Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: http://www.tandfonline.com/loi/iplt20

Clinical utility of remote platelet function measurement using P-selectin: assessment of aspirin, clopidogrel, and prasugrel and bleeding disorders

Philip M Bath, Jane May & Stan Heptinstall

To cite this article: Philip M Bath, Jane May & Stan Heptinstall (2018): Clinical utility of remote platelet function measurement using P-selectin: assessment of aspirin, clopidogrel, and prasugrel and bleeding disorders, Platelets, DOI: 10.1080/09537104.2018.1445839

To link to this article: https://doi.org/10.1080/09537104.2018.1445839

Published online: 18 Apr 2018.

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

platelets

Platelets, Early Online: 1–6
 DOI: https://doi.org/10.1080/09537104.2018.1445839

Taylor & Francis
Taylor & Francis Group

Check for updates

Clinical utility of remote platelet function measurement using P-selectin: assessment of aspirin, clopidogrel, and prasugrel and bleeding disorders

Philip M Bath, Jane May, & Stan Heptinstall

Stroke Trials Unit, Division of Clinical Neuroscience, University of Nottingham, Nottingham, UK

Abstract

Vascular diseases such as myocardial infarction and ischemic stroke are associated with increased platelet function whilst the risk of recurrence is reduced by antiplatelet agents such as aspirin, clopidogrel, and prasugrel. However, some patients exhibit high platelet reactivity, especially with clopidogrel. Existing platelet function tests may not be ideal in that they can be expensive, are often time consuming, and measurements must be made near to the patient and within a few hours of blood collection. Platelet activation leads to translocation of P-selectin from alpha-granules to the cell surface. Following activation with arachidonic acid (which is blocked by aspirin) or adenosine diphosphate (inhibited by clopidogrel) and fixation, samples may be stored or posted to a laboratory performing flow cytometric quantification of platelet P-selectin expression. Acute myocardial infarction and ischemic stroke are associated with high platelet reactivity on clopidogrel in 6–58% of patients when assessed with P-selectin expression, and high reactivity was associated with an increased risk of recurrence after myocardial infarction. Use of P-selectin expression tests may also be of relevance to surgical and veterinary practice and the diagnosis of mild bleeding disorders. The present review explores this topic in further detail.

Keywords

Acute coronary syndrome, bleeding, function, platelet, P-selectin, stroke

History

Received 3 August 2017
Revised 27 January 2018
Accepted 5 February 2018
Published online 16 April 2018

Platelets are small circulating anucleate cells that comprise the main component of particulate hemostasis (1). Their key role in stopping bleeding means that low numbers or platelet dysfunction increases the risk of bleeding. Conversely, accentuated platelet function, as occurs in the presence of vascular risk factors such as hypertension and hyperlipidemia, contributes to acute coronary syndromes and stent thrombosis. As a result, antiplatelet agents such as aspirin and clopidogrel are used widely to reduce the risk of recurrent vascular events (2–4).

In view of the relationship between reduced or accentuated platelet function and subsequent disease and clinical events, multiple approaches have been developed to assess platelet function. Extremely low number of platelets (thrombocytopenia, as occurs in terminal leukemia) or high numbers (thrombocythemia, a myeloproliferative disorder) are easily detected using automated hematology cell counters. Physiologically, platelet size and count are inversely related, and size can be measured using the same cell counters. Small low-function platelets are seen in terminal marrow failure, and large platelets are present and prognostic after acute myocardial infarction and ischemic stroke (5,6). However, these assessments of physical platelet parameters are indirect measures of function, and direct assessments have been developed. Historically, measurement of aggregation in platelet-rich plasma predominated in research studies. This was not ideal in that the approach is time consuming, needs significant technical support, and measurements must be made near to the patient and within a few hours of blood collection.

Over the last 15 years, semi-automated "bedside" commercial tests have been developed that reduce time and operator dependence. Notable devices include Multiplate, VerifyNow, and Biocytex VASP (7–9) and these assays are promoted in an international consensus for platelet function testing in patients on dual antiplatelet therapy (10). Nevertheless, the cost of these devices and their cartridges is relatively high, concordance between their results is not high (7,11), and measurements must be made near to the patient and within a few hours of venepuncture. Hence, the addition of a diagnostic requiring no capital investment that involves local blood sampling but with the flexibility provided by remote measurement is potentially a welcome addition to the armamentarium of measuring platelet function.

P-selectin

P-selectin (CD62P) is a protein that functions as a cell adhesion molecule on the surface of activated platelets and endothelial cells. In both cell types, P-selectin is stored in alpha-granules and is translocated to the cell surface following cell activation. In platelets, activation is driven by stimulation by vascular agonists such as thrombin, collagen, adenosine diphosphate (ADP), thromboxane A_2, and its mimetics and inflammatory agonists including interleukin-4. The relationship between circulating soluble P-selectin and vascular diagnoses and outcomes is well described, for example, in stroke (12), and reflects both platelet

Correspondence: Professor Philip Bath, Stroke Trials Unit, Clinical Sciences Building, City Hospital campus, Hucknall Road, Nottingham NG5 1PB UK. Tel: +44 115 823 1765. Fax: +44 115 823 1767. E-mail: philip.bath@nottingham.ac.uk.uk

and endothelial function. In contrast, measurement of P-selectin on the platelet surface assesses the extent of platelet activation and expression changes in response to the same agonist(s) that cause platelets to aggregate together. One role for P-selectin on activated platelets is that it binds to a specific receptor, P-selectin glycoprotein ligand-1, which is expressed on most leucocytes (neutrophils, monocytes, lymphocytes, eosinophils), thus facilitating platelet–leucocyte conjugation.

Remote assessment of platelet function with P-selectin

Measurement of the surface expression on platelets of P-selectin provides an alternative approach to assessing platelet function and standardized tests are now available for a number of different purposes (Platelet Solutions Ltd., PSL). Blood is taken by conventional venepuncture, placed in warmed reaction tubes (13), stimulated with selected agonist(s) (which leads to expression of P-selectin onto the surface of platelets), fixed with PAMFix (14), posted to a core laboratory, and surface P-selectin measured using flow cytometry (15). One agonist is arachidonic acid (AA), the effects of which are blocked by aspirin (aspirin test). The clopidogrel test detects whether $P2Y_{12}$ antagonists such as clopidogrel, prasugrel, and ticagrelor prevent ADP stimulation of platelets. By comparing data obtained using a number of different agonists, information on the presence of platelet dysfunction or inhibition in an individual can be obtained. Fixation prevents both platelet aggregation and platelet–leucocyte conjugate formation so that all activated single platelets are available for analysis.

In a comparison with light transmission aggregometry, Multiplate, VerifyNow, and Biocytex VASP, the P-selectin-based aspirin and clopidogrel tests were at least as effective in determining the inhibitory effects of antiplatelet therapies including combined aspirin and clopidogrel, and combined aspirin and prasugrel (16). But what does differentiate assessment of platelet expression of P-selectin from these other tests is that samples may be sent by post from where the patient is (hospital, general practice, home) to a core laboratory for measurement, results can then be returned the next day. The test is stable for at least 28 days (9) (and possibly 35 days (17)) allowing between-country as well within-country measurements. This approach is similar to the measurement of other vascular risk factors such as blood cholesterol.

Measurement of platelet expression of P-selectin may be combined with other platelet assessments. For example, P-selectin and CD63 (a marker of dense body secretion) may be assessed on activated platelets in patients with enhanced bruising to diagnose mild bleeding disorders (18).

Resistance

Although antiplatelet agents are highly effective at reducing the risk of recurrent vascular events, a significant minority of patients (≥35%) do not respond adequately to clopidogrel (19–28), a similar but lesser problem also exists for aspirin (8,19,21,23,29). High residual platelet reactivity whilst on treatment (HRPR, amounting to resistance or nonresponse) can follow failure to take or absorb the tablets, or to convert the drug into its active metabolite, or for the metabolite to inhibit platelet function, i.e., a mix of patient, drug, and genetic-related factors. It is widely assumed that nonresponse means that antiplatelet therapy will have only a limited effect on reducing vascular events (antiplatelet failure); hence, patients with HRPR are in essence taking an ineffective medication and so remain at increased risk of recurrent events. Evidence for this comes from a substudy of the clopidogrel in high-risk patients with Acute Non-disabling Cerebrovascular Events trial that showed that the combination of aspirin and clopidogrel was superior to aspirin alone in preventing recurrence after minor ischemic stroke or TIA. However, the presence of the CYP2C19 loss-of-function allele was associated with a failure for clopidogrel to add to aspirin in preventing recurrence (30). Around 14% of patients are CYP2C19-poor metabolizers. A key reason for testing platelet function is to identify patients who exhibit HRPR.

Proton pump inhibitors

Drug interactions are common with antiplatelet agents. Clopidogrel is a prodrug that requires activation by cytochrome P450 (especially CYP2C19) to form an active metabolite. Initially, there was concern that gastroprotection with proton pump inhibitors, which can inhibit CYP2C19, should be avoided. This advice was attenuated to suggesting that omeprazole and esomeprazole should be avoided. A recent meta-analysis found that the PPI–clopidogrel interaction seen *in vitro* has no clinical significance (31). Rather, patients taking PPIs are likely to have more comorbidities and therefore a higher vascular risk. As a result, there is no clinical role for monitoring platelet function when patients are taking clopidogrel and a PPI.

Clinical studies

Volunteers

Several studies have been reported in normal volunteers as part of the development of the aspirin and clopidogrel P-selectin tests (15,32). These show the utility of the aspirin test for assessing aspirin with suppression of AA-stimulated P-selectin expression. Similar findings have been made for the clopidogrel test for ADP-stimulated P-selectin.

Myocardial infarction

Platelets are activated in acute myocardial infarction and chronically afterward and in procedures such as angioplasty and stent insertion. Antiplatelet agents such as aspirin, clopidogrel, and prasugrel are effective in reducing the risk of recurrent events (3,4). In a study of 100 patients with acute coronary syndrome and who were on combined aspirin and clopidogrel, the clopidogrel test found that a majority of patients had a high on-treatment platelet reactivity (Table I) (33). In patients who went on to develop a further cardiovascular event (cardiovascular death, nonfatal myocardial infarction, or stent thrombosis) during the following 12 months, the P-selectin levels were higher than in patients who had no further event. All patients had a suppressed aspirin test and there was no difference in aspirin-related P-selectin expression between patients who developed, or did not develop, a subsequent cardiovascular event (Table I) (33).

In a further study of patients with recent ACS (34), ADP-stimulated P-selectin expression (clopidogrel test) was measured 1 month after starting clopidogrel or prasugrel. HRPR was higher in those taking clopidogrel than those on prasugrel (Table I). However, P-selectin levels were higher in prasugrel users if they had hypertension than if they did not.

A comparison of clopidogrel and prasugrel was made in another study of patients with ACS (9). Prasugrel treatment resulted in significantly greater inhibition than clopidogrel. Three patients with very high P-selectin values were subsequently found to be not taking prasugrel. Additionally, three patients had high AA-stimulated high P-selectin expression and were either nonresponders to aspirin or more likely not taking this drug (9).

Table I. Characteristics of patients and platelet P-selectin expression in response to antiplatelet agents.

Subjects	Study	*N*	Age (years)	Female (%)	Antiplatelet	Clop test	Nonresponse (%)	Asp test	Nonresponse (%)
Normal	Fox (15)	62			None	816 (247)	>500 (90)	950 (295)	
		14			Clop	301 (94)	–		
		12			Asp + Clop	306 (81)			
	Fox (32)	14			None	866 (197)			
		14			Clop	293 (103)			
		10			Asp + Clop	326 (123)			
ACS	Fox (15)	30–42			Asp + Clop	638 (246)		86 (63)	>300 (3)
	Thomas (33)	100	67 (–)	37	Asp + Clop		>860 (58)		>500 (0)
		88			Asp + Clop [1]	777 (281)		95 (56)	
		12			Asp + Clop [2]	953 (246)		100 (35)	
	Laohathai (34)	47			Clop [3]	606 [402]			
		23			Clop [4]	543 [396]			
		43			Pras [3]	356 [161]			
		28			Pras [4]	463 [234]			
	May (9)	102			Asp + Clop	621 (241)		161 (153)	3
		56			Asp + Pras	397 (149)		91 (34)	
IS/TIA	TARDIS (40)	108	66 (10)	30	None	983 (316)	>860 (66)	509 (448)	>500 (35)
		418	69 (10)	36	Asp	944 (298)	>860 (59)	198 (172)	>500 (3)
		42	70 (11)	34	Asp + Dip	1139 (388)	>860 (78)	296 (269)	>500 (12)
		54	69 (11)	35	Clop	578 (276)	>860 (17)	578 (276)	>500 (70)
		33	71 (10)	38	Clop + Asp	758 (296)	>860 (36)	256 (227)	>500 (12)
	Pre-ACRIST (41)	46	64 (14)	47	Clop	534 (273)	>860 (14)	640 (332)	>500 (59)
		16			Asp + Clop	377 (232)	>860 (6)	446 (308)	>500 (38)
Surgery	Keeler pre-op (43)	87	67 [20]	44	None			1303 [397]	
		20	74 [11]	30	Asp			77 [50]	
	Keeler per-op (43)	87			None			1224 [552]	
		20			Asp			282 [836]	
	Endovascular coiling	74	52	77	Asp + Clop	530 (208)	>860 (8)	110 (116)	>500 (1)
Bleeding	Dovlatova (18)	41			None		<900 (5)		<1007 (5)
		9		79	Granule defect		<900 (56)		<1007 (100)
		3		"	TXA_2 defect		<900 (67)		<1007 (67)
		14		"	GI defect		<900 (50)		<1007 (57)
Animals, dogs	Dunning (44)	39			None	954 (634)		1063 (644)	
		1			Asp	2992 (–)		2216 (–)	
		3			Clop	278 (30)		964 (600)	
		2			Asp + Clop	173 (7)		395 (127)	

ACS: Acute coronary syndrome; Asp: aspirin; Clop: clopidogrel; GI: GI signaling defect; Granule: dense granule defect; IS: ischemic stroke; Pras: prasugrel; TIA: transient ischemic attack; TXA_2: thromboxane A_2 defect.
[1] No subsequent vascular event.
[2] Subsequent vascular event.
[3] Without hypertension.
[4] With hypertension.
Nonresponse columns show cut-point for nonresponse and percentage of non-responding subjects; the cut-point detects patients with high platelet reactivity (if on an antiplatelet) or low platelet reactivity (if a bleeding disorder). Data are mean (standard deviation) or median [interquartile range].

Stroke

Platelets are activated in acute stroke and chronically afterward, and antiplatelet agents such as aspirin, clopidogrel, and dipyridamole are effective in reducing the risk of recurrence (4,35,36). The effect of aspirin and clopidogrel on platelet expression of P-selectin was assessed in the large Triple Antiplatelets for Reducing Dependency after Ischaemic Stroke (TARDIS) trial; TARDIS compared the effect of intensive (combined aspirin, clopidogrel, and dipyridamole) versus guideline (combined aspirin and dipyridamole, or clopidogrel alone) in patients with acute stroke or transient ischemic attack (37,38). At baseline, the aspirin test identified patients who declared that they had been taking this antiplatelet before randomization; HRPR was present with aspirin in only 4.7% of patients (Table I) (39,40). Whilst the clopidogrel test similarly identified patients saying that they had been taking this drug, 24.7% of them did not have suppressed platelet expression of P-selectin (39,40). For so-far unexplained reasons, the presence of a second antiplatelet was associated with higher P-selectin expression than if only one antiplatelet, e.g., AA-stimulated P-selectin (aspirin test) was higher in patients taking combined aspirin and clopidogrel than aspirin alone (Table I). The effects of randomized treatment on P-selectin expression and relationship between P-selectin levels and subsequent stroke recurrence and bleeding are to be analyzed.

In the Pre-Accrist study, 62 patients with recent stroke and who were taking clopidogrel were studied; minority were also

taking aspirin. Twenty-three percent of patients did not have suppressed levels for the clopidogrel test (41).

At one neuroscience center, the clopidogrel test is being used off-label to identify nonresponse to clopidogrel in patients having coiling of cerebral aneurysms (42), such patients are changed to prasugrel prior to the procedure if they appear to have HRPR on this $P2Y_{12}$ antagonist (Table I). The use of the test is exemplified in one patient who had unsuppressed P-selectin expression on the aspirin test, the test suppressed when the patient became compliant of medication.

Surgery

Many older patients take aspirin regularly for vascular (or cancer) prophylaxis. The question then arises as to whether this should be stopped temporarily before major surgery to prevent the perceived risk of increased operative blood loss. In an observational study, the effect of stopping aspirin preoperatively in 20 patients having colorectal surgery was assessed and compared with a group who were not on aspirin (43). P-selectin expression was lower on aspirin (Table I) and within 5 days of stopping aspirin prior to surgery. The aspirin test showed that P-selectin expression rose with the length of time between stopping aspirin and surgery. Although the proportion of patients with a complication, including those with hemorrhage, was higher in the aspirin group, P-selectin expression was not associated with complications or bleeding (43).

Veterinary medicine

One study assessed whether P-selectin expression could be used to measure platelet function in dogs (44). The relevance is that pets are increasingly given antiplatelet drugs to prevent vascular disease. Using antibodies against canine P-selectin, it was possible to measure platelet function and the inhibitory effects of antiplatelet agents (Table I). A study is ongoing in cats.

Bleeding disorders

The above clinical disorders all share the use of P-selectin to assess the inhibitory effects of antiplatelet agents. However, P-selectin expression may also be used to diagnose mild bleeding disorders (45) such as defects in dense granule secretion, thromboxane A_2 pathway, GI signaling, response to collagen, or response to thrombin receptor activating peptide (18). The results were comparable to light transmission aggregometry/lumiaggregometry (Table I), a time- and labor-intensive technique that has to be performed shortly after venepuncture.

Clinical P-selectin measurements

The table summarizes results from clinical studies in normal volunteers, patients with vascular or hematological disorders or those having surgery, and in dogs. Whilst comparisons between studies are difficult using summary data, the following observations may be made.

The clopidogrel test is sensitive to the presence of ADP receptor antagonists such as clopidogrel and prasugrel. Typically, volunteers or patients who are not on an antagonist exhibit values above 800 units whilst those on clopidogrel have expression levels below 700 units. Limited data suggest that prasugrel lowers expression more than clopidogrel. Using a predefined cutoff of 860 units (33), a significant proportion of patients on clopidogrel do not suppress (i.e., high platelet reactivity whilst on treatment), this ranging between 6% and 36% (with one study finding it as high as 58% (33)).

The aspirin test showed that individuals who were taking this inhibitor of cyclooxygenase typically had P-selectin expression below 500 units (and often <200 units) whilst those not taking aspirin usually exceeded 500 units (and often >900 units). High platelet reactivity (using a cutoff of >500 units) was uncommon on aspirin, typically affecting <5% individuals.

Future prospects

Use of remote P-selectin measurement offers great promise but several important questions remain. First, P-selectin expression varies considerably between individuals and a key question is what values should be used for deciding that a patient has high platelet reactivity if on an antiplatelet or a low value in the presence of bleeding. Typically, cut-points need to be defined by outcome data so that patients with a value on one side of the cut-point are much more likely to have an event than if on the other side. Practically, this means defining the cut-point using one set of data and then testing it using a second data source. To date, the only cut-point to be defined in this way is the value of >860 units for the clopidogrel test which signals high platelet reactivity on clopidogrel (33).

Second, predictors of high platelet reactivity on clopidogrel need to be identified. It is likely that high platelet reactivity on treatment will be associated with increasing age, male sex, presence of vascular risk factors such as hypertension and diabetes mellitus, and having an acute vascular event. Although a study of patients with acute coronary syndrome found no such difference (33), it was small and the comparisons were probably underpowered statistically.

Third, the relationship between P-selectin expression and outcome needs to be further defined. For example, it can be hypothesized that patients with suppressed P-selectin expression will be less likely to suffer a vascular event when taking antiplatelet therapy than those whose level is not suppressed. This scenario was seen in the small study in acute coronary syndrome, whereby those on clopidogrel who went on to develop a further cardiovascular event had higher platelet P-selectin expression than those who had no further event (33). Equally, increased bleeding might be associated with very suppressed P-selectin expression. This is being studied in a larger sample of patients with recent acute ischemic stroke or TIA who were recruited into the TARDIS trial. Nevertheless, the relationship between assessment of platelet function and subsequent vascular events is challenging to assess and previous studies using other platelet function assessments have found poor correlation between platelet function testing and subsequent events (46).

If high platelet reactivity is related to outcomes, then a fifth question is whether changing treatment reduces not just platelet function but more importantly vascular events. Hence, patients with recent cerebral ischemia and apparent resistance to clopidogrel might best be changed to an alternative antiplatelet regimen such as combined aspirin and clopidogrel. Trials addressing this type of question have yet to be reported.

Sixth, the concept of de-escalation has been reported recently whereby patients taking an expensive antiplatelet agent such as ticagrelor or prasugrel are switched to clopidogrel for reasons of cost or bleeding. It is then necessary to assess whether HRPR is present in which case treatment would be reescalated to the original antiplatelet. The TROPICAL-ACS study used this approach with platelet function assessed using Multiplate (47). The P-selectin approach offers a potential alternative diagnostic for de-escalation and needs further study.

A seventh area for future research is to expand the indication for testing platelet function. The majority of studies quoted here have focused on vascular diseases (9,33,34,39,40) or diagnosis of

bleeding (18). Other potential indications include assessment of risk of bleeding prior to surgery, confirmation that platelet function is appropriately attenuated prior to invasive procedures, and veterinary uses. However, the use of P-selectin measurement for managing other diseases where platelets may contribute to pathogenic processes remains untested, with examples including cancer, dementia, and valvular heart disease (48,49). Similarly, pharmaceutical development may need to demonstrate that novel agents do, or do not, have antiplatelet effects.

Finally, the studies quoted here involved a single core laboratory in Nottingham for flow cytometric quantification of P-selectin expression on the surface of platelets. Flow cytometers are widely available and there is no reason why other laboratories should not perform these assessments, although quality control systems would need to be set-up to ensure that results were comparable between laboratories, as we did previously for international assessment of mean platelet volume (6).

Declaration of Interest

Philip Bath is Stroke Association Professor of Stroke Medicine at University of Nottingham (UoN), chief investigator of the TARDIS and Pre-ACRIST trials, and a NIHR Senior Investigator; he is Medical Advisor at PSL and holds stock in PSL.

Jane May is a founder and contract research coordinator at PSL and holds stock in PSL.

Stan Heptinstall is Emeritus Professor of Haemostasis and Thrombosis at UoN; he coinvented the P-selectin test, is a Founder and Scientific Director at PSL, and holds stock in PSL.

References

1. Holinstat M. Normal platelet function. Cancer Metastasis Rev. 2017;36:195–198.
2. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (caprie). Lancet. 1996;348:1329–1339.
3. Antithrombotic Trialists Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. Br Med J. 2002;324:71–86.
4. Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849–1860.
5. Martin JF, Bath PMW, Burr ML. Increased platelet size following myocardial infarction is associated with subsequent death and non-fatal reinfarction. Lancet. 1991;338:1409–1411.
6. Bath P, Algert C, Chapman N, Neal B, For the PROGRESS collaborative group. Association of mean platelet volume with risk of stroke among 3134 individuals with history of cerebrovascular disease. Stroke. 2004;35:622–626.
7. Harrison P, Segal H, Blasbery K, Furtado C, Silver L, Rothwell PM. Screening for aspirin responsiveness after transient ischemic attach and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. Stroke. 2005;36:1001–1005.
8. Mannu G, Macartney A, Lambert J, Betterncourt-Silva J, Lawn M, Lyall H, Metcalf A, Potter J, Wood J, Clark A, et al. The clinical utility of multiplate analyser measurement in platelet function testing following stroke and transient ischaemic attack. Eur J Haematol. 2015;94:138–144.
9. May J, Dovlatova N, Radhakrishnan A, Heptinstall S, Fox S. Platelet function testing in acs patients using measurement of platelet p-selectin: comparison with other commercial tests. J Thromb Haemostasis. 2015;13:284.
10. Aradi D, Kirtane A, Bonello L, Gurbel PA, Tantry US, Huber K, Freynhofer MK, ten Berg J, Janssen P, Angiolillo DJ, et al. Bleeding and stent thrombosis on p2y12-inhibitors: collaborative analysis on the role of platelet reactivity for risk stratification after percutaneous coronary intervention. Eur Heart J. 2015;36:1762–1771.
11. Harrison P, Segal H, Silver L, Syed A, Cuthbertson F, Rothwell P. Lack of reproducibility of assessment of aspirin responsiveness by optical aggregometry and two platelet function tests. Platelets. 2008;19:119–124.
12. Bath PM, Blann A, Smith N, Butterworth RJ. 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. 1998;9:155–159.
13. Dovlatova N, May J, Fox S. Remote platelet function testing - significant progress towards widespread testing in clinical practice. Platelets. 2015;26:399–401.
14. Heptinstall S, Fox S, May J, White A, Dovlatova N. Pamfix, a fixative developed to enable remote platelet function testing: uses and applications. Curr Top Pharmacol. 2015;19:1–12.
15. Fox SC, May JA, Shah AB, Neubert U, Heptinstall S. Measurement of platelet p-selectin for remote testing of platelet function during treatment with clopidogrel and/or aspirin. Platelets. 2009;20:250–259.
16. Fox SC, May JA, Dovlatova N, Glenn JR, Johnson A, White AE, Radhakrishnan A, Heptinstall S. How does measurement of platelet p-selectin compare with other methods of measuring platelet function as a means of determining the effectiveness of antiplatelet therapy? Platelets. 2018;1–6
17. Adamany J, Dunning M, May J, Fox S, Heptinstall S. Development and refinement of a remote platelet p-selectin test for measurement of platelet function in dogs. Platelets. 2016;27:1.
18. Dovlatova N, Lordkipanidzé M, Lowe GC, Dawood B, May J, Heptinstall S, Watson SP, Fox SC, Group UGS. Evaluation of a whole blood remote platelet function test for the diagnosis of mild bleeding disorders. J Thromb Haemost. 2014;12:660–665.
19. Cattaneo M. Aspirin and clopidogrel. Efficacy, safety and the issue of drug resistance. Arteriosclerosis, Thromb Vasc Biol. 1987;24:1980–1987.
20. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting. Response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation. 2003;107:2908–2913.
21. Tendera M, Wojakowski W. Role of antiplatelet drugs in the prevention of cardiovascular events. Thromb Res. 2003;110:355–359.
22. Heptinstall S, May J, Storey RF, Wilcox RG. Clopidogrel resistance. Catherization Cardiovasc Interventions. 2004;63:397–398.
23. Fontana P, Nolli S, Reber G, De Moerloose P. Biological effects of aspirin and clopidogrel in a randomized cross-over study in 96 healthy volunteers. J Thromb Haemostasis. 2006;4:813–819.
24. Michelson AD, Linden MD, Furman MI, Li Y, Barnard MR, Fox ML, Lau WC, McLaughlin TJ, Frelinger AL. Evidence that pre-existent variability in platelet response to adp accounts for 'clopidogrel resistance'. J Thromb Haemostasis. 2007;5:75–81.
25. Serebruany V, Cherala G, Williams C, Surigin S, Booze C, Kuliczkowski W, Atar D. Association of platelet responsiveness with clopidogrel metabolism: role of compliance in the assessment of "resistance". Am Heart J. 2009;158:925–932.
26. Storey RF. Clopidogrel in acute coronary syndrome: to genotype or not? Lancet. 2009;373:276–278.
27. Vlachojannis G, Dimitropoulos G, Alexopoulos D. Clopidogrel resistance: current aspects and future directions. Hellenic J Cardiol. 2011;52:236–245.
28. Siller-Matula J, Trenk D, Schror K, Gawaz M, Kristensen S, Storey R, Huber K. Response variability to p2y12 receptor inhibitors expectations and reality. JACC: Cardiovasc Intervensions. 2013;6:1111–1128.
29. Fitzgerald R, Pirmohamed M. Aspirin resistance: effect of clinical, biochemical and genetic factors. Pharmacol Ther. 2011;130:213–225.
30. Wang Y, Zhao X, Lin J, Johnston S, Lin Y, Pan Y, Liu L, Wang D, Wang C, Meng X, et al. Association between cyp2c19 loss-of-function allele status and efficacy of clopidogrel for risk reduction among patients with minor stroke or transient ischemic attack. Jama. 2016;316:70–78.
31. Cardoso RN, Benjo AM, DiNicolantonio JJ, Garcia DC, Macedo FY, El-Hayek G, Nadkarni GN, Gili S, Iannaccone M, Konstantinidis I, et al. Incidence of cardiovascular events and gastrointestinal bleeding in patients receiving clopidogrel with and without proton pump inhibitors: an updated meta-analysis. Open Heart. 2015;2:e000248.
32. Fox S, May J, Johnson A, Hermann D, Strieter D, Hartman D, Heptinstall S. Effects on platelet function of an ep3 receptor antagonist used alone and in combination with a p2y12

antagonist both in-vitro and ex-vivo in human volunteers. Platelets. 2013;24:392–400.
33. Thomas M, Wijeyeratne Y, May J, Johnson A, Heptinstall S, Fox S. A platelet p-selectin test predicts adverse cardiovascular events in patients with acute coronary syndromes treated with aspirin and clopidogrel. Platelets. 2014;25:612–618.
34. Laohathai P, Joshi R, Radhakrishnan A, May J, Dovlatova N, Heptinstall S, Fox S. Residual platelet function in acs patients treated with prasugrel is lower than in patients treated with clopidogrel but not in patients with hypertension. J Thromb Haemostasis. 2015;13:518.
35. Committee CS. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (caprie). Caprie steering committee. Lancet. 1996;348:1329–1339.
36. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European stroke prevention study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. J Neurol Sci. 1996;143:1–13.
37. TARDIS Trial Investigators, Krishnan K, Beridze M, Christensen H, Dineen R, Duley L, Heptinstall S, James M, Markus H, Pocock S, et al. Safety and efficacy of intensive vs. Guideline antiplatelet therapy in high-risk patients with recent ischemic stroke or transient ischemic attack: rationale and design of the triple antiplatelets for reducing dependency after ischaemic stroke (tardis) trial (isrctn47823388). Int J Stroke. 2015;10:1159–1165.
38. Bath PM, Woodhouse LJ, Appleton JP, Beridze M, Christensen H, Dineen RA, Duley L, England TJ, Flaherty K, Havard D, et al. Intensive versus guideline antiplatelet therapy in patients with acute cerebral ischaemia (tardis): a randomised, open-label, phase iii, superiority trial. Lancet. 2018;391:850–859.
39. Heptinstall S, Dovlatova N, May J, Robson K, Bath P. Remote platelet function testing using measurements of p-selectin in patients with acute stroke or tia already receiving treatment with antiplatelet agents. J Thromb Haemostasis. 2015;13:695.
40. Bath PM, May J, Flaherty K, Woodhouse LJ, Dovlatova N, Fox SC, England TJ, Krishnan K, Robinson TG, Sprigg N, et al. Remote assessment of platelet function in patients with acute stroke or transient ischaemic attack. Stroke Res Treat. 2017;2017:7365684.
41. Dovlatova N, Heptinstall S, May J, Bath P. Remote platelet function testing using platelet p-selectin as a marker of platelet activation. Findings in patients with stroke or tia treated with clopidogrel. Res Pract Thromb Haemostasis. 2017;1:S1.
42. Heptinstall S. Remote platelet function testing using platelet-bound p-selectin as a marker of platelet activation. Res Pract Thromb Haemost. 2017;1:S1.
43. Keeler B, Simpson J, Fox S, Stavrou C, Briggs R, Patel P, Heptinstall S, Acheson A. An observational study investigating the effect of platelet function on outcome after colorectal surgery. Int J Surg. 2015;17:28–33.
44. Dunning M, May J, Adamany J, Heptinstall S, Fox S. A remote assay for measuring canine platelet activation and the inhibitory effects of antiplatelet agents. J Vet Intern Med. 2018;32:119–127.
45. Dovlatova N. Current status and future prospects for platelet function testing in the diagnosis of inherited bleeding disorders. Br J Haematol. 2015;170:150–161.
46. Harrison P, Frelinger AL, Furman MI, Michelson AD. Measuring antiplatelet drug effects in the laboratory. Thromb Res. 2007;120:323–336.
47. Sibbing D, Aradi D, Jacobshagen C, Gross L, Trenk D, Geisler T, Orban M, Hadamitzky M, Merkely B, Kiss RG, et al. Guided de-escalation of antiplatelet treatment in patients with acute coronary syndrome undergoing percutaneous coronary intervention (tropical-acs): a randomised, open-label, multicentre trial. Lancet. 2017;390:1747–1757.
48. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377:31–41.
49. Patrono C. The multifaceted clinical readouts of platelet inhibition by low-dose aspirin. J Am Coll Cardiol. 2015;66:74–85.