Am J Cardiovasc Dis 2015;5(2):101-109
www.AJCD.us /ISSN:2160-200X/AJCD0008248

Original Article

Subtle renal dysfunction and bleeding risk in atrial fibrillation: symmetric dimethylarginine predicts HAS-BLED score

Nathan EK Procter[1], Jocasta Ball[2], Tamila Heresztyn[1], Vivek B Nooney[3], Saifei Liu[1], Cher-Rin Chong[1], Doan TM Ngo[1], Jeffrey S Isenberg[6], Yuliy Y Chirkov[1], Simon Stewart[4,5], John D Horowitz[1]

[1]Basil Hetzel Institute, The Queen Elizabeth Hospital, University of Adelaide, Australia; [2]Centre for The Heart and Mind, Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia; [3]Basil Hetzel Institute, The Queen Elizabeth Hospital, University of South Australia, Australia; [4]National Health and Medical Research Council (NHMRC) Centre of Excellence to Reduce Inequality in Heart Disease, Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia; [5]Baker IDI Heart and Diabetes Institute, Melbourne, Australia; [6]Heart, Lung, Blood and Vascular Medicine Institute, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.

Received March 19, 2015; Accepted July 1, 2015; Epub August 1, 2015; Published August 15, 2015

Abstract: Background: Risk of substantial haemorrhage represents a critically important limitation to effective antithrombotic treatment in patients with atrial fibrillation (AF). While it is known that this risk is increased in anticoagulated patients either in the presence of anti-aggregatory drugs or concomitant renal insufficiency, there are currently few data on the potential interactions between endogenous platelet aggregability and bleeding risk. Objective: We therefore evaluated in a cohort of AF patients: (1), the putative relationship between platelet aggregability and HAS-BLED score; (2), the potential biochemical bases for such a relationship. Methods: Patients were included as part of SAFETY, a randomised controlled trial evaluating outpatient management of AF patients. Platelet response to ADP was evaluated via whole blood impedance aggregometry; clinical and biochemical correlates of platelet aggregation were sought via univariate and multivariate analysis. Results: Platelet aggregation correlated inversely ($r=-0.220$, $p<0.05$) with HAS-BLED score. Univariate biochemical correlates of decreased platelet aggregation were plasma concentrations of symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA). On multivariate analyses, plasma SDMA concentration ($\beta=-0.318$, $p<0.01$), platelet content of thioredoxin-interacting protein (Txnip, $\beta=0.261$, $p<0.05$) and plasma thrombospondin-1 (TSP-1, $\beta=0.249$, $p<0.05$) concentration were predictive of platelet ADP response. Consistent with previous reports, plasma SDMA concentrations were strongly and inversely correlated with estimated glomerular filtration rate (eGFR, $r=-0.780$, $p<0.001$). Conclusions: These data therefore suggest that (1), physiologically impaired, like pharmacologically impaired, platelet aggregability may increase bleeding risk in anticoagulated AF patients; (2), the biochemical basis for this may include impaired effects of nitric oxide (via Txnip, TSP-1) but also concomitant renal dysfunction.

Keywords: Atrial fibrillation, platelet aggregation, thrombospondin-1, thioredoxin-interacting protein, symmetric dimethylarginine

Introduction

Almost all patients with atrial fibrillation (AF) are at increased risk for thromboembolic events, which can be reduced by the use of oral anticoagulation: this has become part of the standard of care for most patients with AF [1]. On the other hand, anticoagulant therapy, whether with warfarin or with new oral anticoagulants (NOACs), engenders some increase in the risk of major bleeding. The clinical factors predictive of bleeding risk on (warfarin) anticoagulation have been delineated [2-5], but little is known of the physiological and biochemical bases for such observations.

One potential basis for bleeding risk is excessive anticoagulation: this is generally avoided

via effect monitoring for warfarin and by dosage adjustment with the various NOACs. However, many bleeds occur despite apparently optimal anticoagulant dosage [5-8]. In these circumstances, individual predisposition to bleeding must be considered. Of the various components of the HAS-BLED score (hypertension, abnormal renal/liver function, stroke, bleeding history, labile INR, elderly, drugs/alcohol), only renal and hepatic dysfunction constitute potential bases for anticoagulant effect to be increased. Indeed, renal insufficiency was identified as a significant determinant of major and minor bleeding events in sub-analyses of the ROCKET-AF [9], ARISTOTLE [10], and RE-LY [11] trials for patients receiving either warfarin or NOACs. Severe hepatic impairment is a contraindication for the use of NOACs [12] in patients with AF or for prevention of venous thromboembolism.

Platelet aggregability also may modulate bleeding risk in such patients. For example, pharmacologically impaired platelet aggregability represents a well-defined basis for incremental bleeding risk in anticoagulated patients: in trials involving treatment of AF, concomitant use of aspirin/clopidogrel with warfarin or NOACs increases bleeding risk [13, 14]. Furthermore, attempts to utilise NOACs in combination with aspirin and other anti-aggregatory agents in treatment of acute coronary syndromes have been limited by bleeding complications [15-17]. On the other hand, few data are available regarding the potential impact of physiological variability in platelet aggregability regarding bleeding risk in anticoagulated patients: this area represents the objective of the current study. It was observed that extent of platelet aggregation correlated inversely with bleeding risk. Univariate followed by multivariate analyses were subsequently performed to identify clinical and biochemical correlates of diminished platelet aggregability.

Materials and methods

Patient selection

The investigation was conducted as a prospective single center mechanistic sub-study of the recently reported Standard vs. Atrial Fibrillation spEcific managemenT studY (SAFETY) [18, 19], an investigation of non-pharmacological management strategies in patients hospitalized with AF. Patients were considered for inclusion if they were admitted to hospital due to chronic AF. Exclusion criteria for SAFETY were age <45 years, primary diagnosis of valvular heart disease, scheduled catheter ablation of AF, pre-existing NYHA class III-IV heart failure with a documented left ventricular ejection fraction (LVEF) <45%, alcohol-induced AF and terminal illness requiring palliative care. Patients receiving $P2Y_{12}$ receptor antagonists were also excluded from the current sub-study because of potential impact of such agents on capacity to measure platelet response to ADP. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula [20]. The study complied with the *Declaration of Helsinki* and was approved by the Ethics of Human Research Committee of The Queen Elizabeth Hospital. Written informed consent was obtained in all cases.

Clinical data

All patients (n=83) underwent standardized clinical assessment and routine biochemical investigation. Additional cardiac investigations were resting electrocardiogram (ECG, which was used for measures of admission heart rate) and transthoracic echocardiography: LVEF was calculated from biplane images using Simpson's method [21]. Thromboembolic risk was assessed using the CHA_2DS_2VASc (congestive heart failure, hypertension, aged ≥75 years, diabetes mellitus, prior stroke/transient ischemic attack, vascular disease, aged 65-74 years, sex category: female) score [22] and bleeding risk was assessed using the HAS-BLED score [23]. During 12 months follow up from study enrolment, no patients experienced ischemic strokes, while nine patients experienced clinically relevant (TIMI major [24]) bleeding events.

Physiological and biochemical investigations

Blood samples were obtained following admission for biochemical/physiological investigations as follows:

Platelet aggregometry: Platelet aggregometry was performed using whole blood impedance aggregometry as previously described [25]. Briefly, venous blood was collected from an antecubital vein into 10 ml tubes containing 1:10 volume of acid citrate anticoagulant (2 parts 0.1 M citric acid to 3 parts of 0.1 M trisodium citrate). Aggregation was induced with ADP

Table 1. Clinical characteristics of patients with chronic atrial fibrillation admitted to hospital

Socio-demographic profile (n=83)	
Gender, n (% male)	43 (51.8)
Age (years) [median, IQR]	73 [67, 81]
Aged ≥75 years, n (%)	38 (45.8)
Comorbidities	
Congestive heart failure, n (%)	6 (7.2)
Hypertension, n (%)	58 (69.9)
Diabetes mellitus, n (%)	22 (26.5)
Prior stroke/TIA, n (%)	13 (15.7)
Clinical presentation	
Admission heart Rate (bpm) [median, IQR]	81 [66, 112]
LVEF (%) [median, IQR]	59 [52, 65]
Plasma creatinine (μM) [median, IQR]	94 [72, 114]
eGFR ($ml \cdot min^{-1} \cdot m^{-2}$)	65.69±2.47
Plasma CRP (mg/l) [median, IQR]	8.9 [3.4, 34.6]
CHA_2DS_2-VASc score [median, IQR]	3 [2, 4]
HAS-BLED score [median, IQR]	2 [2, 3]

Note: TIA = transient ischemic attack; bpm = beats per minute; LVEF = left ventricular ejection fraction; eGFR = estimated glomerular filtration rate.

(2.5 μM), and responses were recorded for electrical impedance (Ω) via a computer interface system (Aggrolink, Chrono-Log, Havertown, Pennsylvania, USA). The *in vitro* effects of SDMA upon ADP-induced platelet aggregation, SDMA (30 μM) was added to blood samples 10 minutes prior to induction of aggregation using ADP (2.5 μM). Responses were recorded for electrical impedance as outlined above.

Plasma asymmetric and symmetric dimethylarginine concentrations: Peripheral blood was collected into sodium heparin Vacutainer™ tubes and placed immediately on ice. Plasma was stored at -70°C until analysis. Plasma ADMA and SDMA levels were determined by high performance liquid chromatography as reported previously [26].

Plasma thrombospondin-1 concentrations: Plasma levels of TSP-1 were determined by enzyme-linked immunosorbent assay (ELISA, Quantikine, R & D Systems, US). Peripheral blood was collected into sodium heparin Vacutainer™ tubes and placed immediately on ice. Platelet poor plasma was stored at -70°C until analysis. Intra-assay CV was 2.8% and inter-assay CV was 5.0%.

Platelet thioredoxin-interacting protein determination: Platelet Txnip content was determined semi-quantitatively using immunohistochemistry as previously described [27]. Briefly, EDTA-anticoagulated blood was centrifuged to obtain platelet rich plasma, which was smeared onto untreated slides and fixed using 4% (w/v) paraformaldehyde in PBS, then stored at -70°C until assayed. Slides were blocked using 20% (v/v) goat serum in PBS, followed by Txnip detection using rabbit polyclonal anti-human VDUP-1 (Invitrogen, USA), 1% (w/v) BSA in PBS and incubating overnight at 2-4°C. Secondary detection was performed using FITC-conjugated swine anti-rabbit polyclonal IgG (Dako, Denmark), as well as primary detection of platelet CD41 using RPE-conjugated mouse monoclonal anti-human CD41 (Dako, Denmark) in PBS. Fluorescence was developed using 'fluorescent mounting medium' (Dako, Denmark) and images acquired at 400× magnification using an Axio Scope. A1 microscope with apotome and AxioVision 4.8 software (Carl Zeiss, Germany). Images were analyzed for densitometric fluorescence using AxioVision LE software. The intra-assay CV was 8.5% and the inter-assay CV was 18.6%.

Statistical methods

Clinical and biochemical factors were evaluated for their potential influence on platelet aggregability. Patient characteristics were compared by non-paired t-test, Mann-Whitney U test or chi-square (χ^2) test as appropriate. Correlates between clinical parameters and aggregation were evaluated by ANOVA. All data for normally distributed parameters are expressed as mean ± standard error of the mean unless otherwise stated. Skewed data are expressed as median and interquartile range, and linearization of such data was performed by logarithmic transformation or square root conversion. Data were analyzed using the IBM SPSS Statistics 20 and GraphPad Prism 6 software packages.

Results

Clinical correlates of platelet aggregability

Clinical characteristics (**Table 1**) and pharmacotherapy (**Table 2**) were typical for an elderly chronic AF cohort. As previously documented [28-30], platelet response to ADP was more

Table 2. Pharmacotherapy of chronic atrial fibrillation patients admitted to hospital

Pharmacological profile (n=83)	
Anti-thrombotic therapy	
Aspirin, n (%)	27 (32.5)
Warfarin, n (%)	49 (59.0)
Rate and/or rhythm control therapy	
Anti-arrhythmics, n (%)	22 (26.5)
β-receptor antagonists, n (%)	48 (57.8)
Digoxin, n (%)	29 (34.9)
Calcium channel blockers, n (%)	24 (28.9)
RAAS inhibitors	
ACE inhibitors, n (%)	26 (31.3)
Angiotensin receptor antagonists, n (%)	23 (27.7)
Other medications	
Statins, n (%)	44 (53.0)
Metformin, n (%)	13 (15.7)
Nitrates, n (%)	11 (13.3)

Note: ACE = angiotensin-converting enzyme.

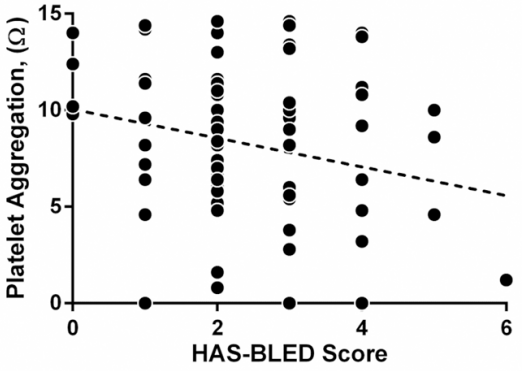

Figure 1. Extent of ADP-induced platelet aggregation correlated significantly with HAS-BLED scores (r=-0.220, p<0.05).

pronounced in female compared to male AF patients (9.9 [6.8, 11.6] Ω vs. 7.2 [5.6, 9.4] Ω, p<0.05). Although there is a theoretical basis for impairment of platelet aggregability by aspirin therapy [31], no significant interaction was observed within this cohort (p=ns, ANOVA). Extent of platelet aggregation correlated inversely with HAS-BLED scores (**Figure 1**), though no significant correlation was observed with CHA_2DS_2VASc scores (data not shown).

Biochemical correlates of platelet aggregability

Biochemical correlates of ADP-induced platelet aggregation are depicted in **Figure 2**. Plasma ADMA and SDMA both correlate with diminished platelet aggregability, whereas eGFR, plasma TSP-1 and platelet Txnip content correlated with increased platelet aggregability. Additionally, plasma ADMA (r=-0.428, p<0.001) and plasma SDMA (r=-0.780, p<0.001) concentrations were both strongly and inversely correlated with eGFR.

Multivariate determinants of platelet aggregability in atrial fibrillation

Clinical and biochemical univariate correlates of ADP-induced platelet aggregation were subjected to backward stepwise multiple logistic regression (**Table 3**). Despite the hyperaggregable response to ADP in females, gender did not represent a multivariate determinant of response. Plasma SDMA concentration, rather than eGFR per se, constituted a strong independent predictor of poor platelet aggregation in response to ADP. Conversely, both plasma TSP-1 concentrations and platelet Txnip content were independently associated with increased platelet response to ADP.

Relationship between determinants of aggregation and HAS-BLED scores

Given that SDMA clearance is largely renal [32], we evaluated the possibility that SDMA predominantly reflected renal function. Indeed, there was a strong direct correlation between plasma SDMA and eGFR (r=-0.780, p<0.001), while the correlation between plasma ADMA and eGFR was somewhat weaker (r=-0.428, p<0.001). Plasma SDMA concentrations, and eGFR, were also significant correlates of HAS-BLED scores (**Figure 3**).

In vitro effects of symmetric dimethylarginine

Additional studies were performed in order to delineate whether the observed relationship between plasma SDMA concentrations and diminished platelet aggregation (**Figure 2B**) might reflect a previously undetected anti-aggregatory effect of SDMA. *In vitro* studies revealed that SDMA in supra-physiological concentrations (≥30 μM) potentiated ADP-induced platelet aggregation in whole blood (**Figure 4**). No evidence of an anti-aggregatory effect was observed.

Discussion

Given that pharmacological inhibition of platelet aggregation engenders increased bleeding

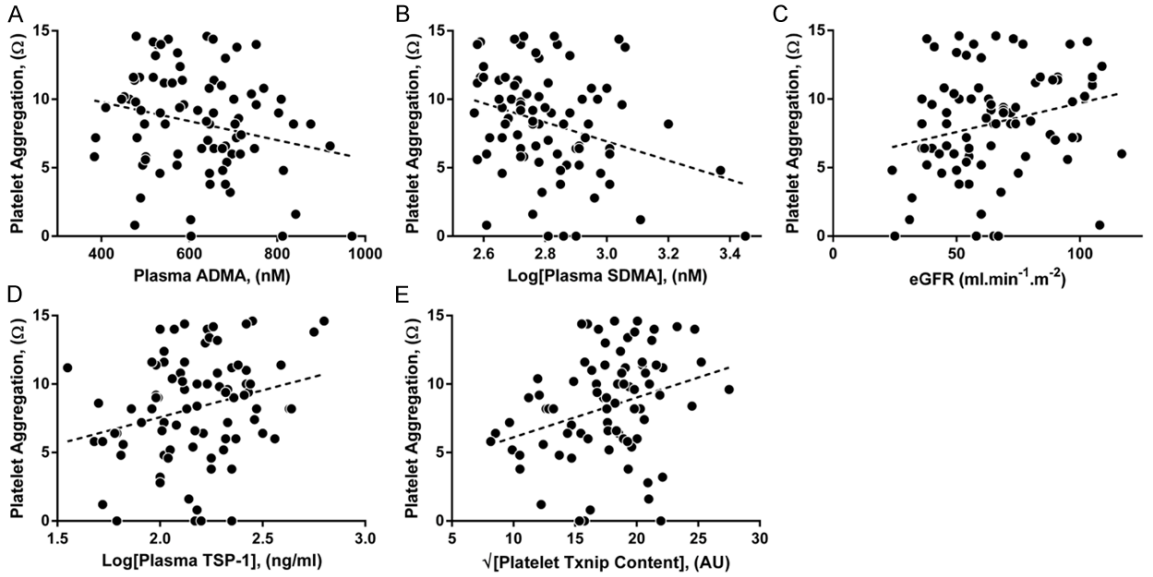

Figure 2. Biochemical correlates of platelet aggregability in chronic atrial fibrillation. (A) Plasma asymmetric dimethylarginine (ADMA, r=-0.226, p<0.05); (B) Plasma symmetric dimethylarginine (SDMA, r=-0.308, p<0.01) concentrations correlated inversely with ADP-induced aggregation; (C) Estimated glomerular filtration rate (eGFR, r=0.250, p<0.05); (D) Plasma thrombospondin-1 (TSP-1) concentrations (r=0.262, p<0.01), and (E) platelet thioredoxin-interacting protein (Txnip) content (r=0.298, p<0.01) were all direct correlates of ADP-induced aggregation.

Table 3. Multivariate correlates of platelet aggregability in chronic atrial fibrillation patients

Multivariate correlates of platelet aggregability		
Factor	β	p
Plasma SDMA concentrations	-0.318	<0.01
Platelet Txnip content	0.261	<0.05
Plasma TSP-1 concentrations	0.249	<0.05

Note: SDMA = symmetric dimethylarginine; Txnip = thioredoxin-interacting protein; TSP-1 = thrombospondin-1.

risk among anticoagulated AF patients [13, 14], we have currently tested the hypothesis that physiological variability in platelet aggregability also might exert a similar influence. To this end, we tested the hypothesis that decreased platelet aggregability corresponded to increased HAS-BLED score. In a cohort of AF patients from SAFETY [18, 19], a significant relationship was indeed found, although the size of the study precluded the comparison of actual bleeding rates.

We sought to identify biochemical bases for impaired platelet aggregability in this cohort. It emerged that elevated SDMA levels, acting presumably as a surrogate for (mildly) impaired renal function rather than on the basis of intrinsic interaction with platelet function, strongly and independently predicted impaired platelet aggregability. Furthermore, both plasma TSP-1 concentration and platelet Txnip levels corresponded with increased platelet aggregability. Therefore the current findings shed new light on the potential bases for bleeding risk in AF patients.

It is well-known that among patients anticoagulated with warfarin, bleeding risk increases sharply as renal function decreases, and that there is a less marked increase with Apixaban [10]. The majority of the relevant events occurred in patients with moderate renal insufficiency, whereas renal function was generally well-preserved in the current cohort. Nevertheless, SDMA levels correlated closely and inversely with eGFR, and SDMA appeared devoid of intrinsic anti-aggregatory effect. Consistent with the current findings, it was recently shown that SDMA levels represented a strong independent predictor of bleeding events in warfarin or Apixaban-treated patients [33]. However, it must be noted that SDMA exerts some pro-oxidant effects [34, 35], and it therefore is possible that its direct actions on vasculature, rather than platelets, may engender bleeding risk.

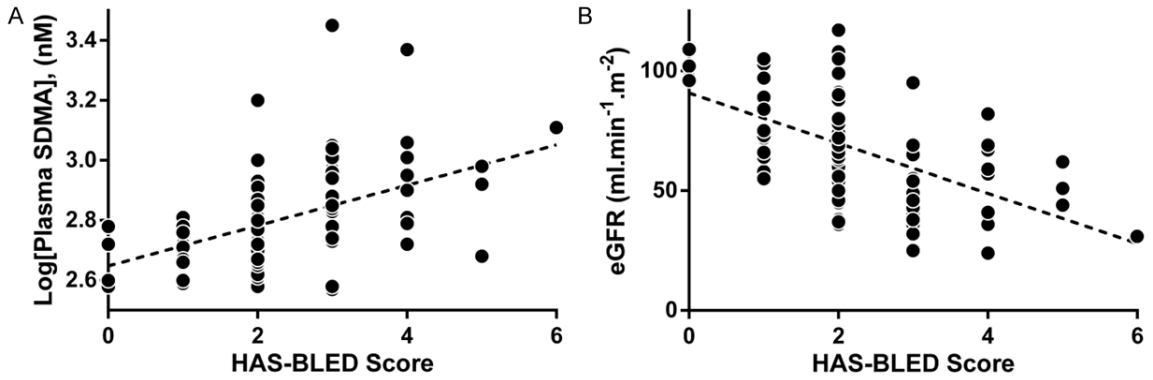

Figure 3. HAS-BLED scores correlated directly with (A), plasma symmetric dimethylarginine (SDMA) concentrations (r=0.478, p<0.001) and inversely with (B), glomerular filtration rate (eGFR, r=-0.573, p<0.001).

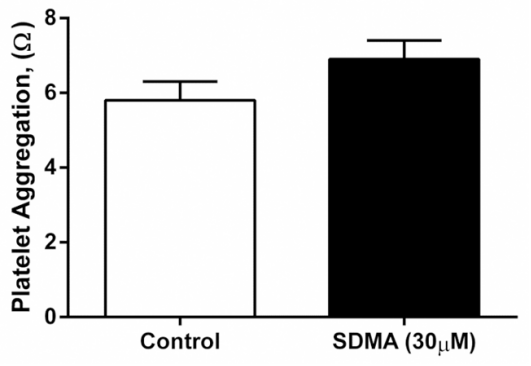

Figure 4. Symmetric dimethylarginine (SDMA) increased platelet aggregatory response to ADP when compared to controls (6.9±0.5 Ω vs 5.8±0.5 Ω, n=7, p<0.01 paired t-test).

TSP-1 has not previously been implicated as a modulator of bleeding risk. However, TSP-1 release from platelet α-granules occurs during platelet activation [36] and TSP-1 suppresses NO signalling by inhibiting its activation of soluble guanylate cyclase: this would be expected to impact on platelet aggregability [37, 38]. Thus, the direct relationship is not surprising.

Similarly, Txnip is a pro-inflammatory molecule [39], the expression of which is suppressed by NO [27, 40]: hence increased Txnip should theoretically lead to hyperaggregability. However, this relationship has not previously been documented.

It must of course be acknowledged that in a study of this size there is no likelihood of being able to evaluate actual bleeding rates. However, the current results are consistent with the (admittedly limited) available clinical data.

The main potential clinical implications of our findings are that, (1) even mild renal dysfunction is likely to constitute a basis for increased bleeding risk in AF patients; (2) pharmacological suppression of Txnip expression [39], for example with calcium channel antagonists [41], or angiotensin-converting enzyme (ACE) inhibitors [42], in AF patients may reduce thromboembolic risk, but increase that of bleeding during anticoagulation.

Acknowledgements

This investigation was supported by NHMRC Program Grant 519823 and the NHMRC Centre of Research Excellence to Reduce Inequality in Heart Disease. Simon Stewart, Jocasta Ball and Cher-Rin Chong are supported by the NHMRC. Jeffrey Isenberg is supported by the NIH grants P01 HL103455-01, 1R01HL108-954-01, 1R01HL112914-01A1 and 2R01HL-089658, and also by the Vascular Medicine Institute, the Institute for Transfusion Medicine and the Hemophilia Center of Western Pennsylvania.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. John D Horowitz, Basil Hetzel Institute, The Queen Elizabeth Hospital, University of Adelaide, Cardiology Unit, 28 Woodville Rd, Woodville, SA 5011, Australia. E-mail: john.horowitz@adelaide.edu.au

References

[1] Friberg L, Rosenqvist M and Lip GY. Net clinical benefit of warfarin in patients with atrial fibril-

lation: a report from the Swedish atrial fibrillation cohort study. Circulation 2012; 125: 2298-2307.
[2] Pisters R, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ and Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. Chest 2010; 138: 1093-1100.
[3] Gage BF, Yan Y, Milligan PE, Waterman AD, Culverhouse R, Rich MW and Radford MJ. Clinical classification schemes for predicting hemorrhage: results from the National Registry of Atrial Fibrillation (NRAF). Am Heart J 2006; 151: 713-719.
[4] Fang MC, Chang YC, Hylek EM, Rosand J, Greenberg SM, Go AS and Singer DE. Advanced age, anticoagulation intensity, and risk for intracranial hemorrhage among patients taking warfarin for atrial fibrillation. Ann Intern Med 2004; 141: 745-752.
[5] DiMarco JP, Flaker G, Waldo AL, Corley SD, Greene HL, Safford RE, Rosenfeld LE, Mitrani G, Nemeth M; AFFIRM Investigators. Factors affecting bleeding risk during anticoagulant therapy in patients with atrial fibrillation: observations from the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) study. Am Heart J 2005; 149: 650-656.
[6] Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med 2009; 361: 1139-1151.
[7] Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD, Ezekowitz JA, Flaker G, Garcia D, Geraldes M, Gersh BJ, Golitsyn S, Goto S, Hermosillo AG, Hohnloser SH, Horowitz J, Mohan P, Jansky P, Lewis BS, Lopez-Sendon JL, Pais P, Parkhomenko A, Verheugt FW, Zhu J, Wallentin L; ARISTOTLE Committees and Investigators. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2011; 365: 981-992.
[8] Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM; ROCKET AF Investigators. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011; 365: 883-891.
[9] Fox KA, Piccini JP, Wojdyla D, Becker RC, Halperin JL, Nessel CC, Paolini JF, Hankey GJ, Mahaffey KW, Patel MR, Singer DE and Califf RM. Prevention of stroke and systemic embolism with rivaroxaban compared with warfarin in patients with non-valvular atrial fibrillation and moderate renal impairment. Eur Heart J 2011; 32: 2387-2394.
[10] Hohnloser SH, Hijazi Z, Thomas L, Alexander JH, Amerena J, Hanna M, Keltai M, Lanas F, Lopes RD, Lopez-Sendon J, Granger CB and Wallentin L. Efficacy of apixaban when compared with warfarin in relation to renal function in patients with atrial fibrillation: insights from the ARISTOTLE trial. Eur Heart J 2012; 33: 2821-2830.
[11] Hijazi Z, Hohnloser SH, Oldgren J, Andersson U, Connolly SJ, Eikelboom JW, Ezekowitz MD, Reilly PA, Siegbahn A, Yusuf S and Wallentin L. Efficacy and safety of dabigatran compared with warfarin in relation to baseline renal function in patients with atrial fibrillation: a RE-LY (Randomized Evaluation of Long-term Anticoagulation Therapy) trial analysis. Circulation 2014; 129: 961-970.
[12] Graff J and Harder S. Anticoagulant therapy with the oral direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban and the thrombin inhibitor dabigatran etexilate in patients with hepatic impairment. Clin Pharmacokinet 2013; 52: 243-254.
[13] Dewilde WJ, Oirbans T, Verheugt FW, Kelder JC, De Smet BJ, Herrman JP, Adriaenssens T, Vrolix M, Heestermans AA, Vis MM, Tijsen JG, van't Hof AW, ten Berg JM; WOEST study investigators. Use of clopidogrel with or without aspirin in patients taking oral anticoagulant therapy and undergoing percutaneous coronary intervention: an open-label, randomised, controlled trial. Lancet 2013; 381: 1107-1115.
[14] Hansen ML, Sorensen R, Clausen MT, Fog-Petersen ML, Raunso J, Gadsboll N, Gislason GH, Folke F, Andersen SS, Schramm TK, Abildstrom SZ, Poulsen HE, Kober L and Torp-Pedersen C. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. Arch Intern Med 2010; 170: 1433-1441.
[15] Cuisset T, Grosdidier C, Loundou AD, Quilici J, Loosveld M, Camoin L, Pankert M, Beguin S, Lambert M, Morange PE, Bonnet JL and Alessi MC. Clinical implications of very low on-treatment platelet reactivity in patients treated with thienopyridine: the POBA study (predictor of bleedings with antiplatelet drugs). JACC Cardiovasc Interv 2013; 6: 854-863.
[16] Patti G, Pasceri V, Vizzi V, Ricottini E and Di Sciascio G. Usefulness of platelet response to clopidogrel by point-of-care testing to predict bleeding outcomes in patients undergoing percutaneous coronary intervention (from the Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty-Bleeding Study). Am J Cardiol 2011; 107: 995-1000.

[17] Alexander JH, Lopes RD, James S, Kilaru R, He Y, Mohan P, Bhatt DL, Goodman S, Verheugt FW, Flather M, Huber K, Liaw D, Husted SE, Lopez-Sendon J, De Caterina R, Jansky P, Darius H, Vinereanu D, Cornel JH, Cools F, Atar D, Leiva-Pons JL, Keltai M, Ogawa H, Pais P, Parkhomenko A, Ruzyllo W, Diaz R, White H, Ruda M, Geraldes M, Lawrence J, Harrington RA, Wallentin L; APPRAISE-2 Investigators. Apixaban with antiplatelet therapy after acute coronary syndrome. N Engl J Med 2011; 365: 699-708.

[18] Carrington MJ, Ball J, Horowitz JD, Marwick TH, Mahadevan G, Wong C, Abhayaratna WP, Haluska B, Thompson DR, Scuffham PA and Stewart S. Navigating the fine line between benefit and risk in chronic atrial fibrillation: rationale and design of the Standard versus Atrial Fibrillation spEcific managemenT studY (SAFETY). Int J Cardiol 2013; 166: 359-365.

[19] Stewart S, Ball J, Horowitz JD, Marwick TH, Mahadevan G, Wong C, Abhayaratna WP, Chan YK, Esterman A, Thompson DR, Scuffham PA and Carrington MJ. Standard versus atrial fibrillation-specific management strategy (SAFETY) to reduce recurrent admission and prolong survival: pragmatic, multicentre, randomised controlled trial. Lancet 2015; 385: 775-784.

[20] Mathew TH; Australasian Creatinine Consensus Working Group. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: a position statement. Med J Aust 2005; 183: 138-141.

[21] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463.

[22] Lip GY, Nieuwlaat R, Pisters R, Lane DA and Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. Chest 2010; 137: 263-272.

[23] Lip GY, Frison L, Halperin JL and Lane DA. Comparative validation of a novel risk score for predicting bleeding risk in anticoagulated patients with atrial fibrillation: the HAS-BLED (Hypertension, Abnormal Renal/Liver Function, Stroke, Bleeding History or Predisposition, Labile INR, Elderly, Drugs/Alcohol Concomitantly) score. J Am Coll Cardiol 2011; 57: 173-180.

[24] Mehran R, Rao SV, Bhatt DL, Gibson CM, Caixeta A, Eikelboom J, Kaul S, Wiviott SD, Menon V, Nikolsky E, Serebruany V, Valgimigli M, Vranckx P, Taggart D, Sabik JF, Cutlip DE, Krucoff MW, Ohman EM, Steg PG and White H. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium. Circulation 2011; 123: 2736-2747.

[25] Willoughby SR, Rajendran S, Chan WP, Procter N, Leslie S, Liberts EA, Heresztyn T, Chirkov YY and Horowitz JD. Ramipril sensitizes platelets to nitric oxide: implications for therapy in high-risk patients. J Am Coll Cardiol 2012; 60: 887-894.

[26] Heresztyn T, Worthley MI and Horowitz JD. Determination of l-arginine and NG, NG- and NG, NG'-dimethyl-L-arginine in plasma by liquid chromatography as AccQ-Fluor fluorescent derivatives. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 805: 325-329.

[27] Sverdlov AL, Chan WP, Procter NEK, Chirkov YY, Ngo DTM and Horowitz JD. Reciprocal regulation of NO signaling and TXNIP expression in humans: impact of aging and ramipril therapy. Int J Cardiol 2013; 168: 4624-30.

[28] Kamath S, Blann AD, Chin BS, Lanza F, Aleil B, Cazenave JP and Lip GY. A study of platelet activation in atrial fibrillation and the effects of antithrombotic therapy. Eur Heart J 2002; 23: 1788-1795.

[29] Becker DM, Segal J, Vaidya D, Yanek LR, Herrera-Galeano JE, Bray PF, Moy TF, Becker LC and Faraday N. Sex differences in platelet reactivity and response to low-dose aspirin therapy. JAMA 2006; 295: 1420-1427.

[30] Otahbachi M, Simoni J, Simoni G, Moeller JF, Cevik C, Meyerrose GE and Roongsritong C. Gender differences in platelet aggregation in healthy individuals. J Thromb Thrombolysis 2010; 30: 184-191.

[31] Weiss HJ, Aledort LM and Kochwa S. The effect of salicylates on the hemostatic properties of platelets in man. J Clin Invest 1968; 47: 2169-2180.

[32] Bode-Boger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M and Reinecke H. Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. J Am Soc Nephrol 2006; 17: 1128-1134.

[33] Horowitz JD, De Caterina R, Heresztyn T, Andersson U, Lopes R, Hylek E, Mohan P, Hanna M, Granger CB, Wallentin L; AFFIRM Investigators. ADMA and SDMA predict outcomes in patients

with chronic atrial fibrillation: an ARISTOTLE substudy. Eur Heart J 2013; 34: 1040-1041.
[34] Schepers E, Glorieux G, Dhondt A, Leybaert L and Vanholder R. Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. Nephrol Dial Transplant 2009; 24: 1429-1435.
[35] Schepers E, Barreto DV, Liabeuf S, Glorieux G, Eloot S, Barreto FC, Massy Z, Vanholder R; European Uremic Toxin Work Group (EUTox). Symmetric dimethylarginine as a proinflammatory agent in chronic kidney disease. Clin J Am Soc Nephrol 2011; 6: 2374-2383.
[36] Esemuede N, Lee T, Pierre-Paul D, Sumpio BE and Gahtan V. The role of thrombospondin-1 in human disease. J Surg Res 2004; 122: 135-142.
[37] Isenberg JS, Martin-Manso G, Maxhimer JB and Roberts DD. Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. Nat Rev Cancer 2009; 9: 182-194.
[38] Isenberg JS, Frazier WA and Roberts DD. Thrombospondin-1: a physiological regulator of nitric oxide signaling. Cell Mol Life Sci 2008; 65: 728-742.
[39] Chong CR, Chan WP, Nguyen TH, Liu S, Procter NE, Ngo DT, Sverdlov AL, Chirkov YY and Horowitz JD. Thioredoxin-interacting protein: pathophysiology and emerging pharmacotherapeutics in cardiovascular disease and diabetes. Cardiovasc Drugs Ther 2014; 28: 347-360.
[40] Schulze PC, Liu H, Choe E, Yoshioka J, Shalev A, Bloch KD and Lee RT. Nitric oxide-dependent suppression of thioredoxin-interacting protein expression enhances thioredoxin activity. Arterioscler Thromb Vasc Biol 2006; 26: 2666-2672.
[41] Chen J, Cha-Molstad H, Szabo A and Shalev A. Diabetes induces and calcium channel blockers prevent cardiac expression of proapoptotic thioredoxin-interacting protein. Am J Physiol Endocrinol Metab 2009; 296: E1133-1139.
[42] Sverdlov AL, Chan WP, Procter NE, Chirkov YY, Ngo DT and Horowitz JD. Reciprocal regulation of NO signaling and TXNIP expression in humans: impact of aging and ramipril therapy. Int J Cardiol 2013; 168: 4624-4630.