# Longitudinal assessment of von Willebrand factor antigen and von Willebrand factor propeptide in response to alteration of antiplatelet therapy after TIA or ischaemic stroke

**W. O. Tobin · J. A. Kinsella · G. F. Kavanagh · J. S. O'Donnell · R. T. McGrath · T. Coughlan · D. R. Collins · D. O'Neill · B. Egan · S. Tierney · T. M. Feeley · R. P. Murphy · D. J. H. McCabe**

**Abstract** The impact of commencing or changing antiplatelet therapy on von Willebrand factor antigen (VWF:Ag) and von Willebrand factor propeptide (VWF:Ag II) levels has not been comprehensively assessed following TIA or ischaemic stroke. In this pilot, longitudinal, observational analytical study, VWF:Ag and VWF:Ag II levels were simultaneously quantified in platelet poor plasma by ELISA in patients within 4 weeks of TIA or ischaemic stroke (baseline), and then 14 days (14d) and >90 days (90d) after altering antiplatelet therapy. Ninety-one patients were recruited. Eighteen were initially assessed on no antiplatelet therapy, and then after 14d ($N = 17$) and 90d ($N = 8$) on aspirin monotherapy; 21 patients were assessed on aspirin and after 14d and 90d on clopidogrel; 52 were assessed on aspirin monotherapy, and after 14d and 90d on aspirin and dipyridamole combination therapy. VWF:Ag, VWF:Ag II levels and VWF:Ag/VWF:Ag II ratio were unchanged at 14d and 90d in the overall study population ($p \geq 0.1$). VWF:Ag and VWF:Ag II levels remained stable at 14d and 90d after commencing aspirin ($p \geq 0.054$), and after changing from aspirin to clopidogrel ($p \geq 0.2$). Following the addition of dipyridamole MR to aspirin, there was a significant reduction in VWF:Ag levels at 14d ($p = 0.03$) and 90d ($p = 0.005$), but not in VWF:Ag II levels ($p \geq 0.3$). The addition of dipyridamole to aspirin led to a persistent reduction in VWF:Ag but not in VWF:Ag II levels, suggesting that dipyridamole may inhibit release of platelet-derived VWF:Ag following TIA or ischaemic stroke.

**Keywords** von Willebrand factor · von Willebrand factor propeptide · TIA · Stroke · Aspirin · Dipyridamole · Clopidogrel · High on treatment platelet reactivity

## Introduction

Activated platelets play a key role in arterial thrombus formation, and because platelets interact with the endothelium, endothelial activation has the potential to cause or exacerbate an ischaemic insult in patients with ischaemic cerebrovascular disease (CVD).

W. O. Tobin · J. A. Kinsella · G. F. Kavanagh · R. P. Murphy · D. J. H. McCabe (✉)
Department of Neurology, The Adelaide and Meath Hospital, Dublin, Incorporating the National Children's Hospital, Trinity College Dublin, Dublin, Ireland
e-mail: dominick.mccabe@amnch.ie

J. S. O'Donnell · R. T. McGrath
Haemostasis Research Group, St James's Hospital, Trinity College Dublin, Dublin, Ireland

T. Coughlan · D. R. Collins · D. O'Neill
Department of Age-Related Health Care, The Adelaide and Meath Hospital, Dublin, Incorporating the National Children's Hospital, Trinity College Dublin, Dublin, Ireland

T. Coughlan · D. R. Collins · D. O'Neill · R. P. Murphy · D. J. H. McCabe
Department of Stroke Service, The Adelaide and Meath Hospital, Dublin, Incorporating the National Children's Hospital, Trinity College Dublin, Dublin, Ireland

B. Egan · S. Tierney · T. M. Feeley
Department of Vascular Surgery, The Adelaide and Meath Hospital, Dublin, Incorporating the National Children's Hospital, Trinity College Dublin, Dublin, Ireland

D. J. H. McCabe
Department of Clinical Neurosciences, UCL Institute of Neurology, Royal Free Campus, London, UK

Von Willebrand factor (VWF:Ag) is a multimeric plasma glycoprotein that is synthesised in vascular endothelial cells and megakaryocytes [3, 28]. Endothelial cells secrete VWF:Ag constitutively into the circulating blood or into the subendothelial matrix, and also release VWF:Ag stored in Weibel-Palade bodies in response to endothelial cell activation [3, 26, 28, 30]. Platelets also secrete VWF:Ag from alpha granules, but the majority of plasma VWF:Ag is thought to originate from the endothelium [26]. VWF:Ag may bind to the platelet surface glycoprotein (Gp) Ib-IX-V receptor complex, thus mediating platelet adhesion to exposed subendothelial collagen and subsequent platelet-rich thrombus formation [3, 28]. VWF:Ag is an important glycoprotein in patients with vascular disease, and elevated VWF:Ag levels have been identified in both the early [2–4, 18, 19, 21, 23, 27] and late phases following an ischaemic cerebrovascular event (CVE) [15, 19, 23], and following an ischaemic or haemorrhagic stroke compared with healthy controls [4, 32].

von Willebrand Factor propeptide (VWF:Ag II) is produced by cleavage of pro-VWF into VWF:Ag and VWF:Ag II [10, 36]. VWF:Ag II undergoes endoproteolytic cleavage to form VWF:Ag in endothelial cells, and is also stored in Weibel-Palade bodies until released during endothelial activation [36]. Levels of VWF:Ag II or the ratio of VWF:Ag/VWF:Ag II in plasma may provide an accurate measure of the degree of acute endothelial cell activation [14, 36], because VWF:Ag II has a shorter half life than VWF:Ag, and returns to baseline levels rapidly after the stimulus to endothelial activation is removed [5, 10, 11, 13, 22, 36]. However, to our knowledge, VWF:Ag and VWF:Ag II levels have not been measured simultaneously after commencing or changing antiplatelet therapy in patients with recent TIA or ischaemic stroke. Because VWF:Ag levels have been shown to have a significant impact on platelet function ex vivo under conditions of moderately high shear stress [33–35], a better understanding of the role of VWF:Ag and VWF:Ag II in ischaemic CVD may improve our understanding of the mechanisms influencing recurrent vascular events in CVD patients on antiplatelet therapy.

## Aims and hypotheses

The aims of this pilot, prospective, longitudinal, case crossover study were to assess the impact of changing antiplatelet agents on plasma VWF:Ag levels, VWF:Ag II levels, and VWF:Ag/VWF:Ag II ratio ex vivo, in patients with recent TIA or ischaemic stroke. We hypothesised that VWF:Ag, VWF:Ag II levels, or VWF:Ag/VWF:Ag II ratio would decrease after altering antiplatelet agents, and that this reduction would be most marked after the addition of dipyridamole to aspirin, due to the proposed inhibitory effects of dipyridamole on both platelets and the endothelium [16, 37].

We also assessed the impact of plasma VWF:Ag and VWF:Ag II levels on platelet adhesion and aggregation on a platelet function analyser (PFA-100®) in patients commencing aspirin and changing from aspirin monotherapy to aspirin–dipyridamole combination therapy. We hypothesised that VWF:Ag and VWF:Ag II levels would be higher in patients with 'high on treatment platelet reactivity' (HTPR) than those without HTPR on the PFA-100 [35].

## Methods

### Study design and inclusion criteria

The overall design of the prospective, longitudinal, observational TRinity AntiPlatelet responsiveness (TRAP) study has been published previously [33–35]. In brief, consecutive eligible patients >18 years old who had a 'stroke specialist-confirmed' TIA or ischaemic stroke within the preceding 4 weeks, and whose treating physician decided to commence or change their antiplatelet therapy, were eligible for inclusion in this component of the TRAP study. Patients were recruited from the rapid access stroke prevention (RASP), neurology, stroke medicine, and vascular surgery services at our university teaching hospital which accepts direct referrals from primary care and emergency department physicians, and hospital consultants in the region.

### Exclusion criteria

We excluded CVD patients with a history of primary intracerebral haemorrhage, myocardial infarction within the preceding 3 months, ongoing unstable angina, unstable symptomatic peripheral vascular disease, major surgery or systemic haemorrhage within the preceding 3 months (haemoglobin decrease of >1 g/dl in 1 day, or requiring transfusion), if they had systemic vasculitis, underlying neoplasia, or a known bleeding or clotting diathesis including known platelet disorders, platelet count $<120 \times 10^9$/l or $>450 \times 10^9$/l, urea >10 mmol/l or GFR <30 ml/min, current infection (clinical signs of infection, white cell count $>12 \times 10^9$/l), or non-steroidal anti-inflammatory drug (NSAID) use other than aspirin within 14 days of recruitment [34].

Written informed consent, or assent where appropriate, was obtained from all subjects. The study was approved by the St. James Hospital/AMNCH local research ethics committee (REC Ref: 2,007/07/MA).

### Clinical and laboratory assessments

Clinical assessment was performed as outlined previously [33, 35]. In brief, all subjects underwent a detailed clinical neurovascular and laboratory assessment at 3 time points, by at least one of the three examiners: at baseline prior to changing antiplatelet therapy, ≥14 days after changing antiplatelet therapy (14d), and ≥90 days after changing antiplatelet therapy (90d). TIA and stroke work-up was performed according to European Stroke Organisation guidelines [9]. The underlying mechanism responsible for TIA or ischaemic stroke was categorised according to the TOAST classification [1].

Adherence to antiplatelet therapy in inpatients was confirmed by history taking and by checking the inpatient drug chart. Adherence in outpatients was assessed by history taking alone, but all outpatients were phoned to stress the importance of medication adherence in the week prior to reassessment. Reassessment was deferred for 14 days in any patients deemed possibly non-adherent to their antiplatelet regimen.

### Blood sampling and laboratory tests

Careful venepuncture was performed as described previously [22–25, 33, 35]. All subjects were rested for at least 20 min before venepuncture to standardise conditions and to minimise platelet activation in vivo. The first three 3 ml 3.2 % citrate-anticoagulated blood samples were used for platelet activation and functional studies, including PFA-100 analysis, as described previously [33, 35]. The next four citrate-anticoagulated samples were used to prepare double-spun platelet poor plasma (PPP) and immediately frozen at −70 °C within 60 min of venepuncture [33, 34].

### Enzyme linked immunosorbent assay (ELISA)

Samples were thawed once at 37 °C for 20 min before quantification of VWF:Ag, utilising a VWF:Ag ELISA. Samples were then refrozen, stored at −70 °C, until thawed once more for the assessment of VWF:Ag II levels, also by ELISA. The concentration of VWF:Ag and VWF:Ag II in each PPP sample was determined, as previously described [14]. In brief, Polyclonal Rabbit Anti-Human VWF antibody (DAKO) was used as coating antibody, and polyclonal rabbit Anti-Human VWF/HRP antibody (DAKO) as detection antibody for the VWF:Ag ELISA. M193902 CLB-Pro 35 coating antibody (Plesmanlaan 125) and M103904HRP CLB-Pro 14.3 detection antibody (Plesmanlaan 125) were used for VWF:Ag II quantification. Following the addition of a colorimetric substrate (ortho-phenylenediamine-dihydrochloride (OPD) Sigma, Ireland), absorbance was measured by spectrophotometry at 490 nm, using a VERSA Max Tuneable Microplate Reader. Standard curves were constructed for VWF:Ag and VWF:Ag II using coagulation reference plasma (Technoclone, Surrey, UK), and VWF:Ag and VWF:Ag II levels in each of the samples were recorded as μg/ml.

### High on-treatment platelet reactivity (HTPR) assessment

The degree of inhibition of platelet function in whole blood was assessed with the PFA-100® platelet function analyser (Dade-Behring, Germany) between 2 and 2.5 h after venepuncture [22, 35]. A novel, scientifically valid definition of HTPR has been established by our group as failure to prolong the relevant closure times on the device from baseline by more than twice the coefficient of variation of the relevant assay, as previously described [33, 35].

High on treatment platelet reactivity in patients commencing aspirin was defined as failure to prolong the C-EPI by >15 % of the patient's own baseline C-EPI closure time [34]. We did not pursue analysis of the relationship between VWF:Ag or VWF:Ag II and PFA-100 C-ADP closure times in patients changing from aspirin to clopidogrel monotherapy, because the C-ADP cartridge does not reliably detect inhibition of platelet function with clopidogrel [12, 17, 33]. HTPR in patients changing from aspirin monotherapy to aspirin and dipyridamole combination therapy was defined as failure to prolong the C-ADP closure times by >14 % of the patient's own baseline C-ADP closure time on aspirin monotherapy [35].

## Statistical methods

Paired or unpaired *t* tests were used for comparison of paired and unpaired parametric variables, respectively. The Wilcoxon signed rank test and the Wilcoxon rank sum test were used for comparison of paired and unpaired non-parametric variables, and the Kruskal–Wallis rank sum test for comparison of multiple non-parametric variables, where appropriate. Chi-squared or Fisher Exact tests were used to compare proportions between groups, where appropriate. $p < 0.05$ was considered to be statistically significant. All statistical calculations were performed using R, version 2.15 [29].

## Results

Ninety-one patients were recruited and assessed at baseline; 90 were followed up at 14d, and 79 of the original 91 patients were reassessed at 90d. The clinical and demographic profiles of the patients are outlined in Tables 1 and 2. There were few patients with large artery disease in this study, because the majority of those patients underwent

**Table 1** Demographic and clinical data in entire patient population and in each treatment subgroup at study entry

| Parameter | All patients ($N = 91$) | 0 − Aspirin ($N = 18$) | Aspirin − Aspirin + Dipyridamole ($N = 52$) | Aspirin − Clopidogrel ($N = 21$) |
|---|---|---|---|---|
| Mean age in years | 60 (±14) | 57 (±15) | 58 (±13) | 70 (±9) |
| Sex (M/F) | 59/32 | 12/6 | 34/18 | 13/8 |
| Prior stroke/TIA | 19 (21 %) | 2 (11 %) | 8 (16 %) | 9 (43 %) |
| IHD | 11 (12 %) | 1 (6 %) | 3 (6 %) | 7 (33 %) |
| Hypertension | 51 (57 %) | 6 (38 %) | 25 (48 %) | 20 (95 %) |
| Diabetes mellitus | 11 (12 %) | 2 (11 %) | 6 (12 %) | 3 (14 %) |
| A fib/flutter at enrolment | 1 (1 %) | 0 (0 %) | 0 (0 %) | 1 (5 %) |
| Family history of stroke | 39 (43 %) | 8 (44 %) | 25 (48 %) | 6 (29 %) |
| Prior DVT/PE | 4 (4 %) | 1 (6 %) | 3 (6 %) | 0 (0 %) |
| Peripheral vascular disease | 4 (4 %) | 0 (0 %) | 2 (4 %) | 2 (10 %) |
| Migraine (with or without aura) | 22 (24 %) | 5 (28 %) | 13 (25 %) | 4 (19 %) |
| Never smoker | 37 (41 %) | 8 (44 %) | 21 (40 %) | 8 (38 %) |
| Statin therapy (at enrolment) | 35 (39 %) | 1 (6 %) | 18 (35 %) | 16 (80 %) |
| Mean NIHSS | 1 | 2 | 1 | 1 |

**Table 2** Aetiological subtyping by TOAST classification in all patients and in each subgroup

| Stroke/TIA subtype | All patients ($N = 91$) | 0 − Aspirin ($N = 18$) | Aspirin − Aspirin + Dipyridamole ($N = 52$) | Aspirin − Clopidogrel ($N = 21$) |
|---|---|---|---|---|
| Large artery atherosclerotic | 3 (3 %) | 0 (0 %) | 2 (4 %) | 1 (5 %) |
| Lacunar | 23 (25 %) | 4 (22 %) | 16 (31 %) | 3 (14 %) |
| Cardioembolic | 17 (19 %) | 5 (28 %) | 9 (17 %) | 3 (14 %) |
| Undetermined aetiology | 42 (46 %) | 6 (33 %) | 22 (42 %) | 14 (67 %) |
| Other determined | 6 (7 %) | 3 (17 %) | 3 (6 %) | 0 (0 %) |

urgent carotid intervention (mainly carotid endarterectomy) and could not undergo laboratory testing at 14 days, because recent surgery within 3 months was an exclusion criterion in the TRAP study. None of the recruited patients had a recurrent vascular event during follow-up in this study.

Alteration of antiplatelet therapy in the overall study patient population did not significantly affect VWF:Ag or VWF:Ag II levels, or the VWF:Ag/VWF:Ag II ratio at 14d or 90d ($p \geq 0.1$) (Table 3).

**Table 3** Plasma markers before (baseline) and 14d and 90d after changing antiplatelet therapy in entire patient population

| | Baseline ($N = 91$) | 14d ($N = 90$) | 90d ($N = 79$) |
|---|---|---|---|
| VWF:Ag (μg/ml) | 10.91 (9.00–15.96) | 11.05 (8.61–14.44) | 10.90 (8.63–14.63) |
| *p* value | | 0.4 | 0.1 |
| VWF:Ag II (μg/ml) | 8.47 (7.20–11.09) | 8.74 (7.08–11.26) | 8.29 (6.80–10.31) |
| *p* value | | 0.2 | 0.2 |
| VWF:Ag/ VWF:Ag II ratio | 1.40 (±0.45) | 1.33 (±0.47) | 1.40 (±0.46) |
| *p* value | | 0.1 | 0.9 |

Values are medians (25–75th percentile) or means (± SD). *p* value refers to comparison between baseline and follow-up at 14d and 90d

## Pre-planned subgroup analyses

### *Changing from no antiplatelet therapy to aspirin monotherapy*

Eighteen patients were assessed at baseline on no antiplatelet medication (Tables 1, 2). Seventeen were reassessed at 14d

**Table 4** Plasma markers before (baseline) and 14d and 90d after commencing aspirin monotherapy

| | Baseline ($N = 18$) | 14d ($N = 17$) | 90d ($n = 8$) |
|---|---|---|---|
| VWF:Ag (μg/ml) | 9.93 (7.19–15.68) | 11.16 (9.54–14.98) | 10.39 (8.09–12.34) |
| $p$ value | | 0.2 | 0.9 |
| VWF:Ag II (μg/ml) | 8.04 (±2.35) | 8.83 (±1.97) | 7.96 (±1.19) |
| $p$ value | | 0.3 | 0.054 |
| VWF:Ag/ VWF:Ag II ratio | 1.41 (±0.52) | 1.48 (±0.71) | 1.31 (±0.43) |
| $p$ value | | 0.6 | 0.5 |

Values are medians (25–75th percentile) or means (± SD). $p$ value refers to comparison between baseline and follow-up at 14d and 90d

**Table 5** Plasma markers on aspirin (baseline), and 14d and 90d after changing to clopidogrel monotherapy

| | Baseline ($N = 21$) | 14d ($N = 21$) | 90d ($N = 21$) |
|---|---|---|---|
| VWF:Ag (μg/ml) | 9.56 (8.25–15.77) | 9.20 (7.62–13.59) | 10.36 (8.09–15.95) |
| $p$ value | | 0.5 | 0.2 |
| VWF:Ag II (μg/ml) | 9.94 (±3.97) | 10.31 (±5.68) | 9.06 (±5.32) |
| $p$ value | | 0.6 | 0.5 |
| VWF:Ag/ VWF:Ag II ratio | 1.18 (±0.41) | 1.14 (±0.41) | 1.43 (±0.53) |
| $p$ value | | 0.5 | 0.6 |

Values are medians (25–75th percentile) or means (± SD). $p$ value refers to comparison between baseline and follow-up at 14d and 90d

on aspirin monotherapy; assessment was not possible in one patient at 14d due to a device error. Only eight of these patients were reassessed at 90d on aspirin monotherapy because the remainder had been changed to an alternate antithrombotic regimen (usually combination antiplatelet therapy or anticoagulation) at the discretion of their treating physician prior to the 90d assessment. There were no significant changes in VWF:Ag levels, VWF:Ag II levels or VWF:Ag/VWF:Ag II ratio at 14d or 90d after commencing aspirin ($p \geq 0.054$) (Table 4). There were no differences in VWF:Ag or VWF:Ag II levels between patients with HTPR and those without HTPR on aspirin monotherapy at 14d or 90d ($p \geq 0.6$).

*Changing from aspirin to clopidogrel monotherapy*

Twenty-one patients were assessed at baseline on aspirin and subsequently at 14d and 90d after changing to clopidogrel monotherapy (Tables 1, 2). There were no significant changes in VWF:Ag levels, VWF:Ag II levels or VWF:Ag/VWF:Ag II ratio at 14d or 90d compared with baseline ($p \geq 0.2$) (Table 5).

**Table 6** Plasma markers on aspirin (baseline), and 14d and 90d after adding dipyridamole MR to aspirin

| | Baseline ($N = 52$) | 14d ($N = 52$) | 90d ($N = 52$) |
|---|---|---|---|
| VWF:Ag (μg/ml) | 12.44 (9.71–17.08) | 11.77 (8.68–14.29) | 11.44 (8.72–14.55) |
| $p$ value | | **0.03** | **0.005** |
| VWF:Ag II (μg/ml) | 9.36 (±3.81) | 9.54 (±3.57) | 12.04 (±4.78) |
| $p$ value | | 0.7 | 0.3 |
| VWF:Ag/ VWF:Ag II ratio | 1.49 (±0.41) | 1.36 (±0.37) | 1.40 (±0.44) |
| $p$ value | | **0.02** | 0.2 |

Values are medians (25–75th percentile) or means (± SD). $p$ value refers to comparison between baseline and follow-up at 14d and 90d Significant $p$ values are shown in bold ($p < 0.05$)

*Addition of dipyridamole MR to Aspirin*

Fifty-two patients were assessed at baseline on aspirin, and subsequently at 14d and 90d after adding dipyridamole modified release (MR) to aspirin (Tables 1, 2). One patient was changed from aspirin monotherapy to the commercially available combination preparation of asasantin retard® twice daily (containing 25 mg of aspirin and 200 mg of dipyridamole MR per tablet); another patient was changed from 75 mg of aspirin monotherapy daily to 75 mg of aspirin in combination with asasantin retard twice daily. All other patients in this study had 200 mg of dipyridamole MR twice daily added to their once daily dose of aspirin. VWF:Ag levels significantly decreased 14d after adding dipyridamole MR to aspirin ($p = 0.03$), and this reduction was maintained at 90d ($p = 0.005$) (Table 6). There was no significant change in VWF:Ag II levels at 14d or 90d compared with baseline values on aspirin monotherapy ($p \geq 0.3$). VWF:Ag/VWF:Ag II ratio transiently decreased at 14d compared to baseline ($p = 0.02$), but this reduction was not sustained at 90d. There were no significant differences in VWF:Ag or VWF:Ag II levels between patients with and those without HTPR on the C-ADP cartridge after changing from aspirin to aspirin–dipyridamole MR combination therapy ($p \geq 0.06$).

## Discussion

This novel, robust, prospective, longitudinal pilot study has shown that the addition of dipyridamole MR to aspirin

leads to a persistent reduction in VWF:Ag levels in both the early and late phases after TIA or ischaemic stroke. These findings could reflect the inhibitory effects of dipyridamole on either platelets or the endothelium because, as stated above, VWF:Ag may be derived from either source [26]. Plasma levels of VWF:Ag II, which is only released from the endothelium, were not influenced by the addition of dipyridamole to aspirin, thus supporting the hypothesis that the reduction in VWF:Ag levels may be mainly mediated by the inhibitory effects of dipyridamole on platelets. Unlike platelet-derived VWF:Ag, endothelial-derived VWF:Ag is linked to ABO blood group antigens [26], and thus the glycan profile of endothelial- versus platelet-derived VWF can be used to differentiate between the cellular source of circulating VWF. However, this study was not designed to distinguish between the impact of dipyridamole on the two potential sub-fractions of VWF:Ag in plasma.

In addition to its beneficial effect of reducing VWF:Ag levels ex vivo [6], there are several postulated mechanisms of action of dipyridamole that may explain its therapeutic effects in the secondary prevention of recurrent vascular events following TIA and ischaemic stroke [7, 20]. Dipyridamole levels in leucocytes appear to increase for at least 30 days after commencing treatment [8, 31], and the drug has several other postulated or proven inhibitory effects on platelets, leucocytes and the endothelium, [16, 34, 35], in addition to its recently described inhibitory effects on thrombin generation [34]. The relative importance of the impact of dipyridamole on each biological pathway is unclear, although an additive, inhibitory effect on different thrombotic/haemostatic pathways ex vivo may contribute to its proven role in secondary prevention after TIA or stroke.

There were no changes in VWF:Ag or VWF:Ag II levels after commencing aspirin monotherapy or after switching from aspirin to clopidogrel monotherapy. Although these findings could potentially reflect a type II error due to the smaller numbers of subjects included in these two subgroups, there was no suggestion whatsoever of a consistent trend in VWF:Ag or VWF:Ag II levels in either subgroup, so it is unlikely that the addition of either aspirin or clopidogrel affects expression of these biomarkers ex vivo. Furthermore, the absence of any change in VWF:Ag levels in the no medication-aspirin monotherapy or aspirin-clopidogrel monotherapy subgroups supports the hypothesis that the results in patients in whom dipyridamole was added to aspirin are not simply reflective of resolution of the acute phase response.

Although previous studies have clearly shown that VWF:Ag levels may influence closure times on the PFA-100 in ischaemic CVD patients [5, 22], we did not find any significant differences in VWF:Ag or VWF:Ag II levels between patients with and those without HTPR on aspirin monotherapy or aspirin–dipyridamole combination therapy using the novel definitions of HTPR employed in this study. Larger studies are warranted to readdress this issue, because the small number of subjects included in these pre-planned subgroup analyses could certainly have led to a type II error.

## Conclusions

These data add to our understanding of the potential diverse, beneficial, anti-thrombotic effects of dipyridamole MR in TIA or ischaemic stroke patients. The addition of dipyridamole to aspirin reduces plasma VWF:Ag levels but not VWF:Ag II levels ex vivo, suggesting that dipyridamole most likely inhibits VWF:Ag release from platelets instead of inhibiting VWF:Ag secretion from the endothelium. Commencing aspirin or clopidogrel does not appear to influence expression of either of these biomarkers. Larger, longitudinal studies are required to determine whether the degree of inhibition of platelet and endothelial function or activation predicts the risk of recurrent vascular events during long-term follow-up [32]. Such data could prompt the design of clinical trials to optimise secondary prevention following TIA or ischaemic stroke [6].

**Acknowledgments** Dr. Tobin's research was funded by the IICN-Serono Fellowship, Meath Foundation, Lundbeck Neurosciences Bursary programme, and by unrestricted educational grant funding from Merck Serono Ireland, Brennan and Company, Ireland, and Biogen Idec Ireland Limited. Dr. Kinsella's research was funded by the Stanley Thomas Johnson Foundation, and by unrestricted educational grant funding from Bayer Schering Ireland, Pfizer Ireland and Elitech UK. Dr. Kavanagh's research was funded by the Health Research Board Ireland. Dr. McCabe's research programme was part-funded by grant support from the Programme for Research in Third Level Institutions in Ireland (Cycle 4), co-funded by the European Regional Development Fund.

**Conflicts of interest** None of the authors report any conflicts of interest or other disclosures. None of the above charities or funding bodies had any influence on study design or conduct, data analysis or interpretation, or had any influence on the decision to submit the final manuscript for publication.

## References

1. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE III (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 24:35–41
2. Bath PM, Blann A, Smith N, Butterworth RJ (1998) Von Willebrand factor, P-selectin and fibrinogen levels in patients with acute ischaemic and haemorrhagic stroke, and their relationship with stroke sub-type and functional outcome. Platelets 9:155–159

3. Bongers TN, de Maat MPM, van Goor ML, Bhagwanbali V, van Vliet HHDM, Gomez Garcia EB, Dippel DWJ, Leebeek FWG (2006) High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability. Stroke 37:2672–2677
4. Catto AJ, Carter AM, Barrett JH, Bamford J, Rice PJ, Grant PJ (1997) von Willebrand factor and factor VIII: C in acute cerebrovascular disease. Relationship to stroke subtype and mortality. Thromb Haemost 77:1104
5. Chakroun T, Gerotziafas G, Robert F, Lecrubier C, Samama MM, Hatmi M, Elalamy I (2004) In vitro aspirin resistance detected by PFA-100 closure time: pivotal role of plasma von Willebrand factor. Br J Haematol 124:80–85
6. De Meyer SF, Stoll G, Wagner DD, Kleinschnitz C (2012) von Willebrand factor: an emerging target in stroke therapy. Stroke 43:599–606
7. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A (1996) European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. J Neurol Sci 143:1–13
8. Eisert WG (2001) Near-field amplification of antithrombotic effects of dipyridamole through vessel wall cells. Neurology 57:S20–S23
9. European Stroke Organisation (ESO) Executive Committee, ESO Writing Committee (2008) Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. Cerebrovasc Dis 25:457–507
10. Frijns CJ, Kasius KM, Algra A, Fijnheer R, Rinkel GJ (2006) Endothelial cell activation markers and delayed cerebral ischaemia in patients with subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 77:863–867
11. Frossard M, Fuchs I, Leitner JM, Hsieh K, Vlcek M, Losert H, Domanovits H, Schreiber W, Laggner AN, Jilma B (2004) Platelet function predicts myocardial damage in patients with acute myocardial infarction. Circulation 110:1392–1397
12. Grau AJ, Reiners S, Lichy C, Buggle F, Ruf A (2003) Platelet function under aspirin, clopidogrel, and both after ischemic stroke. Stroke 34:849–854
13. Harrison P, Segal H, Blasbery K, Furtado C, Silver L, Rothwell PM (2005) Screening for aspirin responsiveness after transient ischemic attack and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. Stroke 36:1001–1005
14. Hollestelle MJ, Donkor C, Mantey EA, Chakravorty SJ, Craig A, Akoto AO, O'Donnell J, van Mourik JA, Bunn J (2006) von Willebrand factor propeptide in malaria: evidence of acute endothelial cell activation. Br J Haematol 133:562–569
15. Kain K, Catto AJ, Young J, Bamford J, Bavington J, Grant PJ (2002) Increased fibrinogen, von Willebrand factor and tissue plasminogen activator levels in insulin resistant South Asian patients with ischaemic stroke. Atherosclerosis 163:371–376
16. Kim HH, Liao JK (2008) Translational therapeutics of dipyridamole. Arterioscler Thromb Vasc Biol 28:s39–s42
17. Kinsella JA, Tobin WO, Cox D, Coughlan T, Collins R, O'Neill D, Murphy RP, McCabe DJ (2013) Prevalence of ex vivo high on-treatment platelet reactivity on antiplatelet therapy after transient ischemic attack or ischemic stroke on the PFA-100(®) and VerifyNow(®). J Stroke Cerebrovasc Dis 22(7):e84–e92
18. Kotzailias N, Elwischger K, Sycha T, Rinner W, Quehenberger P, Auff E, Müller C (2007) Clopidogrel-induced platelet inhibition cannot be detected by the platelet function analyzer-100 system in stroke patients. J Stroke Cerebrovasc Dis 16(5):199–202
19. Kozuka K, Kohriyama T, Nomura E, Ikeda J, Kajikawa H, Nakamura S (2002) Endothelial markers and adhesion molecules in acute ischemic stroke–sequential change and differences in stroke subtype. Atherosclerosis 161:161–168
20. Leonardi-Bee J, Bath PM, Bousser MG, Davalos A, Diener HC, Guiraud-Chaumeil B, Sivenius J, Yatsu F, Dewey ME (2005) Dipyridamole for preventing recurrent ischemic stroke and other vascular events: a meta-analysis of individual patient data from randomized controlled trials. Stroke 36:162–168
21. Lynch JR, Blessing R, White WD, Grocott HP, Newman MF, Laskowitz DT (2004) Novel diagnostic test for acute stroke. Stroke 35:57–63
22. McCabe DJ, Harrison P, Mackie IJ, Sidhu PS, Lawrie AS, Purdy G, Machin SJ, Brown MM (2005) Assessment of the antiplatelet effects of low to medium dose aspirin in the early and late phases after ischaemic stroke and TIA. Platelets 16:269–280
23. McCabe DJ, Harrison P, Mackie IJ, Sidhu PS, Purdy G, Lawrie AS, Watt H, Brown MM, Machin SJ (2004) Platelet degranulation and monocyte-platelet complex formation are increased in the acute and convalescent phases after ischaemic stroke or transient ischaemic attack. Br J Haematol 125:777–787
24. McCabe DJ, Harrison P, Sidhu PS, Brown MM, Machin SJ (2004) Circulating reticulated platelets in the early and late phases after ischaemic stroke and transient ischaemic attack. Br J Haematol 126:861–869
25. McCabe DJH, Harrison P, Mackie IJ, Sidhu PS, Purdy G, Lawrie AS, Watt H, Machin SJ, Brown MM (2005) Increased platelet count and leucocyte-platelet complex formation in acute symptomatic compared with asymptomatic severe carotid stenosis. J Neurol Neurosurg Psychiatry 76:1249–1254
26. McGrath RT, McRae E, Smith OP, O'Donnell JS (2010) Platelet von Willebrand factor—structure, function and biological importance. Br J Haematol 148:834–843
27. Nadar SK, Lip GY, Lee KW, Blann AD (2005) Circulating endothelial cells in acute ischaemic stroke. Thromb Haemost 94:707–712
28. Nishio K, Anderson PJ, Zheng XL, Sadler JE (2004) Binding of platelet glycoprotein Ib+|¦ to von Willebrand factor domain A1 stimulates the cleavage of the adjacent domain A2 by ADAMTS13. Proc Natl Acad Sci USA 101:10578–10583
29. R Development Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna
30. Ruggeri ZM (1997) von Willebrand factor. J Clin Invest 100:S41–S46
31. Serebruany V, Sabaeva E, Booze C, Atar OD, Eisert C, Hanley D (2009) Distribution of dipyridamole in blood components among post-stroke patients treated with extended release formulation. Thromb Haemost 102:538–543
32. Sonneveld MA, van Dijk AC, van den Herik EG, van Loon JE, de Lau LM, van der Lugt A, Koudstaal PJ, de Maat MP, Leebeek FW (2013) Relationship of Von Willebrand factor with carotid artery and aortic arch calcification in ischemic stroke patients. Atherosclerosis 230:210–215
33. Tobin WO, Kinsella JA, Coughlan T, Collins DR, O'Neill D, Murphy RP, Egan B, Tierney S, Feeley TM, McCabe DJ (2013) High on-treatment platelet reactivity on commonly prescribed antiplatelet agents following transient ischaemic attack or ischaemic stroke: results from the Trinity Antiplatelet Responsiveness (TRAP) study. Eur J Neurol 20:344–352
34. Tobin WO, Kinsella JA, Kavanagh GF, O'Donnell JS, McGrath RA, Collins DR, Coughlan T, O'Neill D, Egan B, Tierney S, Feeley TM, Murphy RP, McCabe DJ (2013) Longitudinal assessment of thrombin generation potential in response to alteration of antiplatelet therapy after TIA or ischaemic stroke. J Neurol 260:590–596
35. Tobin WO, Kinsella JA, Collins DR, Coughlan T, O'Neill D, Egan B, Tierney S, Feeley TM, Murphy RP, McCabe DJ (2011) Enhanced ex vivo inhibition of platelet function following

addition of dipyridamole to aspirin after transient ischaemic attack or ischaemic stroke: first results from the TRinity Anti-Platelet responsiveness (TrAP) study. Br J Haematol 152: 640–647
36. van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, Pajkrt D, van Genderen PJJ, Fijnheer R (1999) von Willebrand factor propeptide in vascular disorders: a tool to distinguish between acute and chronic endothelial cell perturbation. Blood 94:179–185
37. Zhao L, Gray L, Leonardi-Bee J, Weaver CS, Heptinstall S, Bath PM (2006) Effect of aspirin, clopidogrel and dipyridamole on soluble markers of vascular function in normal volunteers and patients with prior ischaemic stroke. Platelets 17:100–104