ORIGINAL RESEARCH

Pharmacodynamic Comparison of Ticagrelor Monotherapy Versus Ticagrelor and Aspirin in Patients After Percutaneous Coronary Intervention: The TEMPLATE (Ticagrelor Monotherapy and Platelet Reactivity) Randomized Controlled Trial

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BACKGROUND: To assess differences in platelet inhibition during ticagrelor monotherapy (TIC) or dual therapy with ticagrelor and aspirin (TIC+ASP) in patients after percutaneous coronary intervention using a comprehensive panel of functional tests.

METHODS AND RESULTS: In a single-center parallel group, open label, randomized controlled trial, 110 participants were randomized to receive either TIC (n=55) or TIC+ASP (n=55) for 4 weeks. The primary outcome was the platelet aggregation response with 10 μmol/L thrombin receptor activation peptide-6 (TRAP-6). The secondary outcomes were platelet aggregation responses and binding of surface activation markers with a panel of other activators. The mean percentage aggregation for 10 μmol/L TRAP-6 was similar for the TIC and TIC+ASP groups (mean difference+4.29; 95% CI, −0.87 to +9.46). Aggregation was higher in the TIC group compared with the TIC+ASP group with 1 μg/mL (+6.47; +2.04 to +10.90) and 0.5 μg/mL (+14.00; +7.63 to +20.39) collagen related peptide. Aggregation responses with 5 μmol/L TRAP-6, 5 μmol/L or 2.5 μmol/L thromboxane A2 receptor agonist and surface activation marker binding with 5 μmol/L TRAP-6 or 0.5 μg/mL collagen related peptide were the same between the treatment groups.

CONCLUSIONS: Patients with PCI show similar levels of inhibition of most platelet activation pathways with TIC compared with dual therapy with TIC + ASP. However, the greater aggregation response with collagen related peptide during TIC indicates incomplete inhibition of glycoprotein VI (collagen) receptor-mediated platelet activation. This difference in pharmacodynamic response to anti-platelet medication may contribute to the lower bleeding rates observed with TIC compared with dual anti-platelet therapy in recent clinical trials.

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Key Words: aspirin ■ dual anti-platelet therapy ■ platelet activation ■ ticagrelor monotherapy

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Dual anti-platelet therapy (DAPT) with aspirin and a P2Y12 antagonist to additionally suppress the ADP platelet activation pathway reduces the composite risk of myocardial infarction and cardiovascular death after percutaneous coronary intervention (PCI) when compared with aspirin monotherapy.1,2 Consequently, DAPT is the current standard anti-thrombotic regime.3,4 Potent P2Y12 antagonists improve ischaemic protection when compared with clopidogrel, because they enable more consistent suppression of ADP-mediated platelet activation.5–7 However, the greater anti-thrombotic effect of potent P2Y12 antagonists is accompanied by increased bleeding.8

It has long been recognized that the ADP platelet activation pathway utilizes signalling mediators and effectors that are partially shared with the thromboxane A2 (TxA2) activation pathway, the target of inhibition by aspirin.9–11 In view of this potential redundancy, monotherapy with a potent P2Y12 antagonist has been proposed as an alternative to DAPT, maintaining anti-thrombotic effect whilst avoiding bleeding associated with aspirin.12,13 In healthy volunteers, potent P2Y12 antagonists diminish both ADP and TxA2-mediated platelet aggregation, suggesting that additional aspirin is unnecessary for full platelet inhibition.10,14 However, in other studies a P2Y12 antagonist had minimal impact on the production of the TxA2 metabolite, thromboxane B2, suggesting that the TxA2 pathway is incompletely inhibited.15

Despite the lack of conclusive data from healthy control studies, potent P2Y12 antagonist monotherapy has been compared to DAPT in several large randomized controlled trials in patients who had undergone PCI.16–18 Patients who were switched from DAPT to P2Y12 antagonist monotherapy, between 1 and 3 months after PCI, had non-inferior composite rates of all-cause death or ischaemic events compared with patients remaining on DAPT, suggesting equivalent antithrombotic effect.17–19 Importantly, bleeding in patients switched to P2Y12 antagonist monotherapy was either lower17,19 or non-inferior18 when compared with patients remaining on DAPT.

Translation of the results from these trials into clinical practice remains unclear, primarily because the specific nature of the study designs and subject selection limits generalization of the results to all patients.20 In particular, the possible implementation of P2Y12 antagonist monotherapy to specific patient subgroups is currently hampered by the lack of a mechanistic understanding of the differences between the P2Y12 antagonist monotherapy and DAPT groups observed in the trials.16 In order to quantify differences in the pharmacodynamic effect of P2Y12 antagonist monotherapy compared to DAPT, we randomized patients with PCI to receive either TIC or DAPT with ticagrelor and aspirin (TIC + ASP) and performed a comprehensive panel of platelet functional tests on blood samples.
METHODS

TEMPLATE Trial Design and Interventions

The TEMPLATE (Ticagrelor Monotherapy and Platelet Reactivity) study was a single-center, open-label, parallel group RCT (ISRCTN84335288). The study was approved by a UK Research Ethics Committee (14/SC/1309) and was authorized by the Medicines and Healthcare Regulatory Agency (EUDRACT N° 2013-002734-20). The study protocol has been reported previously.21 The data generated from the trial are available from the corresponding author on request. The request must include a specification of the data requested and justification for that request (i.e., a statement of purpose for which the data are required). The data may not be released unless all Bristol Trials Centre and Sponsor requirements are fulfilled (e.g., protocol, sample size calculation, valid National Research Ethics Service Committee and Health Research Authority approval if appropriate, a collaborative arrangement in place between the Sponsor and the external body).

Patients under the care of the Bristol Heart Institute, Bristol, UK were eligible for inclusion if they were adults aged >18 years receiving DAPT with aspirin and any P2Y12 antagonist after PCI for acute coronary syndrome (ACS) and for which there was a clinical intention to transfer to aspirin monotherapy. Patients were ineligible if there was a contra-indication to ticagrelor, if prior treatment with DAPT had been interrupted because of bleeding, if they were pregnant or breast feeding or if the patient was (or was a partner of) a woman with child-bearing potential unwilling to use contraception. Twelve months after the index PCI, when DAPT would normally be switched to aspirin monotherapy, consenting patients were randomized using an internet-based computer system ensuring allocation concealment. No blinding was used. Patients were allocated in a 1:1 ratio to receive either TIC (TIC group; ticagrelor 180 mg then 90 mg twice per day), or DAPT with ticagrelor and aspirin (TIC+ASP group; ticagrelor 180 mg then 90 mg twice per day plus aspirin 75 mg once per day), for a total of 4 weeks. The ticagrelor doses in the treatment arms were selected to reproduce current standard DAPT given for the first 12 months after PCI. Participants in both groups were then switched to aspirin monotherapy for 4 weeks and returned to standard care. Blood samples were obtained during initial DAPT (blood sample 1), 4 weeks after allocation to the TIC or TIC+ASP groups (blood sample 2) and 4 weeks after transfer to aspirin monotherapy (blood sample 3; Figure 1). Safety outcomes were collected for the duration of the study or until participant withdrawal.

Analysis of Blood Samples

Functional responses of platelet rich plasma obtained from venous blood samples were analyzed by light transmission aggregation using a PAP-8E platelet aggregometer (BioData, Horsham, PA, USA).21 The platelets were stimulated with 10 μmol/L and 5 μmol/L thrombin receptor activation peptide-6 (TRAP-6), 1 μg/mL and 0.5 μg/mL collagen related peptide (CRP), 2.5 μmol/L and 5 μmol/L thromboxane A2 (TXA2) receptor agonist (U46619), 1 μmol/L arachidonic acid (AA) or 10 μmol/L ADP. Reagents were obtained from Hart biologicals (Hartlepool, UK) except for CRP (University of Cambridge, UK). Results were recorded as the maximum amplitude of aggregation, expressed as a percentage of the difference in light transmission between platelet rich plasma and a plasma control.

Platelet surface activation markers were measured in unstimulated platelet rich plasma and in platelet rich plasma after stimulation with 1 μg/mL CRP or 5 μmol/L TRAP-6 by incubated the samples with PE-conjugated anti-CD62P (Becton Dickinson, Oxford, UK) and FITC-conjugated PAC-1 antibodies (Becton Dickinson) for 10 minutes at room temperature and then fixing in 1% paraformaldehyde. Antibody binding was measured on a BD FACSCanto TM II (BD Biosciences) and reported as the median fluorescence intensity (MFI) of antibody binding in unstimulated platelets and the increase in MFI (ΔMFI) of antibody binding after stimulation.
Outcomes
The primary outcome was the maximum amplitude of platelet aggregation after stimulation with 10 μmol/L TRAP-6. Secondary outcomes were the maximum amplitude of aggregation with the remaining activators and the binding of platelet surface activation markers. Outcomes were compared in blood sample 2, taken at the end of the intervention period.

Statistical Analysis
The sample size of 110 participants was chosen to provide a 95% CI of ±0.35 SD for the mean difference in 10 μmol/L TRAP-6 between groups, assuming correlation between pre- and post-treatment platelet function results of 0.5, with no crossover, and allowing for 10% drop-out.

Participants were analyzed on an intention-to-treat basis and analyses were carried out according to a prespecified statistical analysis plan. To minimize the type 1 error rate, it was agreed a priori that the difference in the aggregation response with 10 μmol/L TRAP-6 (primary outcome) and in the aggregation responses with 1 μg/mL CRP, 1 mmol/L AA and 10 μmol/L ADP would be quantified. Differences in aggregation responses for the remaining agonists were quantified posthoc. The binding of platelet surface activation markers was described, but differences were not quantified.

Differences between the treatment groups were assessed using longitudinal mixed effects multiple linear regression models that included data from all blood samples, with patients fitted as random intercepts and sample number as random slopes (depending on model fit, with unstructured covariance matrices). Treatment group, sample number and their interaction were included as fixed effects. Models were compared using the likelihood ratio test. Differences between the treatment groups were expressed as the difference in mean percentage of the maximum amplitude of aggregation in blood sample 2, representing the model-adjusted absolute difference between the responses in the TIC+ASP group as the reference. All P values are 2-sided. Analyses were conducted using Stata Statistical Software Release 15 (StataCorp, College Station, TX).

RESULTS
Recruitment and Follow-up
Between December 2015 and July 2017, all 2967 patients listed for PCI at the study center underwent pre-screening to identify patients who could immediately be excluded because they did not meet the eligibility criteria. The remaining 1186 patients underwent full screening for eligibility and were invited to join the study. Within this group, 182 patients were confirmed as eligible and expressed interest in participation. A total of 115 patients were approached, 112 gave consent and 110 were randomized (55 to TIC and 55 to TIC+ASP; Figure 2). All participants provided the first blood sample at recruitment. Seven participants withdrew during the intervention period (first period of 4 weeks) and 1 withdrew after the trial intervention period (second period of 4 weeks). The reasons for withdrawal were participant request for an unspecified reason (3); participant unable to comply with study visits (2); primary care physician request to withdraw (1) and death of participant (1). One further participant was withdrawn because he was found to be ineligible from medical records that became available only after randomization.

Participant Characteristics
The clinical characteristics of the participants in the 2 groups were similar (Table 1). Overall, 88 participants were male and mean age was 67 years. The median time from PCI to randomization was 366 days (IQR 360, 374). All participants had received DAPT continuously since PCI, comprising aspirin and either ticagrelor

Figure 2. Screening, eligibility, randomization and blood sampling.
ASP indicates aspirin; and TIC, ticagrelor monotherapy.
(44%), clopidogrel (35%) or prasugrel (21%) according to standard institutional treatment protocols.

**Platelet Function 4 Weeks After Allocation to Treatment Group (Sample 2)**

The difference in mean percentage platelet aggregation with 10 μmol/L TRAP-6 (primary outcome) was similar in the 2 groups (aggregation with TIC minus TIC+ASP=+4.29; 95% CI, −0.87 to +9.46; P=0.103; Figures 3A and 4 and Table S1). Considering the secondary outcomes, aggregation was higher in the TIC group compared with the TIC+ASP group with 1 μg/mL CRP (difference +6.47 (+2.04 to +10.90); P=0.004) and with 0.5 μg/mL CRP (difference +14.00 (+7.63 to +20.39); P < 0.001), indicating less platelet inhibition in
the TIC group. There was no difference between the groups in platelet aggregation with 5 μmol/L TRAP-6, 2.5 μmol/L U46619 or with 5 μmol/L U46619.

As expected, platelet aggregation was markedly higher in the TIC group compared with the TIC+ASP group with 1 mmol/L AA (difference +11.68 (+8.55 to +15.95); \( P < 0.001 \)). (Figures 3B and 4) which is a sensitive marker of platelet inhibition with aspirin. Aggregation was similar between the treatment groups with 10 μmol/L ADP (difference +1.15 (−4.89 to +7.19);

\[ P=0.709; \] Figures 3B and 4) and confirmed full suppression of ADP-mediated platelet in both groups.

Participants in both groups had similarly low levels of binding of the activation markers PAC 1 and anti-CD62P in unstimulated platelets. Although after stimulation with 5 μmol/L TRAP-6 or 1 μg/mL CRP, binding of both PAC 1 and anti-CD62P increased markedly, the extent of activation marker binding was similar in the 2 groups (Figure 5 and Table S2).

**Platelet Function at Baseline and After Transfer to Aspirin Alone (Samples 1 and 3)**

No differences in aggregation or surface activation marker binding were found in blood samples 1 and 3 (Figure 3, Figures S1 and S2, Tables S1–S2), consistent with patients in both groups receiving the same classes of anti-platelet drugs at the time of these blood samples.

**Adverse Outcomes**

A total of 258 adverse events occurred during the entire trial period (Table S3), of which 188 occurred during the 4-week trial intervention period (98 events in 39 patients in the TIC group and 90 events in 37 patients in the TIC+ASP group; Table S4). There were 53 bleeding events during the 4-week intervention period: 20 in the TIC group and 33 in the TIC+ASP group. Bleeding was predominantly subcutaneous or dermal in both groups and was mild (all bleeding events were
After 4 weeks of the allocated treatment, patients in both the TIC and TIC+ASP groups had reduced aggregation with 10 μmol/L ADP that was similar between groups, confirming that ticagrelor had fully suppressed the ADP-mediated activation pathway in both groups. Conversely aggregation with 1 mmol/L AA was markedly reduced in the TIC+ASP group compared with the TIC group, indicating the presence of aspirin, which potently inhibits the aggregation response to AA by blocking synthesis of TxA2. Having confirmed strong blockade of the ADP and TxA2 activation pathways by TIC and ASP respectively, we observed that platelet aggregation responses to TRAP-6 (protease activated (thrombin) receptor-1 agonist) and U46619 (TxA2 receptor agonist) were similar between the treatment groups. This finding replicates data from previous studies using platelets from healthy volunteers incubated ex vivo with prasugrel active metabolite or with ticagrelor10,14,15 or from healthy volunteers receiving prasugrel,30 and suggests that strong P2Y12 blockers duplicate the inhibitory effect of aspirin on the thrombin and TxA2 receptor activation pathways. By contrast, we found that the aggregation responses to CRP, which is a specific agonist of the platelet glycoprotein (GP) VI (collagen) receptor, were higher in the TIC group compared with the TIC+ASP group suggesting that TIC is insufficient to completely suppress collagen-mediated platelet activation. This effect was also observed in healthy volunteer studies,14,15,30 although only at high concentrations of the collagen agonist, which has less specificity for the GPVI receptor than CRP.31 The GPVI receptor is essential for normal haemostasis32 and mediates platelet activation through downstream signalling pathways that are dependent on TxA2 synthesis and thereby are highly sensitive to aspirin.33 We were unable to demonstrate a difference between the treatment groups in surface expression of the activation markers PAC 1 and anti-CD62P after stimulation with CRP, possibly reflecting limited sensitivity of this flow cytometry assay to differences in activation responses compared with the gold-standard light transmission aggregation.34

Our findings also provide potential mechanistic explanations for differences in adverse events observed in recent randomized controlled trials of P2Y12 antagonist monotherapy versus DAPT. In particular, they suggest that the lower incidence of bleeding in the P2Y12 antagonist monotherapy groups in the TWILIGHT and SMART-CHOICE trials17,19 may be attributable to partial preservation of platelet GPVI-mediated activation pathways in the monotherapy group, compared with more complete suppression from aspirin in the DAPT group. In the GLOBAL LEADERS study overall bleeding was similar in the P2Y12 antagonist monotherapy and DAPT

DISCUSSION

The TEMPLATE study is the first randomized controlled trial performed to assess differences between the pharmacodynamic effects of TIC and DAPT with TIC + ASP in patients who have previously undergone PCI. The study was performed in order to provide mechanistic insight into the extent of platelet inhibition with TIC. The panel of tests included functional evaluation of the major platelet receptors for thrombin and collagen which are essential for normal haemostasis and initiate pathological thrombus formation in acute coronary syndrome.23–25 We found the pharmacodynamic effect of TIC to be similar to previous studies with groups of patients with acute coronary syndrome.26,27 However our study also showed the primary outcome of platelet aggregation with 10 μmol/L TRAP-6 was the same in patients receiving TIC compared with patients receiving DAPT. However, platelet aggregation with 1 μg/mL or 0.5 μg/mL CRP was higher in patients receiving TIC, indicating less complete platelet inhibition.

Bleeding Academic Research Consortium Type 1.22 One major adverse cardiac (fatal acute coronary syndrome) event occurred during the 4-week trial intervention period in the TIC+ASP group.

Figure 5. Platelet binding of the PAC 1 and anti-CD62P activation markers from blood sample 2. A, baseline activation marker binding without agonist stimulation. B, the increase in activation marker binding after stimulation with thrombin receptor activation peptide 6 (TRAP-6) 5 μmol/L or with collagen related peptide (CRP) 1 μg/mL. The data are expressed as the median (interquartile range) of the median fluorescent intensity of binding of PAC 1 or anti-CD62P.
groups. However, in a posthoc analysis of the acute coronary syndrome cohort, increased bleeding in the DAPT group compared with the P2Y₁₂ antagonist monotherapy group was observed between 1 and 12 months after treatment allocation, supporting the notion that across all patient groups avoidance of aspirin diminishes bleeding. It is also noteworthy that despite different bleeding rates in the treatment groups, thrombotic outcomes were not more frequent in the P2Y₁₂ antagonist monotherapy groups. Several potential explanations for this apparent mismatch have been proposed. However, our data raise the additional possibility that the similar anti-thrombotic effects of P2Y₁₂ antagonist monotherapy and DAPT could reflect their similar inhibitory effect on the platelet thrombin activation pathway, which is an essential component of the stepwise models of pathogenesis of atherothrombosis but may be partially redundant with collagen-mediated platelet activation in normal haemostasis. Considered alongside the clinical outcome data, our findings support comprehensive platelet inhibition with DAPT early after PCI when thrombotic risk is highest. They suggest further the potential for subsequent cessation of aspirin to diminish bleeding by reducing platelet inhibition and by specifically minimising gastrointestinal tract bleeding for which aspirin is a contributory factor because of inhibition of gastroprotective prostanoids. The study necessarily evaluated platelet function ex vivo using tests that are insensitive to regulators of platelet activation released locally in the vasculature such as prostacyclin. Synthesis of this important negative regulator of platelet activation is inhibited by aspirin, thereby offsetting the main anti-thrombotic effect of aspirin that occurs through inhibition of platelet TxA₂ synthesis. This effect is unlikely to be detected in ex vivo platelet function tests thereby potentially leading to an overestimation of overall anti-thrombotic effect of aspirin. The study was insufficiently powered to test whether the differences between the treatment groups were consistent across all patient subgroups such as diabetes mellitus versus no diabetes mellitus or smokers versus non-smokers. Since these characteristics influence baseline platelet reactivity, this is an attractive area of future investigation.

CONCLUSIONS

Monotherapy with the strong P2Y₁₂ antagonist ticagrelor results in less complete inhibition of collagen GPVI-mediated platelet activation when compared with DAPT with TIC+ASP. This finding may partially contribute to the increased bleeding in patients receiving DAPT compared with potent P2Y₁₂ antagonists.

ARTICLE INFORMATION

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Disclosures

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Supplementary Material

Tables S1–S4

Figures S1–S2
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SUPPLEMENTAL MATERIAL
Table S1. Light transmission aggregation responses for the study patients subdivided according to treatment group.

| Agonist       | Blood sample | TIC + ASP Median MA (IQR) | TIC Median MA (IQR) | n  | Coefficient | 95% CI | p-value |
|---------------|--------------|---------------------------|---------------------|----|-------------|-------|---------|
| TRAP-6 10 μM  | 1            | 81.5 (77.0, 91.0)         | 81.0 (75.0, 90.0)   | 54 | -           | -     | -       |
|               | 2            | 81.0 (76.0, 90.0)         | 86.0 (79.0, 93.5)   | 47 | 4.29        | -0.87, 9.46 | 0.103   |
|               | 3            | 86.0 (83.0, 96.0)         | 88.0 (83.0, 97.0)   | 49 | -           | -     | -       |
| TRAP-6 5 μM   | 1            | 73.5 (63.0, 84.0)         | 73.0 (67.0, 81.0)   | 54 | -           | -     | -       |
|               | 2            | 73.0 (68.0, 82.0)         | 81.0 (74.5, 87.0)   | 47 | 7.73*       | -0.13, 15.59 | 0.054   |
|               | 3            | 84.0 (78.0, 91.0)         | 84.0 (79.0, 92.0)   | 49 | -           | -     | -       |
| CRP 1 μg/mL   | 1            | 85.0 (78.0, 92.0)         | 84.0 (78.5, 93.0)   | 54 | -           | -     | -       |
|               | 2            | 85.0 (80.0, 94.0)         | 91.5 (87.0, 100.5)  | 47 | 6.47        | 2.04, 10.90 | 0.004   |
|               | 3            | 92.0 (86.0, 99.0)         | 92.5 (85.0, 97.0)   | 49 | -           | -     | -       |
| CRP 0.5 μg/mL | 1            | 77.0 (61.0, 85.0)         | 76.0 (64.0, 86.0)   | 54 | -           | -     | -       |
|               | 2            | 76.0 (65.0, 82.0)         | 89.0 (82.0, 95.0)   | 47 | 14.00*      | 7.63, 20.39 | <0.001  |
|               | 3            | 85.0 (80.0, 91.0)         | 86.0 (82.0, 96.0)   | 49 | -           | -     | -       |
| U46619 5 μM   | 1            | 78.0 (66.0, 86.0)         | 76.0 (69.0, 85.0)   | 53 | -           | -     | -       |
|               | 2            | 74.0 (66.0, 84.0)         | 74.5 (64.0, 81.5)   | 47 | -1.41*      | -6.01, 3.18 | 0.547   |
|               | 3            | 88.0 (85.0, 93.0)         | 88.5 (86.0, 93.0)   | 48 | -           | -     | -       |
| U46619 2.5 μM | 1            | 49.0 (35.0, 64.0)         | 47.0 (33.0, 61.0)   | 53 | -           | -     | -       |
|               | 2            | 47.0 (35.0, 63.0)         | 44.5 (33.5, 67.0)   | 47 | 0.35*       | -7.73, 8.43 | 0.932   |
|               | 3            | 85.0 (78.5, 91.0)         | 82.0 (77.0, 91.0)   | 48 | -           | -     | -       |
| ADP 10 μM     | 1            | 41.5 (32.0, 56.0)         | 43.0 (33.0, 56.0)   | 54 | -           | -     | -       |
|               | 2            | 34.0 (30.0, 41.0)         | 36.5 (29.0, 42.0)   | 46 | 1.15        | -4.89, 7.19 | 0.709   |
|               | 3            | 77.0 (71.0, 85.0)         | 75.0 (71.0, 83.0)   | 49 | -           | -     | -       |
| AA 1 mM       | 1            | 6.5 (4.0, 9.0)            | 5.0 (3.0, 8.0)      | 54 | -           | -     | -       |
|               | 2            | 5.0 (3.0, 8.0)            | 75.0 (59.0, 83.0)   | 47 | 11.68       | 8.55, 15.95 | <0.001  |
|               | 3            | 7.0 (4.0, 10.0)           | 6.0 (4.0, 8.0)      | 49 | -           | -     | -       |

The data are expressed as the median (interquartile range) of the maximum aggregation responses at the stated agonist concentrations. TRAP-6- thrombin receptor activation peptide 6; CRP- collagen related peptide; U46619- thromboxane A2 receptor agonist ADP- adenosine diphosphate; AA- arachidonic acid. * post hoc analyses
Table S2. Platelet activation marker binding for the study patients subdivided according to treatment group.

| Agonist          | Blood sample | Activation marker | TIC + ASP | TIC |
|------------------|--------------|-------------------|-----------|-----|
|                  |              |                   | Median MFI (IQR) | n | Median MFI (IQR) | n |
| Before stimulation | 1            | PAC-1             | 117 (60) | 53 | 113 (69) | 53 |
|                  | 1            | CD62P             | 24 (21)  | 54 | 23 (17)  | 53 |
|                  | 2            | PAC-1             | 122 (88) | 48 | 112 (82) | 53 |
|                  | 2            | CD62P             | 25 (23)  | 48 | 22 (18)  | 52 |
|                  | 3            | PAC-1             | 166 (117)| 48 | 138 (99) | 49 |
|                  | 3            | CD62P             | 37 (28)  | 48 | 28 (23)  | 50 |
| TRAP-6 5 μM      | 1            | PAC-1             | 1494 (1106)| 52 | 1349 (1131)| 53 |
|                  | 1            | CD62P             | 3056 (2171)| 52 | 3071 (1597)| 53 |
|                  | 2            | PAC-1             | 1286 (1501)| 48 | 1206 (765)| 53 |
|                  | 2            | CD62P             | 3012 (3242)| 48 | 2903 (2789)| 53 |
|                  | 3            | PAC-1             | 3934 (3434)| 48 | 3847 (3588)| 50 |
|                  | 3            | CD62P             | 4511 (5370)| 48 | 3811 (5344)| 50 |
| CRP 1 μg/mL      | 1            | PAC-1             | 2997 (444) | 52 | 2812 (1304) | 53 |
|                  | 1            | CD62P             | 3498 (2731) | 52 | 3378 (2256) | 53 |
|                  | 2            | PAC-1             | 3128 (2323) | 48 | 3056 (1508) | 50 |
|                  | 2            | CD62P             | 3405 (5248) | 48 | 3224 (2718) | 50 |
|                  | 3            | PAC-1             | 4342 (3300) | 47 | 3729 (3398) | 49 |
|                  | 3            | CD62P             | 4614 (5682) | 48 | 4205 (6174) | 49 |

The data are expressed as the median (interquartile range) of the median fluorescent intensity of binding of the PAC-1 or CD62P activation markers at without agonist stimulation and the increase in median fluorescent intensity after stimulation with TRAP-6 5 μM or with CRP 1 μg/mL.
Table S3. Adverse events and serious adverse events for the entire study duration.

|                         | TIC + ASP (N=49) | TIC (N=54) |
|-------------------------|------------------|------------|
|                         | Adverse events   | SAEs       | Adverse events | SAEs   |
|                         | Events/ patients | % patients | Events/ patients | % patients | Events/ patients | % patients |
| Any event               | 115/38           | 69         | 1/1           | 2       | 143/45           | 82         | 6/2        | 4 |
| Bleeding *              | 37/27            | 49         | 0/0           | 0       | 20/16            | 29         | 0/0        | 0 |
| Eye                     | 0/0              | 0          | 0/0           | 0       | 1/1              | 2          | 0/0        | 0 |
| Oral                    | 1/1              | 2          | 0/0           | 0       | 3/3              | 5          | 0/0        | 0 |
| Epistaxis               | 4/4              | 7          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Subcutaneous or dermal  | 23/23            | 47         | 0/0           | 0       | 14/14            | 26         | 0/0        | 0 |
| Post procedural         | 1/1              | 2          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Wound                   | 2/2              | 4          | 0/0           | 0       | 1/1              | 2          | 0/0        | 0 |
| Traumatis               | 2/2              | 4          | 0/0           | 0       | 1/1              | 2          | 0/0        | 0 |
| Other bleeding          | 4/3              | 5          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Gl tract                | 19/9             | 16         | 0/0           | 0       | 42/22            | 40         | 0/0        | 0 |
| Abdominal pain          | 3/3              | 5          | 0/0           | 0       | 3/3              | 6          | 0/0        | 0 |
| Nausea                  | 6/5              | 9          | 0/0           | 0       | 9/6              | 11         | 0/0        | 0 |
| Vomiting                | 3/3              | 5          | 0/0           | 0       | 6/4              | 7          | 0/0        | 0 |
| Diarrhoea               | 6/4              | 7          | 0/0           | 0       | 14/14            | 25         | 0/0        | 0 |
| Constipation            | 0/0              | 0          | 0/0           | 0       | 2/2              | 4          | 0/0        | 0 |
| Dyspepsia               | 1/1              | 2          | 0/0           | 0       | 8/7              | 13         | 0/0        | 0 |
| Liver                   | 2/1              | 2          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Abnormal LFT            | 2/1              | 2          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Skin                    | 4/3              | 5          | 0/0           | 0       | 10/8             | 15         | 1/1        | 2 |
| Skin reaction           | 4/3              | 5          | 0/0           | 0       | 10/8             | 15         | 1/1        | 2 |
| Neurology               | 8/6              | 11         | 0/0           | 0       | 9/8              | 15         | 0/0        | 0 |
| Confusion               | 1/1              | 2          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Dizziness               | 2/2              | 4          | 0/0           | 0       | 1/1              | 2          | 0/0        | 0 |
| Headache                | 3/3              | 5          | 0/0           | 0       | 4/4              | 7          | 0/0        | 0 |
| Parasthesia             | 1/1              | 2          | 0/0           | 0       | 4/4              | 7          | 0/0        | 0 |
| Vertigo                 | 1/1              | 2          | 0/0           | 0       | 0/0              | 0          | 0/0        | 0 |
| Kidney                  | 3/2              | 4          | 0/0           | 0       | 4/4              | 7          | 1/1        | 2 |
| Water retention         | 2/2              | 4          | 0/0           | 0       | 2/2              | 4          | 0/0        | 0 |
| Poor renal function     | 1/1              | 2          | 0/0           | 0       | 2/2              | 4          | 1/1        | 2 |
| Respiratory             | 16/15            | 27         | 0/0           | 0       | 20/20            | 36         | 0/0        | 0 |
| Bronchospasm            | 1/1              | 2          | 0/0           | 0       | 2/2              | 4          | 0/0        | 0 |
| Dyspnoea                | 15/15            | 27         | 0/0           | 0       | 18/18            | 33         | 0/0        | 0 |
| Other                   | 25/17            | 31         | 0/0           | 0       | 36/24            | 44         | 2/2        | 4 |
| Hypersensitivity        | 0/0              | 0          | 0/0           | 0       | 4/3              | 5          | 0/0        | 0 |
| Other events            | 25/17            | 31         | 0/0           | 0       | 32/24            | 44         | 2/2        | 4 |
| MACE                    | 1/1              | 2          | 1/1           | 2       | 2/1              | 2          | 2/1        | 2 |
| Death                   | 1/1              | 2          | 1/1           | 2       | 0/0              | 0          | 0/0        | 0 |
| MI                      | 0/0              | 0          | 0/0           | 0       | 1/1              | 2          | 1/1        | 2 |
| Revascularisation       | 0/0              | 0          | 0/0           | 0       | 1/1              | 2          | 1/1        | 2 |

*All bleeding events were mild and did not require additional clinical intervention (BARC type 1).*
Table S4. Adverse events and serious adverse events during allocation to TIC or TIC+ASP.

| Event Category | TIC + ASP (N=49) | TIC (N=54) |  |
|----------------|------------------|------------|---|
|                | Events/patients  | % patients | Events/patients | % patients | Events/patients | % patients | Events/patients | % patients |
| Any event      | 90/37            | 67         | 1/1              | 2         | 98/39          | 71%        | 1/1              | 2         |
| Bleeding *     | 33/25            | 45         | 0/0              | 0         | 20/16          | 29%        | 0/0              | 0         |
| Eye            | 0/0              | 0          | 0/0              | 0         | 1/1            | 2%         | 0/0              | 0         |
| Oral           | 1/1              | 2%         | 0/0              | 0         | 3/3            | 5%         | 0/0              | 0         |
| Epistaxis      | 4/4              | 7%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Subcutaneous or dermal | 23/23 | 47%       | 0/0              | 0         | 14/14          | 26%        | 0/0              | 0         |
| Post procedural | 1/1              | 2%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Wound          | 2/2              | 4%         | 0/0              | 0         | 1/1            | 2%         | 0/0              | 0         |
| Traumatic      | 2/2              | 4%         | 0/0              | 0         | 1/1            | 2%         | 0/0              | 0         |
| Other bleeding | 0/0              | 0%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| GI tract       | 13/7             | 16%        | 0/0              | 0         | 29/14          | 40%        | 0/0              | 0         |
| Abdominal pain | 3/3              | 5%         | 0/0              | 0         | 3/3            | 6%         | 0/0              | 0         |
| Nausea         | 4/4              | 8%         | 0/0              | 0         | 8/5            | 9%         | 0/0              | 0         |
| Vomiting       | 2/2              | 4%         | 0/0              | 0         | 5/3            | 5%         | 0/0              | 0         |
| Diarrhoea      | 3/3              | 6%         | 0/0              | 0         | 9/9            | 16%        | 0/0              | 0         |
| Constipation   | 0/0              | 0%         | 0/0              | 0         | 2/2            | 4%         | 0/0              | 0         |
| Dyspepsia      | 1/1              | 2%         | 0/0              | 0         | 2/2            | 4%         | 0/0              | 0         |
| Liver          | 1/1              | 2%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Abnormal LFT   | 1/1              | 2%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Skin           | 2/2              | 4%         | 0/0              | 0         | 7/6            | 11%        | 1/1              | 2%         |
| Skin reaction  | 2/2              | 4%         | 0/0              | 0         | 7/6            | 11%        | 1/1              | 2%         |
| Neurology      | 8/6              | 11%        | 0/0              | 0         | 9/8            | 15%        | 0/0              | 0         |
| Confusion      | 1/1              | 2%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Dizziness      | 2/2              | 4%         | 0/0              | 0         | 1/1            | 2%         | 0/0              | 0         |
| Headache       | 3/3              | 5%         | 0/0              | 0         | 4/4            | 7%         | 0/0              | 0         |
| Paraesthesia   | 1/1              | 2%         | 0/0              | 0         | 4/4            | 7%         | 0/0              | 0         |
| Vertigo        | 1/1              | 2%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Kidney         | 2/2              | 4%         | 0/0              | 0         | 2/2            | 4%         | 0/0              | 0         |
| Water retention| 2/2              | 4%         | 0/0              | 0         | 2/2            | 4%         | 0/0              | 0         |
| Poor renal function | 0/0 | 0%       | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Respiratory    | 15/15            | 27%        | 0/0              | 0         | 18/18          | 33%        | 0/0              | 0         |
| Bronchospasm   | 0/0              | 0%         | 0/0              | 0         | 0/0            | 0%         | 0/0              | 0         |
| Dyspnoea       | 15/15            | 27%        | 0/0              | 0         | 18/18          | 33%        | 0/0              | 0         |
| Other          | 15/15            | 27%        | 0/0              | 0         | 13/12          | 22%        | 1/1              | 2%         |
| Hypersensitivity| 0/0              | 0%         | 0/0              | 0         | 2/2            | 4%         | 0/0              | 0         |
| Other events   | 15/15            | 27%        | 0/0              | 0         | 11/11          | 20%        | 1/1              | 2%         |
| MACE           | 1/1              | 2%         | 1/1              | 2%        | 0/0            | 0%         | 0/0              | 0%        |
| Death          | 1/1              | 2%         | 1/1              | 2%        | 0/0            | 0%         | 0/0              | 0%        |
| MI             | 0/0              | 0%         | 0/0              | 0%        | 0/0            | 0%         | 0/0              | 0%        |
| Revascularisation| 0/0              | 0%         | 0/0              | 0%        | 0/0            | 0%         | 0/0              | 0%        |

*All bleeding events were mild and did not require additional clinical intervention (BARC type 1).*
Figure S1. Platelet binding of the PAC 1 and anti-CD62P activation markers from blood sample one.

A. Baseline activation marker binding without agonist stimulation, and, B. the increase in activation marker binding after stimulation with thrombin receptor activation peptide 6 (TRAP-6) 5 µM or with collagen related peptide (CRP) 1 µg/mL. The data are expressed as the median (interquartile range) of the median fluorescent intensity of binding of the PAC 1 or anti-CD62P activation markers.

At this time point all patients were receiving ASP plus a P2Y₁₂ blocker (ticagrelor 44%; clopidogrel 35%; or prasugrel 21%). A. baseline activation marker binding without agonist stimulation, and, B. the increase in activation marker binding after stimulation with thrombin receptor activation peptide 6 (TRAP-6) 5 µM or with collagen related peptide (CRP) 1 µg/mL. The data are expressed as the median (interquartile range) of the median fluorescent intensity of binding of the PAC 1 or anti-CD62P activation markers.
Figure S2. Platelet binding of the PAC 1 and anti-CD62P activation markers from blood sample three.

At this time point all patients were receiving monotherapy with ASP. **A.** baseline activation marker binding without agonist stimulation, and, **B.** the increase in activation marker binding after stimulation with thrombin receptor activation peptide 6 (TRAP-6) 10 µM or with collagen related peptide (CRP) 1µg/mL. The data are expressed as the median (interquartile range) of the median fluorescent intensity of binding of the PAC 1 or anti-CD62P activation markers.