagrelor. The effect of a platelet transfusion on aggregation is affected by the pharmacological characteristics of the antiplatelet agent and is discussed in the following sections.

1.4.1 Pharmacokinetic and pharmacodynamic properties of oral antiplatelet agents

The pharmacokinetic and pharmacodynamic properties of ASA, clopidogrel, prasugrel, and ticagrelor that are relevant for response to platelet transfusions are summarised in Table 2.

ASA irreversibly acetylates and inactivates platelet COX-1, thereby preventing formation of thromboxane A\textsubscript{2} and inhibiting arachidonic acid (AA) induced platelet aggregation.\textsuperscript{34} Non enteric coated ASA reaches a maximum concentration 30-40 minutes after ingestion and has a half-life
of 20 minutes. Elimination occurs by renal excretion.\cite{35} The onset of inhibition of AA mediated aggregation occurs within minutes of ingestion and lasts for the lifespan of the platelet.\cite{36} After ASA is fully bound or metabolised, new platelets which enter the bloodstream from the bone marrow are unaffected by ASA. The offset of ASA occurs as new platelets which have functional COX-1 enter the bloodstream. Full replacement of ASA-treated platelets requires about 10 days because mean platelet survival time is 180 to 220 hours.\cite{37} However, platelet function may be normal after about three days because AA mediated aggregation may be normal with as little as 20% platelets with functional COX-1.\cite{27,38}

Clopidogrel is a tetrahydrothienopyridine prodrug which is activated by \textit{in vivo} hepatic transformation and subsequent hydrolysis.\cite{39} Clopidogrel active metabolite (clopidogrel-AM), specifically the H4 isomer, is a thiol derivative that binds irreversibly to the platelet P2Y\textsubscript{12} receptor and inhibits ADP mediated aggregation.\cite{40} The maximum plasma concentration of clopidogrel occurs 30-60 minutes after ingestion, but due to the time required for transformation, clopidogrel-AM becomes detectable about four hours after ingestion.\cite{41} Clopidogrel has a half-life of six hours, whereas clopidogrel-AM has a half-life of 30 minutes.\cite{41,42} Clopidogrel-AM irreversibly inhibits platelet function for the lifespan of the platelet. The offset of the effect of clopidogrel occurs gradually over about 10 days as new platelets with functional P2Y\textsubscript{12} receptors enter the circulation.\cite{28} However, inter-subject variability in the response to clopidogrel may occur due to variable absorption, drug-drug interactions, drug-food interactions, genetic polymorphisms of Cytochrome P450 (CYP)2C19 and enhanced basal platelet reactivity.\cite{43,44}

Prasugrel is also a thienopyridine prodrug which requires bioactivation mediated by the first step of gut and plasma enzymatic hydrolysis, and a second step of hepatic bioactivation to produce prasugrel active metabolite (prasugrel-AM).\cite{45} Prasugrel-AM binds irreversibly to the platelet P2Y\textsubscript{12} receptor and inhibits ADP mediated aggregation. Prasugrel-AM is detectable in plasma 15 minutes after ingestion and reaches a maximum concentration 30 minutes after ingestion.\cite{46} Prasugrel-AM has a half-life of 3.7 to 7.4 hours.\cite{46} Excretion occurs in urine (70%) and faeces (27%).\cite{47} Platelet inhibition commences 15 minutes after ingestion and peaks at 4 to 6 hours.\cite{48}
The offset of the effect of prasugrel occurs gradually over about 10 days as new platelets with functional P2Y\textsubscript{12} receptors enter the circulation.

Ticagrelor binds to the platelet P2Y\textsubscript{12} receptor reversibly.\textsuperscript{50} When ticagrelor is bound to the P2Y\textsubscript{12} receptor it antagonises ADP mediated activation of G proteins, and after ticagrelor dissociates the receptor is left intact and ongoing reversible binding is permitted through a constant process of desensitisation and resensitisation.\textsuperscript{51, 52} Ticagrelor active metabolite (ticagrelor-AM) has the same mechanism of action as ticagrelor and is an equally potent platelet inhibitor.\textsuperscript{53} Ticagrelor has a rapid onset of action, reaches maximum concentration at 1.5 to 4 hours, and has a mean half-life of 7.7 to 14.1 hours.\textsuperscript{54, 55} Elimination occurs through hepatic metabolism and biliary excretion, with limited renal clearance.\textsuperscript{55} The onset of platelet inhibition occurs at 30 minutes and maximum inhibition occurs at 1 to 40 hours after ingestion.\textsuperscript{56} Ticagrelor produces inhibition of ADP-aggregation of 87 to 89\% during maintenance therapy with 90 mg twice daily which is greater than the magnitude of platelet inhibition that occurs with maintenance dosing of clopidogrel and prasugrel.\textsuperscript{54, 57, 58} The offset of the effect of reversibly-binding ticagrelor occurs as the concentration decreases, with full recovery at about five days after cessation, which is faster than the other oral P2Y\textsubscript{12} receptor antagonists which bind irreversibly.\textsuperscript{27, 37, 54, 59, 60}
### Table 2 Pharmacology of oral antiplatelet agents

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
<td>COX-1 inhibitor</td>
<td>2nd generation thienopyridine</td>
<td>3rd generation thienopyridine</td>
<td>Cyclopentyl-triazolo-pyrimidine</td>
</tr>
<tr>
<td><strong>Route</strong></td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>Prodrug</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Bioavailability</strong></td>
<td>50%</td>
<td>&gt;50%</td>
<td>&gt;80%</td>
<td>36% (25-64%)</td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>COX-1 enzyme</td>
<td>P2Y&lt;sub&gt;12&lt;/sub&gt; receptor</td>
<td>P2Y&lt;sub&gt;12&lt;/sub&gt; receptor</td>
<td>P2Y&lt;sub&gt;12&lt;/sub&gt; receptor</td>
</tr>
<tr>
<td><strong>Binding</strong></td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Irreversible</td>
<td>Reversible</td>
</tr>
<tr>
<td><strong>Drug Responsible for inhibition</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;max&lt;/sub&gt;, h</strong></td>
<td>0.5</td>
<td>N/A</td>
<td>N/A</td>
<td>1.5 – 2</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;0.5&lt;/sub&gt;, h</strong></td>
<td>0.25 – 0.33</td>
<td>6</td>
<td>Not detectable</td>
<td>7.7 – 13.1</td>
</tr>
<tr>
<td>Active metabolite</td>
<td>Salicylate</td>
<td>Responsible for inhibition</td>
<td>( t_{\text{max}}, ) h</td>
<td>( t_{0.5}, ) h</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>N/A</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 – 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>3.7-7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.5-12.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ASA denotes acetylsalicylic acid; COX-1, cyclooxygenase-1; h, hours; N/A, not applicable; \( t_{\text{max}}, \) time to maximum concentration; \( t_{0.5}, \) half-life.
1.4.2 Evidence that donor platelets reverse oral antiplatelet agents

Reversal of the platelet inhibitory effect of aspirin, clopidogrel, and prasugrel has been studied in in vitro mixing studies, where the effect of normal platelets on the aggregation of antiplatelet treated platelets is measured, and in small observational studies where patients receive a platelet transfusion and the change in aggregation is measured. In vitro mixing is a valid method to study antiplatelet agent reversal and is discussed further in section 1.7.2 Validity of in vitro antiplatelet agent reversal studies.

In vitro studies show that ASA can be reversed by transfusing 20-30% new non-aspirinated platelets. Li et al found that 30% normal platelets restored normal platelet function when measured by AA mediated aggregation in subjects treated with ASA 325 mg daily for seven days. The proportion of platelets required to restore platelet aggregation was similar when ASA is administered in combination with a P2Y₁₂ receptor antagonist. Data from an observational study of in vivo platelet transfusions is consistent with the in vitro data. Taylor et al studied 13 patients taking ASA who developed potentially life-threatening haemorrhage or required emergency neurosurgery. An average platelet transfusion dose of 0.13 UI/kg (range, 0.10-0.15 UI/kg) was administered which is equivalent to approximately 30% new platelets. The median aspirin reaction unit (ARU) increased from 420 to 630 (p=0.001), a result corresponding to normal platelet function.

Clopidogrel reversal has been studied in four studies which have been performed by mixing increasing proportions of donor platelets with platelets from subjects who had taken clopidogrel in combination with ASA. Li et al studied blood from six healthy subjects collected 12 hours after a seven-day course of treatment and was the most comprehensive study because it tested more mixing proportions than the other studies. For every 10% addition of untreated platelets, platelet aggregation increased from baseline in a linear manner by approximately 10%.

Prasugrel reversal has been studied in four in vitro studies. The most comprehensive study was that by Zafar et al (2013), in which 25 healthy volunteers took a loading dose of prasugrel 60
mg. Blood was collected 2, 6, 12, and 24 hours after prasugrel ingestion, and donor platelets were added to the prasugrel-treated whole blood within 30 seconds while prasugrel-AM was still stable. The donor platelets had a mean ADP-aggregation (maximum extent) of about 55% and were added in quantities to increase the platelet count of the prasugrel treated blood by 40%, 60%, and 80%. The platelet aggregation in the mixed specimen was then tested by LTA. Six hours after prasugrel ingestion, the ADP induced aggregation of prasugrel treated platelets was 10.8%, which increased to 18.9%, 23.6%, and 28.1% with addition of 40%, 60%, and 80% control platelets, respectively. The results of mixing at 12 and 24 hours were similar to those at 6 hours. This study also informed on the earliest time at which control platelets are effective at reversing prasugrel. Donor platelets had minimal effect on platelet aggregation two hours after ingestion of the prasugrel loading, but were effective at six hours onwards, suggesting that untreated platelets cease to be inhibited by residual prasugrel-AM between two and six hours.86

So far, two clinical studies have examined the efficacy and safety of platelet transfusion in patients treated with antiplatelet agents. The first was a case control study of patients taking ASA and/or clopidogrel who developed gastrointestinal bleeding. The cases who received a platelet transfusion had a higher odds of death than the controls who did not receive a platelet transfusion (OR, 5.57; 95% CI, 1.52 to 27.1). However, there was a significant imbalance between the groups at baseline because the cases had more severe bleeds than the controls. This was evidenced by lower blood pressure and haemoglobin, by higher heart rate and by the proportion admitted to the intensive care unit. The imbalance in the groups at baseline is likely to confound the primary outcome of death.87 The second clinical study was a randomised multicentre trial of platelet transfusion versus no transfusion in patients taking ASA in combination with either clopidogrel or dipyridamole who developed ICH. Platelet transfusion was associated with an increase in death or dependence at three months compared with no transfusion (adjusted common OR, 2.05; 95% CI, 1.18 to 3.56; p=0.0114). However, despite randomisation, a significant imbalance in the groups at baseline existed. The platelet transfusion arm had a significantly larger ICH volume than the no transfusion arm,88 and therefore the results must be interpreted with caution. The results of these trials for clinical
practice are uncertain and may not be generalisable to patients taking potent P2Y$_{12}$ inhibitors such as ticagrelor.

1.5 Patterns of oral antiplatelet “reversal” using platelet transfusion

Platelet transfusions do not directly reverse oral antiplatelet agents, but they can reverse the effect of antiplatelet agents by increasing platelet aggregation. There are three distinct patterns of reversal of the effect of oral antiplatelet agents. The three patterns of reversal are determined by the pharmacological properties of the oral antiplatelet agents and the results of reversal studies:

1. ASA: a platelet transfusion reverses the effect of ASA by replenishing COX-1 that can synthesise thromboxane A2 to support platelet aggregation. A threshold of reversal exists, which is unique to ASA reversal, whereby as little as 20% normal platelets with functional COX-1 is sufficient for normal AA mediated aggregation.$^{38}$

2. Clopidogrel and prasugrel: a platelet transfusion provides platelets with functional P2Y$_{12}$ receptors and increases platelet aggregation. A platelet transfusion is most effective if administered after the free active metabolites of clopidogrel and prasugrel are eliminated from the circulation so that they do not inhibit transfused platelets.

3. Ticagrelor: a platelet transfusion provides platelets with functional P2Y$_{12}$ receptors, to which ticagrelor molecules which dissociate from other platelets or exist free in the serum, can bind. Transfused platelets therefore dilute the ticagrelor molecules and provide haemostatically active platelets.

1.6 Focus on ticagrelor reversal using donor platelets

Ticagrelor reversal with donor platelets needs to be considered separately from the other oral antiplatelet agents because ticagrelor binds to platelet receptors reversibly, has a longer half-life, is associated with a higher risk of bleeding, and can inhibit transfused platelets. Furthermore, published studies of ticagrelor reversal have conflicting results.
1.6.1 Pharmacological properties of ticagrelor that affect reversal

When transfused to a recipient who takes ticagrelor, the effect of donor platelets on ADP-aggregation is determined by the pharmacological characteristics of ticagrelor.

Ticagrelor has the potential to inhibit donor platelets because it binds to the platelet P2Y$_{12}$ receptor reversibly and with a high affinity (dissociation constant 10.5 nM). The binding is non-competitive, whereby ADP and ticagrelor can bind simultaneously to the platelet P2Y$_{12}$ receptor at separate sites. When ticagrelor is bound to the P2Y$_{12}$ receptor it inactivates the receptor by antagonising the ADP-mediated activation of G proteins thereby blocking platelet activation and subsequent aggregation, without causing a permanent conformation change. After ticagrelor dissociates, the P2Y$_{12}$ receptor is left intact and its responsiveness is maintained by a desensitisation and resensitisation process that permits ongoing reversible binding. Therefore, ticagrelor can inhibit donor platelets the entire time it is present in the circulation.

Ticagrelor reversal likely requires a larger quantity of donor platelets compared to the other P2Y$_{12}$ inhibitors because ticagrelor produces significantly greater inhibition of platelet function as measured by LTA. Platelet inhibition with the standard maintenance dose of 90 mg twice daily is about 87%-89%, and with 60 mg twice daily is about 75%. The net platelet inhibition is due to the combined effect of ticagrelor directly inhibiting platelets, as well as ticagrelor-AM, AR-C124910XX, inhibiting platelets by the same mechanism of action with equal potency to ticagrelor. ASA, clopidogrel, and prasugrel do not have the property of both the parent compound and the AM inhibiting platelet function. Greater ticagrelor mediated platelet inhibition is also explained by it binding reversibly to the platelet which permits binding to an inducible pool of receptors on platelets, whereas these inducible receptors may not be blocked effectively by clopidogrel which binds irreversibly and has a shorter half-life.

Donor platelets are inhibited for a longer duration by ticagrelor compared to other oral antiplatelet agents because it has a mean half-life of 7.7 to 14.1 hours. ASA, clopidogrel-AM, and prasugrel-AM have shorter half-lives of 15 minutes, 30 minutes, and four hours, respectively, and can not inhibit donor platelets after their active metabolites are cleared.
Donor platelets are likely to be inhibited rapidly after they are transfused to a patient because ticagrelor binds rapidly. About 50% of platelet P2Y\textsubscript{12} receptors are occupied by ticagrelor after 3.8 minutes and equilibrium is reached within 15 minutes.\textsuperscript{50} The affinity for the receptor is high, with a dissociation constant (K\textsubscript{d}) of 10.5 nM.\textsuperscript{50, 51} Platelet inhibition becomes detectable by LTA 30 minutes after ingestion, and maximum inhibition occurs 1 to 4 hours after ingestion which coincides with maximum plasma concentrations.\textsuperscript{92}

With standard clinical doses of 90 mg twice daily or 60 mg twice daily, ticagrelor circulates in concentrations which exceed the threshold required for platelet inhibition. This would be expected to further increase the quantity of donor platelets required for effective reversal. Ticagrelor concentration during 90 mg twice daily maintenance dosing ranges between 305 ng/mL and 733 ng/mL which exceeds the 25 ng/mL level at which platelet inhibition occurs. Ticagrelor molecules circulate in an approximately 5,000 fold stoichiometric excess to platelet P2Y\textsubscript{12} receptors because there are about 2.88 x 10\textsuperscript{18} ticagrelor molecules present during maintenance dosing (assuming a ticagrelor concentration of 500 ng/mL and molecular weight of 522.567 g/mol), and 4.23 x 10\textsuperscript{14} platelet P2Y\textsubscript{12} receptors present (assuming a platelet count of 200 x 10\textsuperscript{9}/L, a blood volume of 5 L, and that each platelet has a mean of 423+/−28 P2Y\textsubscript{12} receptors at rest).\textsuperscript{91, 93}

The potential for donor platelets to completely reverse ticagrelor is likely to be greater after ticagrelor cessation. Since ticagrelor binds to platelets reversibly, the offset of ticagrelor mediated platelet inhibition after the last dose is rapid and closely follows the decreasing concentration of ticagrelor.\textsuperscript{92} When 24 hours from the last dose have passed, the platelet inhibitory effect of ticagrelor is lower than that of clopidogrel, and full offset occurs about five days after cessation.\textsuperscript{26, 54} Thus, it would become easier to reverse ticagrelor with donor platelets after cessation, particularly from 24 hours after the last dose.

There are seven \textit{in vitro} studies that have examined the reversal of ticagrelor mediated platelet inhibition using donor platelets, as shown in Table 3.
### Table 3 In vitro studies of ticagrelor reversal

<table>
<thead>
<tr>
<th>Study</th>
<th>Specimen, assay</th>
<th>Mix quantities</th>
<th>Comparison/s</th>
<th>Time post ticagrelor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zafar et al</strong> (2017)**&lt;sup&gt;34&lt;/sup&gt; (n=20)</td>
<td>Whole blood, impedance aggregometry induced by ADP 6.5 µM;</td>
<td>Donor platelets added to patients blood to increase the platelet count by</td>
<td><strong>1.</strong> Aggregation of mixed specimen versus prior to mixing</td>
<td><strong>Baseline</strong> 4 hours</td>
<td><strong>Multiplate®</strong> PRU</td>
</tr>
<tr>
<td></td>
<td>25%, 50%, 75% VerifyNow, LTA</td>
<td></td>
<td><strong>1.</strong> Aggregation</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td><strong>2.</strong> Aggregation</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>of mixed 4 hours</td>
<td></td>
<td><strong>67.0 ± 14.3</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>13.4 ± 6.0</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>25%</strong>: <strong>20.9 ± 6.1</strong>*</td>
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<tr>
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<td></td>
<td></td>
<td><strong>22.1 ± 5.9</strong>*</td>
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<td></td>
<td></td>
<td></td>
<td><strong>24.2 ± 6.3</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>75%</strong>: <strong>24.2 ± 6.3</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hansson et al (2017)(^{15}) (n=15)</td>
<td>Whole blood, impedance aggregometry</td>
<td>Donor:Patient &quot;Low dose&quot; 1:2</td>
<td>Aggregation of mixed specimen versus prior to mixing</td>
<td>12 hours Low-dose: 10 ± 7</td>
<td>Baseline: 8 ± 6</td>
</tr>
<tr>
<td>----------------------------------</td>
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<td>-----------------------------------------------</td>
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<td>----------------</td>
</tr>
<tr>
<td>25%: 35.5 ± 15.1*</td>
<td>147.6 ± 65.2*</td>
<td>158.0 ± 43.5*</td>
<td>48 hours 0%: 39.1 ± 21.3</td>
<td>214.8 ± 74.0</td>
<td></td>
</tr>
<tr>
<td>50%: 43.3 ± 16.7*</td>
<td>154.0 ± 51.0</td>
<td>47.5 ± 18.2*</td>
<td>25%: 56.8 ± 19.5*</td>
<td>219.7 ± 52.2</td>
<td></td>
</tr>
<tr>
<td>75%: 47.5 ± 18.2*</td>
<td>47.5 ± 18.2*</td>
<td>50%: 63.8 ± 19.1*</td>
<td>221.1 ± 44.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%: 39.1 ± 21.3</td>
<td>203.9 ± 41.5</td>
<td>75%: 70.3 ± 23.0*</td>
<td>214.8 ± 74.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*\(p<0.05\) versus corresponding 0% mix

Significant increase in aggregation which is time and dose dependent.

Results for impedance aggregometry are presented.
Induced by ADP

<table>
<thead>
<tr>
<th></th>
<th>Low-dose: 12 ± 7</th>
<th>High-dose: 12 ± 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5 µM (U)</td>
<td>48 hours Baseline: 24 ± 19</td>
<td>Low-dose: 21 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-dose: 22 ± 11</td>
</tr>
<tr>
<td>72 hours Baseline: 40 ± 26</td>
<td>Low-dose: 37 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-dose: 33 ± 19</td>
</tr>
<tr>
<td>96 hours Baseline: 52 ± 28</td>
<td>Low-dose: 41 ± 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-dose: 42 ± 19</td>
</tr>
</tbody>
</table>

ADP-Aggregation did not improve when adding platelets.

<table>
<thead>
<tr>
<th>Martin et al95</th>
<th>Whole blood, 75 µL donor platelets, aggregometry</th>
<th>Aggregation of mixed specimen increased</th>
<th>Not defined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole blood: platelet supplementation failed to correct inhibition of ADP-aggregation (median 2 Ω [range, 0.5 to 2.8]) compared to ticagrelor spiked blood (median 2 Ω [range, 1.3 to 2.8]), p&gt;0.05.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(n=6 blood and n=6 PRP) induced by ADP 20 µM; patient platelet count by at least 60% versus prior to mixing PRP: platelet supplementation failed to correct inhibition of ADP-aggregation (median 13.5% [range, 12.5 to 15.5]) compared to ticagrelor spiked PRP (median 15% [range, 10 to 17]), p>0.05.

<table>
<thead>
<tr>
<th>Bonhomme et al(^{80})</th>
<th>PRP, LTA induced by ADP</th>
<th>20%, 40%, 60%, 80% (the ratio of donor platelets to patient platelets)</th>
<th>Aggregation of mixed specimen versus prior to mixing</th>
<th>Median 5.5 hours (IQR 4.3 to 5.9)</th>
<th>PRP-patient: 41.8 ± 9.1%; PRP-20%: 47.7 ± 9.6%; PRP-40%: 41.9 ± 8.9%; PRP-60%: 49.2 ± 9.9%; PRP-80%: 44.6 ± 10.4% (p=0.3 for linear mixed effects model).</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Connor et al(^{83})</td>
<td>PRP, LTA induced by ADP</td>
<td>Patient/Donor: 100%/0%; 70%/30%; 100%/0%; 70%/30%</td>
<td>Aggregation of mixed specimen versus prior to mixing</td>
<td>4 hours</td>
<td>Baseline (prior to ticagrelor): 56.6 ± 11.9%; 100%/0%: 4.8 ± 5.0%; 70%/30%: 8.3 ± 8.8%;</td>
</tr>
</tbody>
</table>
### Hansson et al. (2014)

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Baseline</th>
<th>Aggregation of</th>
<th>2 hours</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood, impedance aggregometry induced by ADP 6.5 μM</td>
<td>Baseline: 1 mL blood + 300 μL PBS; Low: 1 mL blood + 50 μL donor platelets + 250 μL PBS; Medium 1 mL blood + 100 μL donor platelets + 200 μL PBS;</td>
<td>Aggregation of mixed specimen versus prior to mixing</td>
<td>Baseline: 12 U (95% CI 8 to 15); Low: 14 U (11 to 16), p versus baseline &lt;0.05. Medium: 15 (12 to 18), p versus baseline &lt;0.01. High: 16 (13 to 18), p versus baseline &lt;0.01.</td>
<td>Platelet supplementation improved aggregation in samples.</td>
</tr>
</tbody>
</table>

Addition of platelets increased aggregation.

(n=10)

50%/50%; ticagrelor treatment

20%/80%.

50%/50%: 10.9 ± 10.9%;

20%/80%: 22.7 ± 11.5%;

p=0.0052 for trend.
**Hobl et al**<sup>96</sup> (n=20)

|          | Whole blood, impedance aggregometry induced by ADP 6.4 µM | Donor platelets/Subject mixed specimen versus prior to mixing 3 hours | Baseline (prior to ticagrelor): 71 ± 16 U; Whole blood (after ticagrelor, prior to mixing): 16 ± 8 U; 1:10: 31 ± 14 U, p versus pre-mix <0.01; 1:5: 41 ± 14 U, p versus pre-mix <0.01; 1:3: 48 ± 18 U, p versus pre-mix <0.01.
<table>
<thead>
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</thead>
<tbody>
<tr>
<td></td>
<td>Whole blood: 1:10: 1:5: 1:3</td>
<td>mixed specimen 1:10: 1:5: 1:3</td>
<td>1:10: 31 ± 14 U, p versus pre-mix &lt;0.01; 1:5: 41 ± 14 U, p versus pre-mix &lt;0.01; 1:3: 48 ± 18 U, p versus pre-mix &lt;0.01.</td>
</tr>
</tbody>
</table>

Baseline aggregation describes the platelet aggregation of the treated person prior to adding donor platelets.

ADP denotes adenosine diphosphate; CI, confidence interval; IQR, interquartile range; LTA, light transmission aggregometry; n, number; PBS, phosphate buffered saline; PRP, platelet rich plasma; PRU, P2Y<sub>12</sub> reaction units; U, units.
1.6.2 Evidence for ticagrelor reversal

Four in vitro studies demonstrate that the addition of donor platelets to ticagrelor treated platelets increases ADP-aggregation. These studies were performed by mixing increasing proportions of donor platelets with platelets from patients who had taken ticagrelor.

Zafar et al (2017) studied whole blood from 20 patients with CAD who took a loading dose of ticagrelor (180 mg), and then, after a washout period, subsequently took five days of maintenance dose of ticagrelor (180 mg load followed by 90 mg every 12 hours). Blood was collected 4, 6, 24 and 48 hours after the loading dose and the maintenance dose. Donor platelets were mixed with ticagrelor treated blood and the aggregation was measured by LTA and impedance aggregometry. Donor platelets increased the ADP-aggregation in all mixes at all time-points. Complete reversal was observed at 48 hours with the addition of 50% donor platelets. The effect of donor platelets was similar whether they were added after the loading dose or after the maintenance dosing.94

Despite differences in the dose of ticagrelor (loading versus maintenance dose) and time of blood collection, the results of the three other studies were consistent with the study by Zafar et al (2017). In the study by O’Connor et al, mixing of donor platelets four hour after ticagrelor ingestion showed up to about 33% reversal of the effect of ticagrelor in a mixture containing 20% patient platelet rich plasma (PRP)/80% donor PRP.83 In the study by Hobl et al, mixing donor platelets three hours after ticagrelor showed up to about 25% reversal of the effect of ticagrelor in a mixture containing one part donor PRP and three parts patient whole blood.96 In the study by Hansson et al (2014), mixing donor platelets two hours after ticagrelor produced a statistically significant increase in the platelet aggregation above baseline, however there was no aggregation data prior to ticagrelor treatment to gauge the extent of ticagrelor reversal.84

1.6.3 Evidence against ticagrelor reversal

Three in vitro studies conflict with the abovementioned studies because they demonstrate that donor platelets do not increase ADP-aggregation of ticagrelor treated specimens.
Hansson et al (2017) performed a prospective observational study of 15 patients who were treated with ticagrelor. Patients had blood collected at five time-points between 12 and 96 hours after the last dose of ticagrelor and donor platelets were added in vitro. ADP mediated aggregation did not improve at any time-point with addition of donor platelets.⁷⁵ The lack of improvement in aggregation likely occurred because stored donor platelets were used for the in vitro mixing experiments.⁹⁷ Martin et al studied ticagrelor reversal in ticagrelor-spiked specimens from the blood bank. The addition of donor platelets did not increase ADP mediated aggregation.⁹⁵ Bonhomme et al studied 15 patients who were treated with ticagrelor, and collected blood a median of 5.5 hours after ticagrelor. The addition of donor platelets to ticagrelor treated specimens increased the ADP-aggregation by 0.7% per 20% increase in the ratio of donor to patient platelets. Thus, there was no substantial improvement in aggregation in this study either.⁸⁰

The effect of donor platelets has also been studied in vivo in subjects treated with ticagrelor using ADP-mediated platelet aggregation as a surrogate marker for clinically relevant outcomes. In the study by Teng et al, ADP-mediated platelet aggregation was evaluated by LTA in healthy subjects who received an autologous platelet transfusion 24 hours and 48 hours after a loading dose of ticagrelor. This study showed there was no improvement in platelet aggregation when the transfusion was administered 24 hours after ticagrelor, and minimal improvement when the transfusion was administered 48 hours after ticagrelor. The subjects were transfused with one apheresis unit of platelets,⁹⁸ which is equivalent to an in vitro mix containing 80% subject and 20% donor platelets. The in vitro studies suggest that such a quantity is insufficient for ticagrelor reversal.

Three case reports have shown that the effect of ticagrelor has not been reversed by transfused platelets in cumulative amounts up to 14 units of pooled platelets, as assessed by Multiplate®, VerifyNow® P2Y12, vasodilator stimulated phosphoprotein (VASP) and Plateletworks.⁹⁹-¹⁰¹ However, these case reports do not specify the age of the platelets which were transfused.
1.7 Designing the current study

1.7.1 Determining where evidence is lacking

Based on \textit{in vitro} and \textit{in vivo} studies in the literature, there is lack of consensus about the efficacy of platelet transfusion in reversing the effect of ticagrelor. The lack of consensus arises because of conflicting results between studies and the lack of guidance in relating \textit{in vitro} data to clinical transfusion practice. As a result, there is uncertainty on how patients requiring ticagrelor reversal should be managed.

The available \textit{in vitro} data for ticagrelor reversal suggests that donor platelets generally have shown minimal reversal of ticagrelor. However, most of these studies were performed within 12 hours of ticagrelor ingestion, when ticagrelor is still present in the bloodstream and is able to inhibit newly transfused platelets. The maximum quantity of donor platelets tested was 13 units of platelets in one study, but in all other studies the quantity was lower and likely insufficient to reverse ticagrelor.

Different sources of platelets used in the previous studies are likely to contribute to variability in the results. In the studies by Zafar \textit{et al} (2017) and Hansson \textit{et al} (2017), in which donor platelets did and did not reverse ticagrelor, respectively, freshly collected donor platelets were used in the first study and stored donor platelets (median age 1.8 days old) were used in the second study.\textsuperscript{75, 94} Stored donor platelets have an attenuated response to a single agonist, particularly ADP and epinephrine. Despite no increase in aggregation with single agent ADP, the Hansson \textit{et al} (2017) study demonstrated a significant increase in aggregation in response to AA and tartrate resistant acid phosphatase when donor platelets were added to ticagrelor treated blood. Therefore, the lack of increase in aggregation with ADP as a single agent does not negate the ability of donor platelets to increase ADP-aggregation in ticagrelor treated platelets.
1.7.2 Validity of *in vitro* antiplatelet agent reversal studies

The potential for donor platelets to reverse the effect of antiplatelet agents can be investigated by *in vitro* mixing studies. The mixing studies are performed by adding donor platelets to platelets obtained from subjects receiving an antiplatelet agent, and measuring the platelet aggregation. *In vitro* platelet mixing studies are valid for predicting the reversal of antiplatelet agents because they simulate an *in vivo* platelet transfusion, and they directly measure the effect of the antiplatelet agent on its receptor.

*In vitro* mixing studies involve two surrogates for clinical events. The first is that adding donor platelets to platelets obtained from subjects receiving an antiplatelet agent is a surrogate for a platelet transfusion. This is a reasonable surrogate because the transfusion of platelets to a patient results in a mixture of inhibited and uninhibited platelets. The second is that the increase in platelet aggregation after mixing is a surrogate for haemostasis. This is a reasonable surrogate because, based on randomised trials of the P2Y$_{12}$ inhibitors, the degree of inhibition of ADP mediated platelet aggregation correlates with the risk of bleeding. These trials include high versus low dose clopidogrel, prasugrel versus clopidogrel, and ticagrelor versus clopidogrel.\textsuperscript{18, 102, 103}

1.7.3 Key aspects of the current study

To overcome the limitations of existing studies of ticagrelor reversal using donor platelets, the current study was performed to investigate the *in vitro* reversal of ticagrelor at various time-points after the last dose using a wide range of quantities of donor platelets.

Justification for the key aspects of the study are as follows.

*Antiplatelet dosing*

Ticagrelor was administered to subjects at the dose used in clinical practice, which is a loading dose of 180 mg orally followed by a maintenance dose of 90 mg every 12 hours. Five days of treatment was administered because this is a sufficient duration to achieve steady-state levels seen during maintenance treatment.\textsuperscript{57} This study focussed on ticagrelor reversal during
maintenance therapy because it is anticipated that most bleeds in patients who take ticagrelor occur during maintenance treatment rather than after the initial loading dose. ASA was administered in combination with ticagrelor because this is standard clinical practice. The dose of ASA used was 81 mg daily.

*Time of blood collections*

Blood was collected at seven time-points, as follows:

1. Immediately prior to the final dose of a five-day course of ticagrelor, to test reversal at trough ticagrelor concentration during steady-state dosing;
2. Two hours after the last dose of ticagrelor, to test reversal at the time of maximum ticagrelor concentration which corresponds to the nadir platelet aggregation;
3. 10, 24, 48, 72 and 96 hours after the last dose of ticagrelor, to test reversal during the offset of the effect of ticagrelor. 96 hours should be a sufficient duration to study the offset because ticagrelor and AR-C124910XX have a half-life between 7-12 hours and a prior study by Gurbel *et al* indicates that the offset of ticagrelor requires three to five days.²⁸,⁵³

*Source of donor platelets*

Fresh platelets from a single donor were used in all mixing studies. Fresh platelets were preferred because they are more haemostatically active during aggregation testing. Stored platelets were not used because there is a continuous decrease in ADP-aggregation with time, and may introduce bias into the results.⁹⁷,¹⁰⁴

*Quantities of donor platelets*

This study tested a quantity of donor platelets ranging from a mix containing subject 90%/donor 10% to subject 10%/donor 90%, corresponding to 11% and 900% donor platelets relative to subject platelets, respectively. The equivalent number of *in vivo* platelet transfusions for these *in vitro* mixes range between 1 unit and 30 units of apheresis platelets, as detailed in section 3.6.3. Conversion of *in vitro* mixes to number of platelet units. A feasible maximum number of platelet units in a tertiary hospital practice is about four units but this depends on local supply. The
range of platelet units in this study is much broader than in prior studies as the highest quantity of donor platelets tested previously was 400% by O’Connor *et al.* Testing high quantities of donor platelets is important to attempt to define the upper limit of platelets required for ticagrelor reversal, which is likely to be high because ticagrelor circulates in stoichiometric excess to the platelet P2Y<sub>12</sub> receptor.

*Specimen and laboratory assay*

PRP with an adjusted platelet count of 200 x 10<sup>9</sup>/L was used because this is within the platelet count range of 200 to 300 x 10<sup>9</sup>/L which is ideal for LTA. It also ensured consistency in the proportions of the platelets from the subject and donor, thus improving the accuracy of the aggregation results and subsequent extrapolation to the number of units of platelets to transfuse. PRP was tested by LTA because the equipment is available in the research laboratory at the Population Health Research Institute, Hamilton, Canada, and because LTA is considered the gold standard test of platelet function.

*ADP concentration in LTA testing*

An ADP final concentration of 10 µM was chosen for two reasons. First, the aggregation testing was performed using Chrono-log reagents. The reagent information leaflet suggests an ADP final concentration of 10 µM when testing PRP. Second, the ADP concentration of 10 µM in the current study allows comparison with other *in vitro* studies of ticagrelor reversal, in which the ADP final concentration ranged between 6.4 µM to 20 µM, as shown in Table 4.
Table 4 ADP concentration in previous studies of ticagrelor reversal

<table>
<thead>
<tr>
<th>Author</th>
<th>Specimen</th>
<th>Modality</th>
<th>ADP final concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maillard et al(^9)</td>
<td>PRP</td>
<td>LTA</td>
<td>20</td>
</tr>
<tr>
<td>O’Connor et al(^8)</td>
<td>PRP</td>
<td>LTA</td>
<td>20</td>
</tr>
<tr>
<td>Bonhomme et al(^8)</td>
<td>PRP</td>
<td>LTA</td>
<td>10</td>
</tr>
<tr>
<td>Hobl et al(^6)</td>
<td>WB</td>
<td>Multiplate®</td>
<td>6.4</td>
</tr>
<tr>
<td>Hansson et al (2014)(^4)</td>
<td>WB</td>
<td>Multiplate®</td>
<td>6.5</td>
</tr>
<tr>
<td>Zafar et al (2017)(^4)</td>
<td>WB</td>
<td>VerifyNow®</td>
<td>20</td>
</tr>
<tr>
<td>Hansson et al (2017)(^5)</td>
<td>WB</td>
<td>Multiplate®</td>
<td>6.5</td>
</tr>
</tbody>
</table>

ADP denotes adenosine diphosphate; LTA, light transmission aggregometry; PRP, platelet rich plasma; WB, whole blood.

**Defining ticagrelor reversal**

A stringent definition of ticagrelor reversal is increasing the ADP-aggregation of ticagrelor treated platelets from the low level seen during antiplatelet treatment to the pretreatment level, using donor platelets. However, this definition may be difficult to precisely define in a small pilot study with unavoidable variation in LTA. Furthermore, the exact cutoff of ADP-aggregation at which blood clot formation is normal, is not known. A more conservative definition of reversal will therefore be used. Thus, in this study we will use a post-mix aggregation of >90% of the pretreatment aggregation to indicate a major improvement in aggregation that is likely to be clinically important for haemostasis, and call this improvement a “major response”.

“Partial response” will be considered as well, because major response may not be achievable in the first 24 hours after ticagrelor dosing. Partial response will be defined as a post-mix ADP-aggregation >50% of the pretreatment aggregation, which is likely to be clinically helpful for haemostasis when a major response is not achieved.
Aims and hypothesis

2.1 Rationale

Ticagrelor plays a key role in the reduction of thrombotic events in patients with CAD, but increases the risk of bleeding. When bleeding during ticagrelor treatment occurs, management is difficult because ticagrelor does not have an antidote and its antiplatelet effects persist for days after the last dose. Platelet transfusion provides new platelets with functional P2Y₁₂ receptors which has the potential to reverse the antiplatelet effect of ticagrelor and promote haemostasis. However, the optimal timing of transfusion and quantity of platelet units have not been defined.

2.2 Aims

Our aims are to characterise the quantity and time dependent effects on ADP-aggregation when donor platelets are mixed in vitro with platelets from subjects treated with ticagrelor and ASA. Specifically, this project aims to determine the minimum quantity of donor platelets required for major (>90%) reversal of ticagrelor, and the minimum quantity of donor platelets required for 50% reversal of ticagrelor at time-points up to 96 hours after the last dose.

2.3 Outcomes

The primary outcomes are to determine the minimum quantity of donor platelets required to achieve 1) a major response and 2) 50% reversal at each time-point. A major response is defined as ≥90% improvement in mean aggregation of the mixed specimen when compared to the pretreatment ADP-aggregation. 50% reversal of ticagrelor is defined as the mid-point of the pretreatment ADP-aggregation and the ADP-aggregation immediately prior to the last dose of ticagrelor.

The secondary outcome was to measure the effect of free ticagrelor in platelet poor plasma (PPP) on the ADP-aggregation of donor platelets.
2.4 Hypothesis

Major reversal of ticagrelor using donor platelets will not be possible in the first 24 hours, but will be possible after 24 hours.

This hypothesis will be tested by comparing ADP-aggregation post-mixing to the pretreatment ADP-aggregation for all mixtures at all time-points. If the hypothesis is correct, the ADP-aggregation post mixing will not achieve major reversal (>90%) at the 2 and 10 hour time-points post ticagrelor. However, major reversal should be possible at 24, 48, 72, and 96 hours post ticagrelor.

2.4.1 Previous observations facilitating hypothesis generation

In contrast to ASA, clopidogrel-AM and prasugrel-AM, reversing ticagrelor with platelet transfusions is likely to be more challenging because it binds reversibly to the platelet and has a longer half-life, therefore is able to inhibit donor platelets long after the last dose. Furthermore, ticagrelor circulates in stoichiometric excess to the platelet P2Y₁₂ receptor and causes greater inhibition of ADP-aggregation during maintenance dosing.⁵⁷, ¹⁰⁶

Complete reversal of ticagrelor in the first 24 hours is likely to be difficult because previous in vitro studies show that donor platelets do not completely reverse ticagrelor within this time period. There are five in vitro studies in which reversal was studied between two and six hours after ticagrelor was administered. One of these studies showed that the addition of donor platelets did not significantly increase aggregation.⁸⁰ The four other studies showed that donor platelets improved ADP-aggregation in a dose-dependent manner, however, only partial reversal was achieved.⁸³, ⁸⁴, ⁹⁴, ⁹⁶ The highest aggregation that was achieved after the addition of donor platelets was about 50% of the level seen prior to ticagrelor administration. The maximum quantity of donor platelets in these studies was four times the number of treated platelets. Complete reversal was not achieved in any study of reversal within 24 hours of ticagrelor dosing.
Ticagrelor reversal is likely to be feasible from about 24 hours after the last dose because the concentration of ticagrelor decreases in the bloodstream. In the ONSET/OFFSET study, in which antiplatelet treatment was ceased and the offset of the antiplatelet agent was measured, the platelet inhibitory effect of ticagrelor diminished more rapidly than clopidogrel. From 24 hours after the last dose, platelet inhibition in patients who had taken ticagrelor was lower than those who had taken clopidogrel, despite ticagrelor causing greater platelet inhibition than clopidogrel between zero and 24 hours after dosing.28 The key reason for the rapid offset of ticagrelor is that it binds reversibly to the platelet P2Y\textsubscript{12} receptor and the inhibition of the receptor closely follows drug exposure. Thus, during elimination of ticagrelor the inhibition of the platelet P2Y\textsubscript{12} receptor decreases quickly and offset of platelet inhibition is complete by about three to five days which is when ticagrelor is fully eliminated.50,57 In contrast, clopidogrel has a slower offset of action because it binds irreversibly to platelets and its offset occurs as new platelets enter the bloodstream, which takes ten days for full turnover.37,59
Methods

3.1 Setting and recruitment

The study was conducted at the Population Health Research Institute, Hamilton, Canada. Healthy volunteers were recruited by the Research Assistant who explained the requirements of the study and provided a Participant Information Sheet and Consent Form. Potential participants who were interested in the study subsequently met with the Principal Investigator to discuss the study requirements in detail. Written, informed consent was obtained prior to entering the study.

3.2 Inclusion and exclusion criteria

The inclusion criteria are:

1. Age >18 years.
4. Willing and able to provide written informed consent.

The exclusion criteria are:

1. Abnormal platelet aggregation prior to treatment.
2. Thrombocytopenia prior to treatment (platelet count <150 x 10^9/L).
3. Known coagulation disorder such as von Willebrand disease or haemophilia.
4. Known allergy or intolerance to ASA or ticagrelor or any of the excipients.
5. Consumption of ASA containing products within the preceding 14 days.
6. Consumption of antiplatelet or anticoagulant medications within the preceding 14 days.
7. Consumption of medications within the preceding 14 days that potentially interfere with ticagrelor metabolism through CYP3A4, CYP3A or P-glycoprotein, including ketoconazole, clarithromycin, nefazodone, ritonavir, atazanavir, diltiazem, amprenavir, aprepitant, erythromycin, fluconazole, verapamil, rifampicin, dexamethasone, phenytoin,
carbamazepine, phenobarbital, cyclosporin, simvastatin, atorvastatin, tolbutamide, digoxin.

8. Pregnant or trying to conceive.
10. Gastrointestinal bleeding, ulcers, intracranial bleeding, or trauma within the previous six months.
11. Surgery within the previous 30 days.

To participate in the study, all inclusion criteria and none of the exclusion criteria had to be met.

3.3 Study flow

Subjects who were potentially eligible for the study had a venous blood specimen collected prior to treatment ("pretreatment"). The blood was collected into one K$_2$ ethylenediaminetetraacetic acid (EDTA) 7.2 mg tube and three sodium citrate 3.2% tubes (Becton, Dickinson and Company [BD] Vacutainer™, Franklin Lakes, New Jersey, United States of America). A full blood picture was measured from the K$_2$ EDTA tube using a Becton Dickinson COULTER® A€•T diff 2™ Haematology Analyser. Platelet aggregation was tested in PRP prepared from the sodium citrate tubes. Women of childbearing potential had a serum tube collected for testing of quantitative beta human chorionic gonadotropin (beta-HCG).

To continue in the study, the results of the pretreatment blood test had to be normal, defined as follows:

1. Platelet count 150 to 400 x 10$^9$/L;
2. Platelet aggregation in response to:
   2.1 ADP-mediated aggregation 71% to 88%;
   2.2 AA-mediated aggregation of 74% to 99%;
   2.3 Collagen-mediated aggregation 70% to 94%, (defined by the manufacturer$^{107}$);
3. Negative pregnancy test (serum beta-HCG <5 IU/L).

The study design is shown in Figure 2.
Eligible subjects took ticagrelor (AstraZeneca, Brilinta®, Mississauga, Canada) 180 mg loading dose, followed by 90 mg twice daily, in combination with ASA (Bayer Inc., Aspirin®, Mississauga, Canada) 81 mg daily for a total of five days. The last doses of both were administered at 0800 on study day 6. Blood was collected immediately prior to the last doses, and at 2, 10, 24, 48, 72, and 96 hours after the last dose of ticagrelor and ASA. An untreated single donor had blood collected at the same time-points as each subject, and was the
3.4 Blood collection and plasma preparation

Venostasis was minimised by collecting venous blood either without a tourniquet or after releasing the tourniquet at the commencement of blood collection. Blood was collected with a 21-gauge needle and drawn directly into BD Vacutainer™ tubes.

Plasma was prepared by centrifugation of blood in sodium citrate 3.2% tubes in an Eppendorf 5810 R Centrifuge (Eppendorf AG, Hamburg, Germany). PRP was prepared by centrifuging whole blood at 200 g for 10 minutes, without brake. The PRP was removed from the sodium citrate tubes and stored in a test tube. PPP was prepared by centrifuging the remaining specimen in the tubes from which PRP was removed, at 1500 g for 15 minutes, without brake. The PPP was removed from the tubes and stored in a test tube.

All platelet aggregation studies were performed on PRP that had a platelet count adjusted to 200 x 10⁹/L. To prepare PRP adjusted to 200 x 10⁹/L, the platelet count of PRP was measured and autologous PPP was added to obtain a final platelet count of 200 x 10⁹/L. The formula for preparing PRP with a platelet count of 200 x 10⁹/L is shown in Appendix 1. Plasma specimens were kept at room temperature throughout the duration of testing.

3.5 Platelet aggregation testing

Platelet aggregation in PRP was measured by LTA. Two aggregometers were used, Chrono-log models 490-2D and 560-VS (Chrono-log Corporation, Havertown, Pennsylvania, United States of America) as shown in Figure 3.

The difference in optical light transmission between PRP and PPP was set as 100%. PRP was warmed to 37°C for three minutes before adding the agonist. In the baseline study, the agonists
used and their final concentrations were ADP 10 μM, AA 1 mM, and collagen 2 μg/L. In the mixing studies, only ADP was used. Platelet aggregation was monitored for six minutes after adding the agonist. Results were considered non-evaluable if haemolysis, insufficient signal, or unstable baseline were present. Specimens were tested in duplicate.

**Figure 3 Light transmission aggregometers**

Platelet aggregation testing was performed in a Chrono-log 490 2D optical aggregometer (centre) and Chrono-log 560-VS in optical mode (right). The output was connected to a computer (left) with the AGGRO/LINK® software package. Each aggregometer had two wells, and using the aggregometers allowed up to four specimens to be tested simultaneously in wells 1 to 4 (left to right in picture). To minimise inter-well variability, each well was used for the same test throughout the study. Well 1 was used for testing PRP mixes. Well 2 was used for simultaneously testing the duplicate of Well 1. Well 3 was used for testing specimens for stability. Well 4 was used for testing the subject PPP/donor PRP mixes.

### 3.6 Mixing studies

The mixing studies were performed at seven time-points between study days 6 and 10 inclusive. For each mixing study, the subject and donor had venous blood collected into five 4.5 mL sodium citrate 3.2% tubes. Plasma was prepared from the subject and donor specimens.
Mixed specimens were prepared by pipetting different quantities of PRP from subject and donor into cuvettes. The experiment design for each mixing study is shown in Figure 4.
Blood collected from subject and donor

PRP and then PPP prepared by centrifugation

Platelet count in PRP measured

PRP with platelet count adjusted to $200 \times 10^9$/L prepared

Subject and donor PRP pipetted in cuvettes

15-minute incubation at room temperature

Warm to $37^\circ$C for 3 minutes in aggregometers

Add ADP

Aggregation measured for 6 minutes

Every mixing study involved preparing 38 specimens (1 subject, 1 donor, 9 PRP mixes, 4 PPP mixes, 4 drifts, all in duplicate). To complete testing of all specimens within three hours of blood collection, up to four aggregometry wells were used simultaneously for their dedicated specimen, and the subsequent specimens were prepared and incubated while the previous was still being tested.

ADP denotes adenosine diphosphate; PPP, platelet poor plasma; PRP, platelet rich plasma.
3.6.1 PRP mixing studies

Subject PRP and donor PRP were mixed in nine proportions. The smallest quantity of donor platelets was subject 90%/donor 10% (11% donor platelets relative to subject), and the largest was subject 10%/donor 90% (900% donor platelets relative to subject) as shown in Table 5. Mixed specimens were incubated at room temperature for 15 minutes prior to LTA testing to allow ticagrelor sufficient time to redistribute. Thereafter, the specimens were warmed and tested.

Table 5 Quantities in PRP mixes

<table>
<thead>
<tr>
<th>PRP mix</th>
<th>PRP volumes (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject</td>
</tr>
<tr>
<td>Subject</td>
<td>250</td>
</tr>
<tr>
<td>Subject 90%/Donor 10%</td>
<td>225</td>
</tr>
<tr>
<td>Subject 80%/Donor 20%</td>
<td>200</td>
</tr>
<tr>
<td>Subject 70%/Donor 30%</td>
<td>175</td>
</tr>
<tr>
<td>Subject 60%/Donor 40%</td>
<td>150</td>
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<tr>
<td>Subject 50%/Donor 50%</td>
<td>125</td>
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</tr>
<tr>
<td>Donor</td>
<td>0</td>
</tr>
</tbody>
</table>

“PRP mix” is the mix description used throughout the paper, and the corresponding volumes are shown. The final volume in all cuvettes was 250 µL.

PRP denotes platelet rich plasma.
3.6.2 PPP mixing studies

Subject PPP was mixed with donor PRP to test whether free ticagrelor in PPP inhibits donor platelets. The smallest quantity of subject PPP was subject 10%/donor 90%, and the largest was subject 30%/donor 70% as shown in Table 6. Adding more than 30% subject PPP was not performed because it resulted in dilution of the platelet count in the mixed specimen to less than 200 x 10^9/L in most cases. Additional calculations were required to prepare PRP for these studies so that a final platelet count of 200 x 10^9/L was maintained, as described in Appendix 2.

Table 6 Quantities in Subject PPP/Donor PRP mixes

<table>
<thead>
<tr>
<th>Subject PPP/Donor PRP mix</th>
<th>Plasma volumes (µL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject</td>
<td>Donor</td>
</tr>
<tr>
<td>Subject 30%/Donor 70%</td>
<td>75</td>
<td>175</td>
</tr>
<tr>
<td>Subject 20%/Donor 80%</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Subject 10%/Donor 90%</td>
<td>25</td>
<td>225</td>
</tr>
<tr>
<td>Donor only</td>
<td>0</td>
<td>250</td>
</tr>
</tbody>
</table>

The final volume in all cuvettes was 250 µL.

PPP denotes platelet poor plasma; PRP, platelet rich plasma.

3.6.3 Conversion of in vitro mixes to number of platelet units

Conversion of the in vitro mixing quantities to the number of units of platelets to transfuse in vivo can be estimated using the platelet count of the recipient and the number of platelets in each unit, as shown in Table 7.
Table 7 Conversion of in vitro mix quantities to number of platelet units to transfuse

<table>
<thead>
<tr>
<th>In vitro mixes</th>
<th>Apheresis or pooled units</th>
<th>Random donor units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 90%/Donor 10%</td>
<td>0.4</td>
<td>2</td>
</tr>
<tr>
<td>Subject 80%/Donor 20%</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Subject 70%/Donor 30%</td>
<td>1.5</td>
<td>8</td>
</tr>
<tr>
<td>Subject 60%/Donor 40%</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Subject 50%/Donor 50%</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Subject 40%/Donor 60%</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Subject 30%/Donor 70%</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Subject 20%/Donor 80%</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>Subject 10%/Donor 90%</td>
<td>30</td>
<td>150</td>
</tr>
</tbody>
</table>

Conversions were based on two assumptions. The first assumption is that an individual has $1 \times 10^{12}$ platelets in the body if they have a normal platelet count of $200 \times 10^9$/L and a blood volume of five litres (total of $[200 \times 10^9$/L $\times 5$ L] = $1 \times 10^{12}$ platelets in body). The second assumption is that one unit of either apheresis or pooled platelets contains about $3 \times 10^{11}$ platelets.\textsuperscript{108} It is be expected that there are $(10^{12}/3 \times 10^{11})$ 3.3 units of apheresis platelets in an individual. Therefore an in vitro mix of subject 90%/donor 10% corresponds to the transfusion of $(10/90 \times 3.3)$ 0.4 apheresis units while an in vitro mix of subject 10%/donor 90% corresponds to the transfusion of $(90/10 \times 3.3)$ 30 units of platelets in that recipient. Some institutions may supply platelets as platelet concentrates from a random donor which contain fewer (about $0.55 \times 10^{11}$) platelets per unit, and therefore five platelet concentrates contain a similar number of platelets to one apheresis unit.\textsuperscript{109}

3.7 Sample size calculation

We assumed that the minimum clinically important difference that would be helpful to detect was a 10% absolute difference in ADP-aggregation after mixing. We also assumed that the
standard deviation of ADP-induced aggregation was about 10% based on an experiment by O'Connor et al which used similar methods.\textsuperscript{63} Using these assumptions, a sample size of 10 subjects was calculated to provide 80% power at a 2-sided alpha of 5% to detect a 10% absolute difference in mean ADP-aggregation.

**3.8 Stability of specimens**

The ADP-aggregation of subject and donor PRP was tested at the beginning and at the end of each testing period. Aggregation results at the end of the testing period that were similar to at the start indicated a stable platelet specimen.

**3.9 Statistical analysis**

The quantitative variable of interest is ADP-aggregation maximum amplitude (\%) that occurred during a test of six minutes duration in the LTA. Normally distributed data were summarised as mean ± standard deviation. Categorical data were summarised as count and percentage. ADP-aggregation data were presented by the time since the last doses of ticagrelor and ASA, and by the mixing proportions within each time-point.

Comparisons of ADP-aggregation were performed using a longitudinal mixed-effect analysis which assessed repeated measures over time. One-way ANOVA compared aggregation results within each time-point, and if statistically significant, t-tests were used for pairwise comparisons.

The primary outcomes were the minimum quantity of donor platelets required to achieve a major (≥90% improvement in mean aggregation) response and 50% reversal. Major response was defined as the mix with the lowest proportion of donor platelets when the ADP-aggregation of the mixed specimen is >90% of the subject pretreatment ADP-aggregation. 50% reversal was defined as the mix with the lowest proportion of donor platelets when the ADP-aggregation of the mixed specimen is the mid-point of subject pre-treatment aggregation and the nadir of subject ADP-aggregation. The minimum quantity of donor PRP was defined as the first mix proportion that resulted in a lack of statistically significant difference between the ADP-aggregation of the mix, and 90% of the pretreatment ADP-aggregation (for major reversal) or
the mid-point between the subject pretreatment ADP-aggregation and the nadir ADP-aggregation (for 50% reversal). P-values <0.05 were considered statistically significant. Analyses were performed using SAS® Studio 3.6.

The secondary outcome was to measure the effect of free ticagrelor in PPP on the ADP-aggregation of donor platelets. The ADP-aggregation of donor platelets was compared to mixes with increasing quantities of subject PPP.

3.10 Ethical and legal conduct of the study

Authorisation to Conduct Clinical Research was granted by the Population Health Research Institute (08-Nov-2016). The study commenced after receiving approval from multiple regulatory authorities. The Hamilton Integrated Research Ethics Board approved the study on 30-Nov-2016 (project number 2334). Health Canada provided a No Objection Letter on 21-Dec-2016 (File number HC6-24-c200615). The study was listed on www.clinicaltrials.gov on 26-Dec-2016 (identifier NCT03005704). The University of Western Australia Human Research Ethics Committee approved the study on 26-Apr-2017 (approval RA/4/1/9101). Written informed consent was obtained from all subjects. The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice Guidelines.
Results

4.1 Characteristics of study population

Twelve participants entered the study. Ten subjects took the full course of ticagrelor and ASA, and had blood collected at all the pre-specified times. One participant became the donor who repeatedly provided untreated platelets for all mixing studies. Data from one subject was excluded because the cuvettes were tested in an incorrect sequence in the aggregometers. All participants had a normal platelet count, haemoglobin, haematocrit, and platelet aggregation induced by ADP, AA and collagen prior to commencing ASA and ticagrelor. No adverse events occurred. The baseline characteristics of the 10 subjects are shown in Table 8.

Table 8 Baseline characteristics

<table>
<thead>
<tr>
<th>Subjects (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Female</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>Platelets (x 10^9/L)</strong></td>
</tr>
<tr>
<td><strong>Haemoglobin (g/L)</strong></td>
</tr>
<tr>
<td><strong>Haematocrit</strong></td>
</tr>
<tr>
<td><strong>Aggregation (maximum %)</strong></td>
</tr>
<tr>
<td>ADP</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>Collagen</td>
</tr>
</tbody>
</table>

Quantitative data are presented as mean ± standard deviation.

AA denotes arachidonic acid; ADP, adenosine diphosphate.
The same donor provided the platelets for all mixing studies, by having blood collected at the same time-points as each subject. The ADP-aggregation of donor PRP was normal. The mean ADP-aggregation of the subjects prior to antiplatelet treatment was not different from the mean ADP-aggregation of the donor at any time-point, as shown in Table 9.

### Table 9 Platelet function in donor PRP and subject PRP pre-treatment

<table>
<thead>
<tr>
<th>Time post ticagrelor</th>
<th>ADP-aggregation, maximum amplitude (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor</td>
<td>Subjects</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>N/A</td>
<td>76.4 ± 6.3</td>
</tr>
<tr>
<td>Prior to last dose</td>
<td>78.8 ± 5.6</td>
<td>N/A</td>
</tr>
<tr>
<td>2 hours</td>
<td>80.3 ± 5.1</td>
<td>N/A</td>
</tr>
<tr>
<td>10 hours</td>
<td>76.0 ± 4.7</td>
<td>N/A</td>
</tr>
<tr>
<td>24 hours</td>
<td>75.6 ± 8.3</td>
<td>N/A</td>
</tr>
<tr>
<td>48 hours</td>
<td>73.5 ± 7.3</td>
<td>N/A</td>
</tr>
<tr>
<td>72 hours</td>
<td>75.9 ± 10.5</td>
<td>N/A</td>
</tr>
<tr>
<td>96 hours</td>
<td>73.7 ± 7.6</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Data are presented as mean and standard deviation.

*p denotes comparison between ADP-aggregation of donor PRP and subject PRP prior to treatment.

ADP denotes adenosine diphosphate; N/A, not applicable.

### 4.2 Pharmacodynamic effect of ticagrelor

Immediately prior to the last dose of a five-day course of ticagrelor and ASA, the ADP-aggregation was 36.5 ± 11.5%, (p versus pretreatment <0.0001). The nadir ADP-aggregation of
34.6 ± 11.1% occurred two hours after the last dose (p versus pretreatment <0.0001). After two hours, the ADP-aggregation progressively increased at each successive time-point.

Spontaneous offset of the effect of ticagrelor was complete at 72 hours when the ADP-aggregation was 71.8% (p versus pretreatment 0.1794), shown in Figure 5.

**Figure 5 Pharmacodynamic effect of ticagrelor**

The platelet inhibitory effect of ticagrelor was measured by ADP-aggregation in PRP by LTA. Data are presented as mean and error bars represent standard deviation. The p denotes the comparison of mean pretreatment ADP-aggregation and ADP-aggregation at each time-point.

ADP denotes adenosine diphosphate; LTA, light transmission aggregometry; PRP, platelet rich plasma.

### 4.3 Effect of donor platelets on ADP-aggregation

Mixing subject PRP and donor PRP led to a stepwise increase in ADP-aggregation which was proportional to the increasing quantity of donor platelets. The stepwise increase was seen when
the pharmacodynamic effect of ticagrelor was present between two and 48 hours, but not at 72 or 96 hours. Aggregation results are displayed in graphical form in Figure 6 and numerical form in Appendix 3.

4.4 Primary and secondary outcomes

The primary outcome of major reversal was defined as the minimum quantity of donor platelets required to achieve an ADP-aggregation >90% of the pretreatment ADP-aggregation, which is about 68% (76.4% x 0.9). Major reversal of the effect of ticagrelor was not achieved at two hours post the last dose. Major reversal at 10 hours occurred in the subject 10%/donor90% mix (73.4% versus 76.4%; p=0.5069). Major reversal at 24 hours was achieved in the subject 40%/donor 60% mix (66.4% versus 76.4%; p=0.0550), and at 48 hours, in the subject 80%/donor20% mix (72.6% versus 76.4%; p=0.283). The minimum quantity of donor platelets required to achieve 50% reversal of the effect of ticagrelor, was defined as the mid-point of pretreatment ADP-aggregation (76.4%) and ADP-aggregation immediately prior to the last dose of ticagrelor (36.5%), which is about 56%. 50% reversal was achieved at two hours in the subject 30%/donor 70% mix (54.8%), at 10 hours in the subject 40%/donor 60% mix (58.5%), at 24 hours in the subject 80%/donor 20% mix (55.6%). 50% reversal was achieved at 48, 72, and 96 hours without donor platelets due to the spontaneous offset of the effect of ticagrelor.
Figure 6 Reversal of the antiplatelet effect of ticagrelor
Subjects were treated with ticagrelor in combination with aspirin for five days and had blood collected immediately prior to the last dose, and at 2, 10, 24, 48, 72, and 96 hours after the last dose. ADP-aggregation was measured in PRP using light transmission aggregometry.

Data are presented as ADP-aggregation, maximum amplitude (mean ± standard deviation).

ADP denotes adenosine diphosphate; PRP, platelet rich plasma.

* denotes p<0.05 when compared to the subject ADP-aggregation pretreatment.
4.5 Inhibition of donor platelets by free circulating ticagrelor

When subject PPP was mixed with donor PRP, the ADP-aggregation decreased in the specimens collected prior to the last dose, and at 2, 10, and 24 hours. The greatest inhibition of ADP-aggregation from PPP was seen at two hours post the last dose of ticagrelor, where the absolute change in donor ADP-aggregation was -6.1% (p=0.0108), -11.7% (p<0.001), -20.6% (p<0.001) in the mixes containing 10%, 20%, and 30% subject PPP, respectively. There was no significant change in donor platelet aggregation when subject PPP was mixed with donor platelets at 48, 72, or 96 hours after the last dose of ticagrelor. Aggregation results are displayed in graphical form in Figure 7 and in numerical form in Appendix 4.

Figure 7 Inhibition of donor platelet aggregation by subject PPP

Data are presented as mean ADP-aggregation (maximum amplitude).
ADP denotes adenosine diphosphate; PPP, platelet poor plasma; PRP, platelet rich plasma.

4.6 Stability of specimens

Stability of PRP throughout the testing period was assessed by comparing the ADP-aggregation immediately after PRP preparation with that at the conclusion of testing. The difference was not statistically significant for either donor or subject and is shown in Table 10.
**Table 10 Stability of specimens**

<table>
<thead>
<tr>
<th>PRP</th>
<th>Difference</th>
<th>Test for $\mu_0=0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>-1.0 ± 5.8</td>
<td>$p=0.1570$</td>
</tr>
<tr>
<td>Subject</td>
<td>-1.5 ± 6.0</td>
<td>$p=0.1849$</td>
</tr>
</tbody>
</table>

“Difference” denotes the ADP-aggregation immediately after PRP preparation minus the ADP-aggregation at the conclusion of testing.

Data are presented as mean ± standard deviation.

$\mu_0$ denotes mean of group; PRP, platelet rich plasma.
Discussion

5.1 Key findings

The aims of this study were to examine the effect of donor platelets on ticagrelor reversal over a wide range of time-points and using a wide range of quantities of donor platelets.

Without the addition of donor platelets, platelet ADP-aggregation in subjects treated with ticagrelor recovers spontaneously by about 30% at 24 hours, about 80% at 48 hours, and that it recovers completely at 72 hours after the last dose of ticagrelor.

When the platelet inhibitory effect of ticagrelor is present, the addition of donor platelets to subject platelets produces a stepwise increase in ADP-aggregation. The response to donor platelets is determined by the quantity of donor platelets added and the time-point after the last dose of ticagrelor at which they are administered. At 2 and 10 hours after ticagrelor, partial reversal of ticagrelor occurred, but no cases of complete reversal occurred which is likely due to ticagrelor circulating in stoichiometric excess to the platelet P2Y12 receptors, and that excess unbound ticagrelor in plasma is able to bind reversibly to donor platelets. Between 24 and 72 hours after ticagrelor, major reversal was possible.

Donor platelets had no effect on ADP-aggregation when they were administered after the offset of ticagrelor, at 72 and 96 hours.

Free ticagrelor in PPP has the potential to inhibit donor platelets, particularly between 2 and 24 hours after the last dose of ticagrelor.

5.2 Validity of results

The results are likely to be valid because the mean inhibition of ADP-aggregation observed in this study is comparable to that seen in clinical practice, and the effect of donor platelets are consistent with the pharmacokinetic and pharmacodynamic effect of ticagrelor.
The pharmacodynamic response of healthy subjects to ticagrelor in this study is similar to the response in patients with CAD. For example, in the PEGASUS-TIMI 54 trial, the effect of ticagrelor 90 mg twice daily on ADP-aggregation by LTA was studied in 58 patients with prior MI, and compared to 64 patients who took placebo. The baseline characteristics of the patients in the trial were typical for patients with cardiovascular disease, as the mean age was 63 years, 90% were male, mean body mass index 29 kg/m², and cardiovascular risk factors were present including diabetes mellitus in 28%, hypertension in 50%, and cigarette smoking in 16%. The mean ADP-aggregation prior to treatment with ticagrelor in patients with CAD is similar to healthy subjects in the current study, 73 ± 10% versus 76.4 ± 6.3%, respectively. Furthermore, the mean ± standard deviation ADP-aggregation in patients with CAD is similar to healthy subjects in the current study during maintenance treatment with ticagrelor (36 ± 14% versus 36.5 ± 11.5%, respectively) and at the nadir level (two hours) after ticagrelor (29 ± 11% which is comparable to 34.6 ± 11.1%).

5.3 What was previously known, and contribution of this study

When measured by LTA, the offset of the effect of ticagrelor in patients with CAD in the ONSET/OFFSET Study occurred by about five days. However, aggregation was not measured at four days in this study, therefore it is possible that the offset of ticagrelor is earlier than five days. In the current study with healthy volunteers, the offset of ticagrelor occurred at three days. This difference is small and unlikely to be significant. The findings of the current study of healthy volunteers suggest that the offset of ticagrelor is similar to patients with CAD and that the findings are likely generalisable to patients with CAD.

Previous reports of ticagrelor reversal using donor platelets show conflicting results. Only two studies, both published after the current study commenced, tested reversal at multiple time-points. In the first, Zafar et al (2017) showed that donor platelets increase ADP-aggregation at all time-points between 4 to 48 hours. In the second, Hansson et al (2017) showed that donor platelets had no effect on ADP-aggregation between 12 and 96 hours after the last dose of ticagrelor.
Unlike the studies by Zafar et al. (2017) and Hansson et al. (2017), the current study examined the effect of a variable mix of donor platelets which ranged from 11% to 900% donor platelets relative to the ticagrelor treated platelets, and followed the response over 96 hours. Our results are consistent with the study by Zafar et al. (2017), as the addition of donor platelets led to a time- and dose-dependent response between 4 to 48 hours after the last dose of ticagrelor. It is feasible to partially reverse the effect of ticagrelor within 24 hours of the last dose, and to completely reverse ticagrelor 24 hours or more after the last dose.

The current study adds a substantial amount of new information to the previous studies. Previous studies were limited to up to three mixing proportions and a maximum quantity of four parts donor platelets to one part ticagrelor treated platelets. The current study had nine mixing proportions and the maximum quantity of donor platelets was nine parts donor platelets to one part ticagrelor treated platelets. The current study tested ticagrelor reversal at seven different time-points, up to 96 hours, which informs on ticagrelor reversal from during maintenance dosing, until complete offset. One previous study with positive results tested reversal at four different time-points up to 48 hours, at which time ticagrelor is still present in the circulation. Other studies tested reversal at one time-point, usually within six hours of ticagrelor, at which time reversal is not feasible. The current study defines the quantity of donor platelets required for partial and complete ticagrelor reversal at multiple time-points after the last dose of ticagrelor, which was not done in previous studies.

The findings of the current study extend the findings from previous reports and suggest that:

- In the first 10 hours after the last dose, transfusion with three units will reverse ticagrelor by 20-25%, and of six units will reverse ticagrelor by 25-50%
- By 24 hours after the last dose, transfusion of three units will reverse ticagrelor by 65%, and of six units will reverse ticagrelor by 100%
- Between 24 and 48 hours, progressively fewer platelets are required to reverse ticagrelor
- From 72 hours, donor platelets have no effect on ADP-aggregation.
In other words, platelet transfusion for ticagrelor reversal is difficult within the first 24 hours after taking ticagrelor, effective at 24 and 48 hours, and unnecessary at and after 72 hours.

### 5.4 Clinical importance of this study

Ticagrelor reversal is desirable for patients who take ticagrelor and require urgent surgery, or who develop life-threatening bleeding. This study provides information which helps clinicians to use their clinical judgement to decide about transfusing platelets for ticagrelor reversal when indicated clinically.

In the absence of a specific antidote, platelet transfusion is a feasible treatment for ticagrelor reversal. Prior studies have not determined the optimal quantity or optimal timing of platelet transfusion. Determining the optimal quantity of platelets to transfuse is important because transfusing too few platelets may be ineffective for ticagrelor reversal, while transfusing too many platelets may increase the thrombotic risk for patients and waste scarce human blood product resources. Determining the optimal time at which to transfuse platelets is also important because transfusing platelets too early may be ineffective for ticagrelor reversal due to inhibition of transfused platelets by circulating ticagrelor, while transfusing platelets too late may prolong bleeding or delay surgery.

This study has detected three distinct time periods during ticagrelor reversal, in which donor platelets are partially effective, effective, or unnecessary. Donor platelets are partially effective from immediately prior to the last dose to 10 hours after ticagrelor, because the ADP-aggregation increases but does not recover to the pretreatment level. Donor platelets are effective for ticagrelor reversal between 24 and 72 hours after ticagrelor, because ADP-aggregation can be restored to the pretreatment level with about six apheresis units of platelets at 24 hours, and about one unit at 48 hours. Donor platelets are unnecessary from 72 hours after ticagrelor because this is the time at which the offset of ticagrelor occurs.

The results of this study inform on the quantity of donor platelets required for reversal at multiple time-points as shown in Table 11. For patients who take ticagrelor and require urgent reversal,
the equivalent of six apheresis units of donor platelets produces 50% reversal at 10 hours and at least 90% reversal at 24 hours after the last dose of ticagrelor. For patients who are able to wait for the spontaneous offset of ticagrelor, a lower quantity of transfused platelets will achieve reversal. For example, if a patient last ingested ticagrelor 10 hours ago, major reversal would theoretically require 30 units of apheresis platelets which would not be a feasible quantity in most centres, and only partial reversal would be possible at this time. Alternatively, if the patient is able to wait until 24 hours after the last dose, major reversal would be possible with about six units of apheresis platelets and 50% reversal would be possible with one unit.
Table 11 Estimated number of units of platelets required for ticagrelor reversal

<table>
<thead>
<tr>
<th>Hours after last ticagrelor dose</th>
<th>Major (&gt;90%) reversal</th>
<th>50% reversal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apheresis</td>
<td>Single donor</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>180</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The assumptions are that the recipient has a platelet count of 200 x 10⁹/L and blood volume five litres. One apheresis or pooled unit contains about 3 x 10¹¹ platelets per unit. One single donor unit contains about 0.55 x 10¹¹ platelets per bag.¹⁰⁸

Reversal of the antiplatelet effect of ASA was not directly examined in this study. However, other in vitro studies which studied ASA reversal have shown that transfusion of about 1-2 units of apheresis platelets are sufficient to completely normalise AA mediated aggregation.²⁷ Complete ASA reversal would be highly likely to occur in a patient who receives a platelet transfusion for ticagrelor reversal, because the quantity transfused for ticagrelor reversal would also be sufficient for ASA reversal, and that ASA works through a different pathway. ASA reversal is likely to be beneficial for haemostasis in patients who are receiving ticagrelor as well.

5.5 Implications for future research

Designing a clinical research study of the efficacy of platelet transfusion for ticagrelor reversal is challenging.
A randomised study of platelet transfusion versus no transfusion would be the gold standard method to compare the efficacy of platelet transfusion versus no transfusion in patients who take ticagrelor. ASA reversal with platelet transfusion has been studied in a multicentre randomised fashion in patients with intracranial bleeding. However, ticagrelor is associated with more severe bleeding problems than ASA and it may be considered unethical to withhold potentially effective haemostatic treatments, such as platelet transfusion, from patients with life-threatening bleeding who are randomised to a control arm. Therefore, a randomised controlled trial is unlikely to be feasible.

A cohort study is another potential option to test the efficacy of platelet transfusion, where patients who take ticagrelor and ASA and who develop bleeding are transfused platelets. The outcomes of bleeding severity and increase in platelet aggregation could be compared to a group of controls who do not receive platelet transfusion. A cohort study of ticagrelor reversal would be more feasible than a randomised controlled trial, however there is the potential for the results to be biased due to the lack of randomisation.

Since designing a clinical study of ticagrelor reversal will be difficult, the results of the current in vitro study inform clinicians on the expected response to different quantities of donor platelets. Clinicians can then use their judgement along with knowledge of the time at which the last dose of ticagrelor was taken, and the risk of bleeding, to transfuse platelets to a patient.

5.6 Potential limitations

The current study has four potential limitations.

ADP-aggregation was used as a surrogate to measure the haemostatic effect of transfused platelets. The level of ADP-aggregation required for blood clot formation is not known. However, this is a reasonable surrogate because ADP-aggregation predicted bleeding risk in clinical trials that compared the efficacy and safety of high versus low dose clopidogrel, ASA and clopidogrel versus ASA, and of ticagrelor versus clopidogrel. ADP-aggregation and AA-aggregation
by LTA has also been used to investigate the reversal of the effect of ASA and clopidogrel using donor platelets.\textsuperscript{27, 83, 84}

Using one concentration of ADP to induce platelet aggregation is a potential limitation because it examines a single point in the ADP dose-response curve. A more comprehensive study of ticagrelor reversal could have been performed using threshold, submaximal and saturating doses of ADP. However, studying multiple doses of ADP within three hours of blood collection was not feasible because of the time required to perform the aggregation studies, and the results obtained using the single concentration are still effective at measuring the effect of ticagrelor on the platelet ADP receptor.

This study was performed in healthy volunteers instead of patients with symptomatic CAD. However, the results are relatable to patients with CAD because the pharmacodynamic effect of ticagrelor is consistent in both populations. Age and gender is not considered an indication for changing the dose of ticagrelor because the effect of ticagrelor on ADP-aggregation is substantial regardless of age or gender.\textsuperscript{111} The offset of the effect of ticagrelor in the current study was 72 hours which is only a small difference from, and unlikely to be clinically relevant to, patients with CAD in the ONSET/OFFSET trial in which the offset of ticagrelor was between three and five days (not measured at day four).\textsuperscript{28}

Our study does not inform on the thrombotic risk of platelet transfusion. However, the results enlighten the clinician on the different quantities of platelets required for partial and major reversal of the effect of ticagrelor, and are most valuable in the management of patients in whom the risk of ongoing bleeding is expected to outweigh the risk of thrombosis.
Conclusion

The benefits of ticagrelor therapy in patients with CV disease include decreased risk of death from vascular causes, MI, stroke, and all cause mortality. These benefits outweigh the risk of bleeding. However, the increased risk of bleeding attributable to ticagrelor treatment remains a substantial problem because bleeding is associated with morbidity, mortality, and the discontinuation of ticagrelor in some patients. When patients develop life threatening bleeding or require urgent surgery, reversing ticagrelor with platelet transfusion may be considered to mitigate the morbidity and mortality associated with bleeding.

This study informs on the potential of donor platelets to reverse ticagrelor when measured by ADP-aggregation, at different time-points and with different quantities of donor platelets. The efficacy of donor platelets to normalise ADP-aggregation in patients receiving ticagrelor increases with a higher quantity of donor platelets and the passage of time since the last dose of ticagrelor.

Application of these findings to the clinical setting aids with the optimal prescription of a platelet transfusion to reverse ticagrelor. Donor platelets can partially reverse ticagrelor from 10 hours after the last dose, and produce major reversal from 24 hours after the last dose. For example, the addition of the equivalent of six apheresis units of platelets produces a 50% relative reversal at 10 hours and major reversal at 24 hours.

Platelet transfusion remains the most feasible and effective method to reverse the effect of ticagrelor until an antidote is developed in the future. Based on the current findings, it would be reasonable for clinicians to consider platelet transfusion in patients who take ticagrelor and experience life threatening bleeding or who require urgent surgery. Major reversal of ticagrelor within the first 24 hours is not feasible with platelet transfusion, but partial reversal during this time may be beneficial because subpopulations of normal platelets can act as a seed for platelet aggregation.⁼¹²
Further studies are required to evaluate the impact of platelet transfusion on clinical outcomes in patients taking ticagrelor. A randomised design would be the most likely to inform on efficacy and safety, and is possible because a randomised study has been performed before, albeit with an imbalance in the severity of the bleeding at baseline.

It is likely that platelet transfusions will play a larger role in the future for patients who take ticagrelor. Further information on cut-off targets for platelet function tests and clinical outcomes from randomised multicentre trials of platelet transfusion versus no transfusion are needed.
References


Appendices

Appendix 1 Formula for preparing PRP with platelet count adjusted to \(200 \times 10^9/L\)

<table>
<thead>
<tr>
<th>PRP_{200} = [1 \text{ mL } \text{ PRP}<em>{\text{UNADJUSTED}}] + \frac{\text{PRP}</em>{\text{UNADJUSTED}} - 200}{200}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where</td>
</tr>
<tr>
<td>• PRP_{200} = platelet rich plasma with platelet count of (200 \times 10^9/L)</td>
</tr>
<tr>
<td>• PRP_{\text{UNADJUSTED}} = platelet rich plasma with unadjusted platelet count, i.e., removed from specimen tube after centrifugation</td>
</tr>
</tbody>
</table>

PRP with a platelet count adjusted to \(200 \times 10^9/L\) was used for two reasons. First, a constant platelet count facilitated a calculation of the ratio of donor to subject to donor PRP, which was used to estimate the quantity of units of platelets to transfuse \textit{in vivo}. Second, a platelet count of \(200 \times 10^9/L\) is suitable for testing platelet function by LTA.
Appendix 2 Formula for preparing PRP with platelet count adjusted to 200 x10⁹/L in PPP mixing studies

\[
\text{PRP}_{\text{PPP mix}} = \frac{0.25}{(1 + \frac{\text{PRP}_{\text{UNADJUSTED}} - \text{PRP}_{\text{TARGET ADJUSTED}}}{\text{PRP}_{\text{TARGET ADJUSTED}}})}
\]

\[
\text{PPP}_{\text{PPP mix}} = \left(\frac{\text{PRP}_{\text{UNADJUSTED}} - \text{PRP}_{\text{TARGET ADJUSTED}}}{\text{PRP}_{\text{TARGET ADJUSTED}}}\right) \left(\frac{0.25}{1 + \text{PRP}_{\text{PPP mix}}}\right)
\]

Where

- \(\text{PRP}_{\text{UNADJUSTED}}\) = platelet count (x 10⁹/L) in platelet rich plasma removed from specimen tube after centrifugation
- \(\text{PRP}_{\text{PPP mix}}\) = platelet rich plasma volume in mL
- \(\text{PRP}_{\text{TARGET ADJUSTED}}\) is:
  - 222 for subject PPP 10%/donor PRP 90% mix
  - 250 for subject PPP 20%/donor PRP 80% mix
  - 285 for subject PPP 30%/donor PRP 70% mix

Preparing PRP for the subject PPP and donor PRP mixes required a formula different from that used in the PRP mixes shown in Appendix 1. The reason for the different formula is that a higher platelet count in donor PRP was required so that after mixing with subject PPP the final platelet count was 200 x 10⁹/L. The first step was to calculate the volume of donor PRP, and the second was to calculate the volume of donor PPP, which were mixed to provide the donor PRP for the donor PRP/subject PPP mixes. After the donor PRP was prepared, it was mixed in the cuvette with subject PPP and the aggregation was tested.
### Appendix 3 Raw ADP aggregation data

**ADP-aggregation, maximum amplitude (%)**

*(p for comparison to subject pretreatment level)*

<table>
<thead>
<tr>
<th>Subject:Donor PRP mix</th>
<th>Pretreatment dose</th>
<th>Prior to last</th>
<th>2</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject before mix</td>
<td>76.4 ± 6.3</td>
<td>36.5 ± 11.5</td>
<td>34.6 ± 11.1</td>
<td>38.5 ± 11.2</td>
<td>48.5 ± 14.6</td>
<td>67.2 ± 8.6</td>
<td>71.8 ± 5.5</td>
<td>75.4 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.0108)</td>
<td>(0.1794)</td>
<td>(0.7532)</td>
<td></td>
</tr>
<tr>
<td>90% / 10%</td>
<td>-</td>
<td>41.2 ± 10.9</td>
<td>35.7 ± 9.1</td>
<td>39.8 ± 10.0</td>
<td>50.9 ± 15.3</td>
<td>68.1 ± 11.3</td>
<td>71.4 ± 6.9</td>
<td>76.7 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.0205)</td>
<td>(0.1405)</td>
<td>(0.8503)</td>
<td></td>
</tr>
<tr>
<td>80% / 20%</td>
<td>-</td>
<td>45.3 ± 10.4</td>
<td>37.9 ± 9.0</td>
<td>42.2 ± 9.2</td>
<td>55.6 ± 13.4</td>
<td>72.6 ± 10.3</td>
<td>76.2 ± 9.1</td>
<td>76.4 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.2838)</td>
<td>(0.9645)</td>
<td>(0.9875)</td>
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</tr>
<tr>
<td>Percentage Distribution</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
<td>Value 5</td>
<td>Value 6</td>
<td>Value 7</td>
<td>Value 8</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>70% / 30%</td>
<td>49.7 ± 10.7</td>
<td>38.5 ± 7.1</td>
<td>43.1 ± 10.2</td>
<td>59.7 ± 15.3</td>
<td>73.7 ± 6.8</td>
<td>77.9 ± 5.1</td>
<td>77.4 ± 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>0.0016</td>
<td>0.4458</td>
<td>0.6462</td>
<td>0.7413</td>
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<tr>
<td>60% / 40%</td>
<td>50.0 ± 9.8</td>
<td>40.9 ± 10.2</td>
<td>46.7 ± 12.1</td>
<td>62.4 ± 12.6</td>
<td>75.1 ± 6.5</td>
<td>78.1 ± 6.5</td>
<td>76.1 ± 5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>0.0074</td>
<td>0.7238</td>
<td>0.6147</td>
<td>0.9248</td>
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<tr>
<td>50% / 50%</td>
<td>53.0 ± 9.7</td>
<td>45.1 ± 7.2</td>
<td>49.3 ± 10.3</td>
<td>65.6 ± 13.8</td>
<td>75.5 ± 7.7</td>
<td>77.0 ± 7.4</td>
<td>80.9 ± 6.0</td>
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<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>0.0376</td>
<td>0.7991</td>
<td>0.8589</td>
<td>0.1589</td>
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</tr>
<tr>
<td>40% / 60%</td>
<td>56.5 ± 12.9</td>
<td>48.6 ± 5.9</td>
<td>58.5 ± 12.5</td>
<td>66.4 ± 10.4</td>
<td>77.7 ± 5.2</td>
<td>78.5 ± 6.1</td>
<td>78.4 ± 5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>0.0001</td>
<td>0.0550</td>
<td>0.7132</td>
<td>0.5245</td>
<td>0.5195</td>
<td></td>
</tr>
<tr>
<td>30% / 70%</td>
<td>63.0 ± 11.5</td>
<td>54.8 ± 8.4</td>
<td>60.1 ± 13.2</td>
<td>67.6 ± 6.8</td>
<td>78.3 ± 8.2</td>
<td>77.5 ± 5.8</td>
<td>76.0 ± 8.8</td>
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</tr>
<tr>
<td></td>
<td>0.0050</td>
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<td>0.0004</td>
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<td>0.7334</td>
<td>0.8875</td>
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<tr>
<td>20% / 80%</td>
<td>67.3 ± 13.0</td>
<td>60.2 ± 11.0</td>
<td>64.5 ± 8.7</td>
<td>70.8 ± 6.9</td>
<td>77.2 ± 7.1</td>
<td>79.0 ± 6.4</td>
<td>77.4 ± 7.3</td>
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</tr>
<tr>
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<td>0.0545</td>
<td>(&lt;0.0001)</td>
<td>0.0086</td>
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<td>0.8210</td>
<td>0.4330</td>
<td>0.7532</td>
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</tr>
<tr>
<td>10% / 90%</td>
<td>71.7 ± 10.7</td>
<td>69.0 ± 6.3</td>
<td>73.4 ± 6.4</td>
<td>77.4 ± 7.7</td>
<td>79.6 ± 7.4</td>
<td>74.5 ± 11.7</td>
<td>74.3 ± 10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3176</td>
<td>0.0499</td>
<td>0.5069</td>
<td>0.8382</td>
<td>0.3590</td>
<td>0.5737</td>
<td>0.5195</td>
<td></td>
</tr>
<tr>
<td>Donor</td>
<td>78.8 ± 5.6</td>
<td>80.3 ± 5.1</td>
<td>76.0 ± 4.7</td>
<td>75.6 ± 8.3</td>
<td>73.5 ± 7.3</td>
<td>75.9 ± 10.5</td>
<td>73.7 ± 7.6</td>
<td></td>
</tr>
</tbody>
</table>
Data are presented as mean ± standard deviation.

ADP denotes adenosine diphosphate; ASA, acetylsalicylic acid; PRP, platelet rich plasma.
Inhibition of donor platelet function by subject PPP

<table>
<thead>
<tr>
<th>Subject</th>
<th>Prior to last</th>
<th>2</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP mix PRP:Donor dose</td>
<td>0% / 100%</td>
<td>-1.7 ± 4.0</td>
<td>-6.1 ± 6.3</td>
<td>-0.7 ± 3.54</td>
<td>1.5 ± 4.2</td>
<td>0.6 ± 7.0</td>
<td>0.9 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>20% / 80%</td>
<td>-6.6 ± 9.1</td>
<td>-13.6 ± 10.4</td>
<td>-4.4 ± 8.7</td>
<td>-0.4 ± 4.3</td>
<td>0.7 ± 2.9</td>
<td>2.2 ± 4.3</td>
</tr>
</tbody>
</table>

ADP-aggregation (maximum amplitude, %)

(p for comparison to donor aggregation prior to mix)
<table>
<thead>
<tr>
<th></th>
<th>(p=0.2009)</th>
<th>(p=0.0011)</th>
<th>(p=0.1166)</th>
<th>(p=0.900)</th>
<th>(p=0.7688)</th>
<th>(p=0.5883)</th>
<th>(p=0.4573)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% / 70%</td>
<td>-9.6 ± 11.9</td>
<td>-20.6 ± 9.3</td>
<td>-8.2 ± 10.1</td>
<td>-4.3 ± 4.4</td>
<td>-2.1 ± 5.6</td>
<td>-0.6 ± 6.4</td>
<td>0.6 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>(p=0.0552)</td>
<td>(p&lt;0.0001)</td>
<td>(p=0.0103)</td>
<td>(p=0.1362)</td>
<td>(p=0.5428)</td>
<td>(p=0.7541)</td>
<td>(p=0.8409)</td>
</tr>
</tbody>
</table>

Data are presented as mean and standard deviation.

ADP denotes adenosine diphosphate; ASA, acetylsalicylic acid; PPP, platelet poor plasma; PRP, platelet rich plasma.