Coming safely to a stop: A review of platelet activity after cessation of antiplatelet drugs

Dr Isobel Ford, Ph.D.

Senior Research Fellow
Division of Applied Medicine
School of Medicine & Dentistry
University of Aberdeen
Polwarth Building
Foresterhill
Aberdeen
Scotland
AB25 2ZD
U.K.

Telephone: +44 1224 437843
Email: i.ford@abdn.ac.uk

Word count: 3755

Keywords: antiplatelet therapy; cessation; clopidogrel; P2Y12 inhibitors; platelet activation; rebound

Abbreviations:

ADP adenosine diphosphate

DAPT dual antiplatelet therapy

NSTEMI non-ST elevation myocardial infarction

PCI percutaneous coronary intervention

RCT randomised controlled trial

STEMI ST-elevation myocardial infarction

Isobel Ford is an employee of the University of Aberdeen. The research for the writing of this review received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Coming safely to a stop: A review of platelet activity after cessation of antiplatelet drugs

Abstract

The platelet P2Y12 antagonists are widely used, usually in combination with aspirin, to prevent atherothrombotic events in patients with acute coronary syndromes, during PCI and after placement of arterial stents.

Inhibition by clopidogrel or prasugrel lasts for the lifetime of the affected platelets and platelet haemostatic function gradually recovers after stopping the drug, as new unaffected platelets are formed.

The optimal durations for dual antiplatelet therapy are prescribed by clinical guidelines. Continuation beyond the recommended duration is associated with an increased mortality, mainly associated with major bleeding.

Fear of a "rebound" of prothrombotic platelet activity on stopping the drug has provoked much discussion and many studies. However, review of the available literature reveals no evidence for production of hyper-reactive platelets after cessation of clopidogrel in stable patients. Any increase in acute coronary and other vascular events after stopping seems most likely therefore to be due to premature discontinuation or disruption of treatment while thrombotic risk is still high. No difference in rebound was found with the newer P2Y12 inhibitors, although ticagrelor and prasugrel are more potent platelet inhibitors than clopidogrel.

Recent RCTs confirm it is safe to stop the thienopyridine and continue with aspirin alone in most patients after the duration of treatment recommended by the guidelines. Decisions on when to stop therapy in individuals, however, remain challenging and there is a growing rationale for platelet testing to assist clinical judgement in certain situations such as patients stopping DAPT before surgery or in individuals at highest bleeding or thrombotic risk.

Background

Medical therapy to reduce platelet activation is a mainstay in prevention of atherothrombotic events in cardiovascular disease. For many years aspirin was the drug of choice, but randomised controlled trials from the 1990s onwards demonstrated the efficacy of clopidogrel in reduction of major acute coronary and cerebrovascular events and vascular deaths (CAPRIE 1996). Clopidogrel in combination with aspirin improved outcomes after acute coronary syndrome, and after percutaneous coronary intervention (PCI) with or without placement of stents (Bhatt et al 2006, Chen et al 2005; Steinhubl et al 2003; Yusuf et al 2001).

Two newer P2Y12 inhibitors are in clinical use. Prasugrel, another irreversible thienopyridine inhibitor (Wiviott et al 2007), and ticagrelor, a reversible, non-competitive inhibitor (Wallentin et al.2009), are now preferred over clopidogrel for acute myocardial infarction for their more rapid and more potent antiplatelet action (Windecker et al.2014). However, the association with higher bleeding risk, contra-indication in certain categories of patient, and higher cost, mean that clopidogrel is still widely-used throughout the world, and following PCI in stable coronary artery disease. For the purposes of this review I will concentrate on clopidogrel.

Action of Clopidogrel

Clopidogrel is a prodrug of the thienopyridine class. Conversion to the active metabolite in the liver is dependent on cytochrome P450 isoforms including CYP3A and CYP2C19. There is well-recognised variability in responsiveness to clopidogrel, which is at least partly attributable to polymorphisms of these genes (Simon et al NEJM 2009), as well as to drug interactions and adherence to treatment. The main therapeutic target for the active molecule is the platelet P2Y12 receptor for adenosine disphosphate (ADP). Through a Gi-coupled signalling pathway, receptor occupancy induces activation of the alpha IIb beta 3 integrin receptor (also known as Glycoprotein IIb-IIIa), exposing the receptor for fibrinogen and resulting in platelet aggregate formation (Braun et al 2007;Schror 1998). Inhibition of the P2Y12 is irreversible and so, as with aspirin blockade of cyclo-oxygenase, this pathway of activation is inhibited for the lifetime of the platelets (Schror 1998). Function gradually

recovers after stopping the drug because of new, unaffected platelets being produced from megakaryocytes, while ageing platelets are gradually removed. Normal platelet lifespan is 7-10 days, but disease and ageing may influence turnover times and therefore affect the time to recovery of platelet function after a single antiplatelet dose.

Duration of Dual Antiplatelet Therapy

Evidence-based, national and international clinical guidelines prescribe the optimum durations for antiplatelet therapy, and these have evolved considerably over the past decade as more trial results emerge (Jneid et al 2012; Windecker et al 2014). The European Society of Cardiology (Windecker et al 2014) currently states that after acute myocardial infarction – STEMI or NSTEMI, dual antiplatelet therapy (DAPT) with aspirin plus a P2Y12 inhibitor be continued for 12 months after the acute event; whereas for patients with stable coronary artery disease who receive PCI and a drug-eluting stent, clopidogrel is recommended for 6 months, and for a minimum of one month after placement of a bare metal stent. It is usually recommended that patients continue on aspirin for life, unless contraindicated, for example by drug intolerance or bleeding.

Because of the pivotal role of platelets in haemostasis and thrombosis, maintenance of the balance between inhibition and activation is essential. Thus, all antithrombotic agents introduced to date carry the side-effect of increased bleeding risk. As the thrombotic risk diminishes over time, for example after successful PCI to unblock the diseased coronary artery, or healing of the vessel wall after placement of stents, the risk of mortality from bleeding becomes disproportionately higher with continued use of DAPT. However, the European guidelines (Windecker et al 2014) also state that a longer duration can be used in those at high ischaemic risk and low bleeding risk, and a shorter duration in people at lower risk of recurrence or higher bleeding risk. In addition, the need may arise to withdraw antiplatelet drugs earlier than recommended, before surgery for example, or if there is intolerance to the drug, or bleeding events.

What happens clinically on stopping clopidogrel?

The publication of a series of case-reports and retrospective studies sounded the alarm over an apparently increased rate of ischaemic events in the period after withdrawal of clopidogrel

(Ong et al 2005; Pfisterer et al 2006; Ho et al 2008; Ho et al 2010). Ho et al (2008) reported a clustering of adverse events in patients with previous acute coronary syndrome in the first 90 days after stopping clopidogrel. Despite the observational and retrospective nature of these studies, an "Advisory" statement was issued on premature stopping of DAPT (Grines et al 2007) (see Gaglia and Waksman 2011 for systematic review).

What happens to platelets when clopidogrel is withdrawn?

These studies provoked discussion of a "rebound" in platelet activity. The theory was proposed that suppression by clopidogrel might lead to a biological adaptation of platelets or megakaryocytes, conferring enhanced sensitivity to ADP or other stimuli. Such a change would manifest itself after cessation of the drug and recovery of a non-inhibited, hyperactive platelet population (Lordkipanidze et al 2009; Sambu et al 2011). This gave rise to some small studies that claimed to find a rebound of platelet responsiveness at varying times after stopping clopidogrel treatment.

An observational study of 28 patients with coronary stent implantation, who had been treated with clopidogrel for 12 months, found that ADP-stimulated platelet aggregation was increased at two weeks after withdrawal of clopidogrel, compared to on-treatment levels, and remained elevated at six weeks, as would be expected [Diehl et al]. However on testing at 17 weeks after stopping, aggregation was lower than at the earlier time-points, and was now no different from that in a group of untreated stable CAD controls.

Mylotte et al. (2011) studied 32 patients with drug-eluting stents (DES). Between on-treatment values and the sample taken at one week following withdrawal, there was an increase in aggregation response to a range of doses of ADP, epinephrine, and thrombin-receptor-activating peptide (TRAP), but not arachidonic acid (AA). There was a further increase in aggregation induced by the lower, but not higher, doses of ADP at one month. By three months after cessation however, aggregation with ADP and epinephrine had decreased, returning to levels similar to those found at seven days, and this was interpreted as a transient rebound effect occurring at one month post-cessation.

The prospective CESSATION study enrolled 28 patients who stopped clopidogrel therapy one year following insertion of a DES. Using the more unusual technique of

thromboelastography platelet mapping (Short TEG), there was gradual recovery of ADP-induced aggregation up to one week, but levels remained similar at two and four weeks, and so no evidence was found for a rebound in ADP pathways of aggregation (Sambu et al 2011b).

Thirty-seven patients with clinically stable coronary artery disease who had been taking clopidogrel and aspirin (for varying lengths of time) were compared with a control group of stable CAD patients who were not taking clopidogrel (Lordkipanidze et al 2014). There were no differences in the Verify Now P2Y12 test, but there was a difference in ADP-stimulated aggregation between the clopidogrel and control groups at seven and 28 days after stopping. Difficulties in comparing these two groups directly include the considerable individual variability in platelet response, and the reasons for being prescribed clopidogrel or not, which most likely include severity of arterial disease and the estimated thrombotic and bleeding risk.

On the other hand, in a study of 200 patients who had taken clopidogrel for 12 months after stenting, there was no evidence for rebound [Djukanovic 2011]. Although again there was no pre-treatment test, platelet aggregation by a whole blood multi-electrode impedance assay (Multiplate®) merely reached a plateau around ten days after stopping and was not significantly different at 45 or 90 days.

However, as none of these studies included a pre-treatment baseline measurement, it was not possible to say whether platelet responsiveness was merely returning to pre-treatment levels.

Platelet studies with a pre-treatment baseline

Several prospective studies have been performed that included detailed platelet function analysis at a pre-treatment baseline as well as on-treatment, and with follow-up for a least a month after stopping clopidogrel. In a placebo-controlled cross-over study of 15 healthy subjects who took clopidogrel plus aspirin or placebo plus aspirin for 7 days each**,** a comprehensive range of platelet measurements was made. These comprised activation markers GPIIb/IIIa and P-selectin by flow cytometry, aggregometry in platelet-rich plasma and in whole blood, all with a range of doses of ADP and other agonists; counting of the immature platelet fraction, and soluble CD40Ligand assay. Once platelet function had

recovered - by around eight days after stopping - there was no further increase in stimulated platelet aggregation or activation markers and no significant differences in any of the tests at 11, 15 or 45 days post cessation compared to baseline [Frelinger et al 2010]. These data showed conclusively that a short-term course of clopidogrel did not increase regeneration nor alter the reactivity of newly-produced platelets, at least in healthy subjects.

In a prospective, double-blind placebo controlled trial, Ford et al (2014) randomised 171 patients with stable coronary artery disease or peripheral arterial disease to 28 days of clopidogrel (75mg) or placebo. Patients continued to take their 75mg daily aspirin therapy throughout the study. A range of platelet tests was carried out at pre-treatment baseline, on-treatment (just before stopping clopidogrel or placebo), and at seven, 14 and 28 days after stopping. ADP-stimulated platelet aggregation (5 and 10 micromolar ADP), platelet activation markers by flow cytometry, Verify NowP2Y12 and VASP-P, were all significantly lower on clopidogrel treatment compared with baseline, showing appropriate responses to the drug. Unstimulated markers of platelet activation were unchanged. Values returned to baseline levels by seven days after discontinuation. Apart from the on-treatment measurements, there were no statistically significant differences between those taking clopidogrel or placebo at any of the follow-up timepoints. Furthermore, there was no evidence for a treatment-time interaction in any of the statistical models, thereby confirming that platelet responsiveness remained stable over time after stopping clopidogrel (Ford et al 2014).

Two studies used the approach of comparing the recovery of platelet activity in patients who were allocated to abrupt cessation of clopidogrel or to a tapering regimen at the planned completion of their post-stent treatment, with the aim of attenuating any rebound. Neither study found any evidence for a difference in platelet activity after complete cessation (Sibbing et al 2010; Yedidya et al 2012).

In summary, in studies with adequate numbers, baseline pre-treatment measurements and inclusion of a placebo arm, therefore, there has been no evidence for a rebound of platelet responsiveness to ADP after stopping clopidogrel. Instead, these studies show a gradual return to original levels of platelet activation and responsiveness. It is unlikely that any rebound effect would be missed in a 30-day follow-up as this is more than adequate for a complete turnover of the platelet population. Furthermore, most of the reported excess of thrombotic events occurs within the first 30 days of stopping (Lemesle et al 2011). It has also

been argued that increased sensitivity of the P2Y12 receptor may develop over longer-term therapy. However, the incidence of post-clopidogrel thrombotic events is highest in patients who discontinued the drug within one month of commencing [Lemesle et al 2011], a finding more consistent with discontinuation before healing of the arteries was complete.

Most of the controlled laboratory studies selected patients with stable coronary artery disease, and it could be argued that a greater effect might be seen in patients with ACS or drug-eluting stents as they are at higher thrombotic risk. Patients with acute events would unfortunately be more difficult to study rigorously because of the expected long interval between pre-treatment sample and stopping clopidogrel. Caution should of course be exercised in extrapolating the results to patients with a recent acute event.

Could there be adaptation of other platelet pathways to clopidogrel?

Reflecting the vital function of haemostasis and thrombus formation, platelets carry many different surface receptors that recognise various stimulatory agonists, and several signalling pathways for activation. As well as the established target of P2Y12, it is apparent that clopidogrel may inhibit aggregation stimulated by other agonists, including arachidonic acid and TRAP (Ford et al 2013). Some studies have found that, although there was no rebound effect on ADP pathways of activation or aggregation, the "aspirin-specific" tests, i.e. using arachidonic acid as platelet stimulus, appeared less inhibited after stopping clopidogrel but maintaining the aspirin dose (Djukanovic et al 2014; Hobson et al 2009, Sambu et al 2011). However, detection appears to be method-dependent, as it is not seen with light transmission aggregometry (Ford et al 2013, Ford et al 2014, Frelinger et al 2010), and Good et al [2015] have recently shown that there is no additional suppression of thromboxane B2 production when clopidogrel is added to aspirin therapy. Nevertheless, such synergistic effects probably contribute to the efficacy of dual compared with single antiplatelet therapy.

Reactive inflammation following cessation of clopidogrel has also been proffered as an explanation for an increase in MACE (Angiolillo et al 2006). The results are conflicting however and are likely to reflect changes in disease state rather than an association with stopping clopidogrel (Sambu et al 2011; Wykrzykowska et al 2009). It is unclear in any case whether clopidogrel therapy directly affects any inflammatory markers although, in common

with aspirin, appearing to be associated with lower C-Reactive Protein (CRP) (Woodward et al. 2004; Husted et al. 2010).

Despite lacking a nucleus, platelets contain the translational mechanisms needed to manufacture proteins (Weyrich et al 1998), and so an adaptation response of either megakaryocytes or platelets to chronic antiplatelet therapy is not implausible (Lordkipanidze 2009). However there is no evidence to support any pro-thrombotic changes in receptor number or signalling capacity of platelets in the weeks after antiplatelet therapy in humans, and in any case this would not explain why any such changes were not detectable by the range of functional tests employed in the controlled research studies.

Newer P2Y12 Inhibitors and Platelet Activity

Ticagrelor (a reversible inhibitor) and prasugrel induce more potent inhibition of platelet function than clopidogrel (Storey et al 2011) and, in the case of prasugrel, a more delayed recovery of platelet function after stopping (Price et al 2012). Studies so far have found no sign of rebound on stopping either prasugrel or ticagrelor (Angiolillo et al 2011;Gurbel et al 2009; Gurbel et al 2011; Jakubowski et al 2011), and as their modes of action are similar this would seem rational. Reversible inhibitors of P2Y12 allow more rapid return of haemostatic activity when stopped. These would be considered to be beneficial properties if urgent surgery or intervention were required, or if bleeding problems arose. On the other hand, issues of poor compliance could be more serious as a missed dose would produce intermittent reversal of platelet inhibition.

Is there a true clinical rebound?

A systematic review (Gaglia and Waksman 2011) found most early studies to be flawed, and more recent detailed analyses have cast doubt on the existence of a clinical rebound particular to clopidogrel._A retrospective analysis (Collet et al 2009) of the CHARISMA trial data (Bhatt et al 2006) found that in almost 3000 patients who stopped taking medication before the end of the study, there were increased rates of ischaemic events and overall mortality, but the rate was actually lower in the clopidogrel plus aspirin group compared with the placebo plus aspirin group (Collet et al 2009). Bleeding events were higher than in those who

completed, but no different between those who had been taking clopidogrel or placebo, and the investigators concluded there was no evidence for a clinically-detectable rebound.

Controversy still remains over the ideal duration, but the data now emerging from randomised trials demonstrate that extending the recommended time might be of no overall benefit and even detrimental (El Hayek et al 2014; Mauri et al 2014; Schulz-Schupke et al 2015;Valgimigli et al 2013). In the DAPT Trial (Mauri et al 2014), the risk of stent thrombosis was reduced but all-cause mortality and the incidence of severe or moderate bleeding were significantly higher in the group that continued a thienopyridine to 30 months. Interestingly the rate of stent thrombosis increased in the three months after stopping in both the group that continued on clopidogrel and the group that had changed to placebo.

Are the Reasons for Stopping Antiplatelet Drugs Important?

In most studies, the timing and the reasons for stopping antiplatelet therapy were unknown. The PARIS registry study (patterns of non-adherence to antiplatelet regimens in stented patients) (Mehran et al 2013) followed patients for two years and analysed the outcomes according to pre-specified reasons for stopping:

1) "discontinuation", defined as physician-recommended cessation;
2) "interruption", defined as temporary cessation of DAPT of less than 14 days due to surgical procedures;
3) "disruption", defined as withdrawal of antiplatelet treatment due to bleeding or non-compliance.

In 5031 patients, the rate of DAPT cessation by 24 months was 57.3%, mostly as physician-recommended discontinuation (40.8%), with disruption in 14.4% and interruption in 10.5%. Three-quarters of all events occurred in patients while still taking DAPT, but compared with those on treatment, the interruption and disruption group had a higher risk of major acute coronary events (MACE). In contrast, those with physician-recommended cessation had lower MACE risk than those who remained on DAPT at 24 months. The highest risk ratio in the disruption group occurred in the first 7 days after stopping. This is consistent with the study of Lemesle et al (2011) who found that those stopping earliest had the highest risk of death, MI, and stent thrombosis at 30 days.

The evidence gives reassurance that discontinuation of DAPT in low-risk patients should be considered safe, especially after 6 months from stent implantation (Franchi and Angiolillo 2014). Unanticipated premature disruption of treatment however is likely to place the patient at higher risk. Patients in this latter group are more likely to have stopped because of drug intolerance, bleeding issues or illness. Although there is no sign that clopidogrel leads to production of hyper-reactive platelets, after drug withdrawal uninhibited platelets in circulation will become activated by encountering areas of atherosclerotic vessel wall, or by increased shear stresses in narrowed arteries, or by the prothrombotic stimulus of unhealed stents.

The Future of Platelet Testing to Assess Risk and Guide Therapy

Determining the right time to stop antiplatelet therapy in individuals remains a major issue for clinical judgement. The case for routine platelet testing at the time of PCI to guide therapy has not been supported by the recent GRAVITAS and TRIGGER-PCI trials (Price et al 2011; Trenk et al 2012), although in the study of Aradi et al (2015), switching those with highest on-treatment platelet reactivity from clopidogrel to prasugrel appears more promising. Testing to stratify those patients at higher risk for either bleeding or thrombotic events could be more useful (Collet and Montalesco 2013) and there is proven value in specific situations, such as when surgery is required.

In the perioperative period, decision making on interruption of DAPT and removal of cardioprotection is complicated by the increase in both bleeding risk and hypercoagulability. (Patel and Fleisher 2014). Where it is deemed safe to do so, discontinuation of P2Y12 inhibitors five days before cardiac surgery is advised to allow recovery of platelet function, although it is generally accepted that aspirin can be continued (Ferraris et al 2012). Marked individual variability in return of adequate haemostatic activity is evident however (diDedda et al 2014) and time to recovery is determined, *inter alia*, by the level of platelet reactivity before drug exposure and the extent of platelet inhibition immediately after discontinuation (Price et al 2012). A strong rationale is emerging for platelet testing; particularly suitable are the rapid whole blood methods requiring minimal sample preparation, such as the Multiplate (multi-electrode impedance aggregometry) and the point of care test, Verify Now (Reviewed by Petricevic et al 2015, Kong et al 2014). In addition, in cases where surgery cannot be delayed, testing may be used to predict the need for blood products (Emeklibas et al 2013).

Conclusion

"Drug discontinuation effects are part of the pharmacology of a drug" (Reidenberg 2011) and as such are an important part of safety evaluation. The term "rebound" has been applied to denote an unexpected increase in adverse clinical events at the end of a course of treatment, and has been applied to a range of drugs (Graves et al 1997). There has also been some misuse of the term "rebound" in the literature to denote simply a return to the status quo of the pre-drug state. The current evidence indicates that there is nothing unusual about clopidogrel in this regard: platelet responsiveness gradually recovers to the pre-drug levels after stopping. The most likely explanation for post-clopidogrel ischaemic events is premature withdrawal of required protection while the damaged vessels continue to present a highly prothrombotic stimulus. A role exists for platelet testing to assist in clinical decision making.

References

Angiolillo, D.J, Fernandez-Ortiz, A., Bernardo, E., Ramirez, C., Sabate, M., Jimenez-Quevedo, P., et al. (2006) Clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes and coronary artery disease. *Diabetes* 55: 780-784.

Angiolillo DJ, Badimon JJ, Saucedo JF, Frelinger AL, Michelson AD, Jakubowski JA, et al. (2011) A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type2 diabetes mellitus and coronary artery disease: Results of the Optimizing anti-Platelet Therapy In diabetes MellitUS (OPTIMUS)-3 Trial. *European Heart Journal* 32: 838–846.

Aradi D, Tornyos A, Pintér T, Vorobcsuk A, Kónyi A, Faluközy J, et al. (2014) Optimizing $P2Y_{12}$ Receptor Inhibition in Patients With Acute Coronary Syndrome on the Basis of Platelet Function Testing: Impact of Prasugrel and High-Dose Clopidogrel *Journal of the American College of Cardiology* 63: 1061-1070.

Bhatt DL, Fox KAA, Hacke W, Berger PB, Black HR, Boden WE, Cacoub P, Cohen P, Cohen EA, Creager MA, Easton JD, Flather MD, Haffner SM, Hamm CW, Hankey GJ, Johnston SC, Mak KH, Mas JL, Montalescot G, Pearson TA, Steg PG, Steinhubl SR, Weber MA, Brennan DM, Fabry-Ribaudo L, Booth J, Topol EJ and CHARISMA Investigators. (2006) Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. 2006; 354: 1706–1717. *N Engl J Med* 354: 1706-1717.

Braun OO, Amisten S, Wihlborg A, Hunting K, Nilsson D and Erlinge D (2007) Residual platelet ADP reactivity after clopidogrel treatment is dependent on activation of both the unblocked $P2Y_1$ and the $P2Y_{12}$ receptor and is correlated with protein expression of $P2Y_{12}$. *Purinergic Signalling* 3: 195–201.

CAPRIE Steering Committee (1996) A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE) *Lancet* 348: 1329-1339.

Collet J and Montalescot G (2013) Personalized antiplatelet therapy: The wrong approach? *Archives of Cardiovascular Disease* 106: 481-486.

Collet J, Montalescot G, Steg PG, Steinhubl S, Fox KA, Hu TF, et al. (2009) Clinical outcomes according to permanent discontinuation of clopidogrel or placebo in the CHARISMA trial *Archives of Cardiovascular Disease (2009) 102, 485—496* 102: 485-496.

COMMIT (ClOpidogrel and Metoprolol in Myocardial Infarction Trial) collaborative group. (2005) Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* 366: 1607-1621.

Di Dedda U, Ranucci M, Baryshnikova E, Castelvecchio S and Surgical and Clinical Outcome Research (SCORE) Group. (2014) Thienopyridines resistance and recovery of platelet function after discontinuation of thienopyridines in cardiac surgery patients. *European Journal of Cardio-Thoracic Surgery* 45: 165-170.

Diehl P, Halscheid C, Olivier C, Helbing T, Bode C and Moser M (2011) Discontinuation of long term clopidogrel therapy induces platelet rebound hyperaggregability between 2 and 6 weeks post cessation. *Clinical Research in Cardiology* 100(9): 765-771.

Djukanovic N, Todorovic Z, Obradovic S, Njegomirovic S, Zamaklar-Trifunovic D, Protic D, et al. (2014) Clopidogrel cessation triggers aspirin rebound in patients with coronary stent. *J Clin Pharm Ther* 39: 69-72.

Djukanovic N, Todorovic Z, Obradovic S, Zamaklar-Trifunovic D, Njegomirovic S, Milic NM, et al. (2011) Abrupt Cessation of One-Year Clopidogrel Treatment Is Not AssociatedWith Thrombotic Events. *Journal of Pharmacological Sciences* 117: 12-18.

El-Hayek G, Messerli F, Bangalore S, Hong MK, Herzog E, Benjo A, et al. (2014) Meta-Analysis of Randomized Clinical Trials Comparing Short-Term Versus Long-Term Dual Antiplatelet Therapy Following Drug-Eluting Stents. *American Journal of Cardiology* 114: 236-242.

Emeklibas N, Kammerer I, Bach J, Sack F and Hellstern P (2013) Preoperative hemostasis and its association with bleeding and blood component transfusion requirements in cardiopulmonary bypass surgery. *Transfusion* 53: 1226-1234.

Ferraris VA, Saha SP, Oestreich JH, Song HK, Rosengart T, Reece B, et al. (2012) 2012 update to the Society of Thoracic Surgeons guideline on use of antiplatelet drugs in patients having cardiac and non-cardiac operations. *Annals of Thoracic Surgery* 94: 1761-1781.

Ford I, Scott NW, Herd V, Mitchell LR, Williams DJP and Brittenden J (2014) A Randomized Controlled Trial of Platelet Activity Before and After Cessation of Clopidogrel Therapy in Patients With Stable Cardiovascular Disease. *Journal of the American College of Cardiology* 63: 233-239.

Ford I, Scott NW, Herd V, Mitchell LR, Williams DJP and Brittenden J (2013) Clopidogrel therapy has additional inhibitory actions on cyclo-oxygenase and thrombin receptor-mediated pathways in platelets. *Journal of Thrombosis and Haemostasis* 11, supplement 2:495-495. Available online at: http://onlinelibrary.wiley.com/doi/10.1111/jth.12284/abstract

Franchi F and Angiolillo DJ (2014) Comment on Cessation of dual antiplatelet treatment and cardiac events after percutaneous coronary intervention (PARIS) study. http://www.escardio.org/communities/ACCA/publications/recommended-readings/Pages/PARIS-study.aspx

Frelinger AL,III., Barnard MR, Fox ML and Michelson AD (2010) The Platelet Activity After Clopidogrel Termination (PACT) Study. *Circulation Cardiovascular Interventions* 3: 442-449.

Gaglia MA and Waksman R (2011) Systematic review of thienopyridine discontinuation and its impact upon clinical outcomes. *European Heart Journal* 32: 358–2364.

Good RIS, McGarrity A, Sheehan R, James TE, Miller H, Stephens J, et al. (2015) Variation in thromboxane B2 concentrations in serum and plasma in patients taking regular aspirin before and after clopidogrel therapy. *Platelets* 26: 17-24.

Graves T, Hanlon JT, Schmader KE, Landsman PB, Samsa GP, Pieper CF, et al. (1997) Adverse Events After Discontinuing Medications in Elderly Outpatients. *Archives of Internal Medicine* 157(19): 2205-2210.

Grines CL, Bonow RO, Casey DE, Gardner TJ, Lockhart PB, Moliterno DJ, et al. (2007) Prevention of Premature Discontinuation of Dual Antiplatelet Therapy in Patients With Coronary Artery Stents. A Science Advisory From the American Heart Association, American College of Cardiology, Society for Cardiovascular Angiography and Interventions, American College of Surgeons, and American Dental Association, With Representation From the American College of Physicians. *Journal of the American College of Cardiology* 49: 734-739.

Gurbel PA, Bliden P, Antonino MJ, Gesheff M, Jeong Y and Tantry US (2011) Is there a rebound after cessation of antiplatelet therapy? First comparison of irreversible vs. reversible P2Y12 receptor blocker. (Abstract). *Journal of Thrombosis and Haemostasis* 9 (Supplement s2): O-MO-119. http://onlinelibrary.wiley.com/doi/10.1111/jth.2011.9.issue-s2/issuetoc

Gurbel PA, Bliden KP, Butler K, Tantry US, Geshef T, Wei C, et al. (2009) Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation* 120: 2577-2585.

Ho PM, Peterson ED, Wang L, Magid D J, Fihn SD, Larsen GC, et al. (2008) Incidence of death and acute myocardial infarction associated with stopping clopidogrel after acute coronary syndrome. *Journal of the American Medical Association* 299: 532-539.

Ho PM, Tsai TT, Wang TY, Shetterley SM, Clarke CL, Go AS, et al. (2010) Adverse Events After Stopping Clopidogrel in Post–Acute Coronary Syndrome Patients. Insights From a Large Integrated Healthcare Delivery System *Circulation Cardiovascular Quality and Outcomes* 3:303-308.

Hobson AR, Qureshi Z, Banks P and Curzen N (2009) Effects of clopidogrel on "aspirin specific" pathways of platelet inhibition. *Platelets* 20: 386-390.

Husted S, Storey RF, Harrington RA, Emanuelsson H and Cannon CP (2010) Changes in Inflammatory Biomarkers in Patients Treated With Ticagrelor or Clopidogrel. *Clinical Cardiology* 33: 206-212.

Jakubowski, J,A, Li YG, Payne CD, Small DS and Winters KJ (2011) Absence of "rebound" platelet hyperreactivity following cessation of prasugrel. *Thrombosis and Haemostasis* 106: 174-176.

Jneid H, Anderson JL, Wright RS, Adams CA, Bridges CA and Casey DE (2012) 2012 ACCF/AHA focused update of the guideline for the management of patients with unstable angina/non– ST-elevation myocardial infarction. (Updating the 2007 Guideline and Replacing the 2011 Focused Update). A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology* 60: 645-681.

Kong R, Trimmings A, Hutchinson N, Gill R, Agarwal S, Davidson S, et al. (2014) Consensus recommendations for using the Multiplate(R) for platelet function monitoring before cardiac surgery . *International Journal of Laboratory Hematology*: 10.1111/ijlh.12279. [Epub ahead of print].

Lemesle G, Torguson R, Bonello L, de Labriolle A, Maluenda G, Ben-Dor I, et al. (2011) Relation between clopidogrel discontinuation and early cardiovascular events after percutaneous coronary intervention with drug-eluting stents. *Eurointervention* 6: 1053-9.

Lordkipanidzé M, Diodati JG, Schampaert E, Palisaitis DA and Pharand C (2014) Evidence of platelet sensitization to ADP following discontinuation of clopidogrel therapy in patients with stable coronary artery disease. [Epub ahead of print]. *Platelets*: PMID: 25167467.

Lordkipanidzé M, Diodati JD and Pharand C (2009) Possibility of a rebound phenomenon following antiplatelet therapy withdrawal: A look at the clinical and pharmacological evidence *Pharmacology & Therapeutics* 123: 178-186.

Mauri L, Kereiakes DJ, Yeh RW, Driscoll-Shempp P, Cutlip DE, Steg PG, et al. (2014) Twelve or 30 Months of Dual Antiplatelet Therapy after Drug-Eluting Stents. *New England Journal of Medicine* 371: 255-266.

Mehran R, Baber U, Steg PG, Ariti C, Weisz G, Witzenbichler B, et al. (2013) Cessation of dual antiplatelet treatment and cardiac events after percutaneous coronary intervention (PARIS): 2 year results from a prospective observational study. *Lancet* 3382: 1714-1722.

Mosfegh K, Redondo M, Julmy F, Wuillemin WA, Gebauer MU, Haeberli A, et al. (2000) Antiplatelet effects of clopidogrel compared with aspirin after myocardial infarction:Enhanced inhibitory effects of conbination therapy. *Journal of the American College of Cardiology* 36: 699-705.

Mylotte D, Peace AJ, Tedesco AT, Mangiacapra F, Dicker P, Kenny D, et al. (2011) Clopidogrel discontinuation and platelet reactivity following coronary stenting. *Journal of Thrombosis and Haemostasis* 9: 24-32.

Ong ATL, McFadden EP, Regar E, deJaegere PPT, van Domburg RT and Serruys PW (2005) Late Angiographic Stent Thrombosis (LAST) Events With Drug-Eluting Stents. *Journal of the American College of Cardiology* 45: 2088–2092.

Patel PA and Fleisher LA (2014) Aspirin, Clopidogrel, and the Surgeon. *Advances in Surgery* 48: 211-222.

Petricevic M, Kopjar T, Biocina B, Milicic D, Kolic K, Boban M, et al. (2015) The Predictive Value of Platelet Function Point-of-Care Tests for Postoperative Blood Loss and Transfusion in Routine Cardiac Surgery:A Systematic Review *Thoracic and Cardiovascular Surgery* 63: 2-20.

Pfisterer M, Brunner-La Rocca HP, Buser PT, Rickenbacher P, Hunziker P, Mueller C, et al. (2006) Late Clinical Events After Clopidogrel Discontinuation May Limit the Benefit of Drug-Eluting Stents. An Observational Study of Drug-Eluting Versus Bare-Metal Stents *Journal of the American College of Cardiology* 48: 2584-2591.

Price MJ, Berger PB, Teirstein PS, et al. (2011) Standard- vs high-doseclopidogrel based on platelet function testing after percuta-neous coronary intervention: the GRAVITAS randomized trial. *Jama* 305: 1097-1105.

Price MJ, Walder JS, Baker BA, Heiselman DE, Jakubowski JA, Logan DK, et al. (2012) Recovery of platelet function after discontinuation of prasugrel or clopidogrel maintenance dosing in aspirin-treated patients with stable coronary disease: The Recovery trial. *Journal of the American College of Cardiology* 59: 2238-2243.

Reidenberg MM (2011) Drug Discontinuation Effects Are Part of the Pharmacology of a Drug *The Journal of Pharmacology and Experimental Therapeutics* 339: 324–328.

Sambu N, Warner T and Curzen,N (2011a) Clopidogrel withdrawal: Is there a rebound phenomenon? *Thrombosis and Haemostasis* 105: 211-220.

Sambu N, Dent H, Englyst N, Warner TD, Leadbeater P, Roderick P, et al. (2011b) Effect of clopidogrel withdrawal on platelet reactivity and vascular inflammatory biomarkers 1 year after drug-eluting stent implantation: results of the prospective, single-centre CESSATION study. *Heart* 97: 1661-1667.

Schror K (1998) Clinical pharmacology of the adenosine diphosphate (ADP) receptor antagonist, clopidogrel *Vascular Medicine* 3: 247-251.

Schulz-Schüpke S, Byrne RA, Ten Berg JM, Neumann FJ, Han Y and Adriaenssens Tea (2015) ISAR-SAFE: a randomized, double-blind, placebocontrolled trial of 6 versus 12 months of clopidogrel therapy after drug-eluting stenting. *European Heart Journal*: Epublished ahead of print January 2015.DOI:10.1093/eurheartj/ehu523

Sibbing D, Stegherr J, Braun S, Mehilli J, Schulz S, Seyfarth M, et al. (2010) A Double-Blind, Randomized Study on Prevention and Existence of a Rebound Phenomenon of Platelets After Cessation of Clopidogrel Treatment. *Journal of the American College of Cardiology* 55: 558-565.

Simon T, Verstuyft C, MAry-Krause M, Quteineh L, Druet E, Meneveau N, et al. (2009) Genetic Determinants of Response to Clopidogrel and Cardiovascular Events *New England Journal of Medicine* 360: 363-375.

Steinhubl S, Berger PB, Mann JT,III., Fry ETA, DeLago A, Wilmer C, et al. (2002) Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: A randomized controlled trial *Journal of the American Medical Association* 288: 2411-2420.

Storey RF, Bliden KP, Ecob R, Karunakaran A, Butler K, Wei C, et al. (2011) Earlier recovery of platelet function after discontinuation oftreatment with ticagrelor compared with clopidogrel in patients with high antiplatelet responses *Journal of Thrombosis and Haemostasis* 9: 1730-1737.

Trenk D, Stone GW, Gawaz M, Kastrati A, Angiolillo DJ and Muller U, et al. (2012) A Randomized Trial of Prasugrel Versus Clopidogrel in Patients With High Platelet Reactivity on Clopidogrel After Elective Percutaneous Coronary Intervention With Implantation of Drug-Eluting Stents. Results of the TRIGGER-PCI (Testing Platelet Reactivity In Patients

Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel) Study. *Journal of the American College of Cardiology* 59: 2159-2164.

Valgimigli M, Park S, Kim H, Park KW, Park D, Tricodi P, et al. (2013) Benefits and risks of long-term duration of dual antiplatelet therapy after drug-eluting stenting: A meta-analysis of randomized trials. *International Journal of Cardiology* 168: 2579-2587.

Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H and Held,C. et al. For the PLATO Investigators. (2009) Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes. *New England Journal of Medicine* 361: 1045-1057.

Weyrich AS, Dixon DA, Pabla R, Elstad MR, McIntyre TM, Prescott SM, et al. (1998) Signal-dependent translation of a regulatory protein, Bcl-3, in activated human platelets. *Proceedings of the National Academy of Sciences USA* 95: 5556-5561.

Windecker S, Kolh P, Alfonso F, Collet J, Cremer J, Falk V, et al. (2014) 2014 ESC/EACTS Guidelines on myocardial revascularization. The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) *European Heart Journal* Epub ahead of print September 2014 doi:10.1093/eurheartj/ehu278

Wiviott SD, Braunwald E, McCabe CH, Montalescot G and Ruzyllo, W. et al. For the TRITON–TIMI 38 Investigators (2007) Prasugrel versus Clopidogrel in Patients with Acute Coronary Syndromes. *New England Journal of Medicine* 357: 2001-2015.

Woodward M, Lowe GDO, Francis LMA, Rumley A and Cobbe, S.M. on behalf of the CADET Study Investigators. (2004) A randomized comparison of the effects of aspirin and clopidogrel on thrombotic risk factors and C-reactive protein following myocardial infarction: the CADET trial. *Journal of Thrombosis and Haemostasis* 2: 1934-1940.

Wykrzykowska JJ, Warnholtz A, de Jaeger P, Curzen N, Oldroyd KG, Collet J-P et al. (2009) Effect of clopidogrel discontinuation at 1 year after drug eluting stent placement on soluble CD40L, P-Selectin and CRP levels: DECADES (Discontinuation Effect of Clopidogrel After Drug Eluting Stent): a multicenter, open-label study. *Journal of Thrombosis and Thrombolysis* 28: 410-417.

Yedidya I, Netzer A, Vaduganathan M, Solodky A, Kornowski R and Lev EI (2012) Clopidogrel tapering as a strategy to attenuate platelet rebound phenomenon in patients with bare-metal stents. *Journal of Thrombosis and Thrombolysis* 33: 16-21.

Yusuf S, Mehta SR, Zhao F, Gersh BJ, Commerford PJ, Blumenthal M, et al. (2001) Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *New England Journal of Medicine* 345: 494-502.