Proton pump inhibitors, genetic polymorphisms and response to clopidogrel therapy

Himawan Fernando1,2; Anthony M. Dart1,2; Karlheinz Peter1,2; James A. Shaw1,2,*
1Department of Cardiovascular Medicine, Alfred Hospital, Melbourne, Victoria, Australia; 2Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia

Summary
Clopidogrel has become part of the mainstay of therapy for acute coronary syndromes and in patients post stenting. Clopidogrel is a prodrug and is metabolised by liver enzymes, particularly CYP2C19, into its active form. A considerable proportion of patients have a poor response to clopidogrel and this may be due to several factors. Genetic polymorphisms involved in clopidogrel’s absorption, metabolism and activity at the platelet may interfere with its antiplatelet actions. Further, proton pump inhibitors (PPI) may interfere with clopidogrel’s actions by functionally reducing the ability of CYP2C19 to convert clopidogrel to its active metabolite. By attenuating clopidogrel’s actions, both polymorphisms and drug interactions may increase the risk of thrombotic events during clopidogrel therapy. This review will explore the current evidence relating to the association between PPIs, genetic polymorphisms and poor response to clopidogrel. Routine genetic testing cannot be recommended for patients receiving dual antiplatelet therapy (DAPT). However, it may have a role for patients with an episode of stent thrombosis, prior to planned high-risk stenting or major bleeding. Regarding concomitant clopidogrel and PPI therapy, it is recommended that only patients with previous gastrointestinal (GI) bleeding or multiple risk factors for GI bleeding should be prescribed gastroprotection. This is due to the uncertainty surrounding the clinical significance of this interaction given the discordant biochemical and clinical data, conflicting results from observational studies and the limitations of the COGENT study. Pantoprazole seems least likely to interact with clopidogrel and most suitable for use in patients receiving DAPT.

Keywords
Clopidogrel, proton pump inhibitors, CYP2C19, genetic polymorphism, clopidogrel resistance

Introduction
Inter-individual response to clopidogrel varies significantly and poor response has been termed clopidogrel resistance (1–3). A recent consensus guideline has defined cut-off values for several platelet function tests that represent a poor response to clopidogrel by evaluating many studies which address this topic (4). Importantly, clopidogrel resistance is clinically relevant as these values have been correlated with adverse cardiovascular outcomes (1, 5–15). Identification of factors reducing the efficacy of clopidogrel therapy is essential as a significant number of patients take dual antiplatelet therapy (DAPT) for its protective role in acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI).

In the search to investigate factors contributing to clopidogrel resistance concomitant proton-pump inhibitor (PPI) use and genetic polymorphisms have attracted significant attention from the cardiology community (see Fig. 1). This review will outline the metabolism and actions of clopidogrel followed by assessment of the impact of PPIs and polymorphisms on clopidogrel’s efficacy with a view of determining their clinical significance.

Pharmacokinetics and pharmacodynamics of clopidogrel
A small proportion of clopidogrel’s oral dose is rapidly absorbed from the intestine (16–17) (see Fig. 2). Bioavailability is affected by efflux transport proteins such as P-glycoprotein (PG1) present on intestinal luminal cells that return clopidogrel into the intestinal lumen following drug absorption (18).

Clopidogrel, a prodrug undergoes two hepatic enzyme steps to produce the active metabolite (R-130964) (see Fig. 2). The first of these steps is oxidation to form 2-Oxo-clopidogrel. This intermedi-
The active compound is then hydrolysed to form R-130964. The thiol-group of this compound binds to cysteine residues of the P2Y12 receptor resulting in irreversible receptor shape change (19–21). Thus ADP binding and activity through this receptor is inhibited.

The cytochrome P450 family, particularly CYP2C19, CYP1A2 and CYP2B6 are involved in formation of 2-Oxo-clopidogrel and CYP2C19, CYP3A4/5, CYP2C9 and CYP2B6 in conversion of this intermediate to the active metabolite (R-130964) (22, 23). At the same time, however, there are esterases within the liver that convert clopidogrel and 2-Oxo-clopidogrel to inactive metabolites (24). These competing processes are estimated to consume a significant proportion of the absorbed dose such that only a
small amount is converted to active form (25). Clopidogrel and its inactive metabolites are excreted in urine (40%) and faeces (35–60%) (16).

Inhibition of platelet aggregation with 75 mg of clopidogrel per day dose typically reaches steady-state 3–7 days after initiation (26). However, use of a loading dose leads to an earlier steady-state and antithrombotic effects 6 hours (h) following loading are comparable to 10 days of maintenance dosing (27).

Inhibition of the P2Y12 platelet receptor reduces final stages of platelet aggregation and stabilisation primarily by inhibiting activation of the glycoprotein IIb/IIIa receptor (GP IIb/IIIa). It also leads to down-regulation (phosphorylation) of intracellular signalling molecules such as vasodilator-stimulated phosphoprotein (VASP) downstream of the P2Y12 receptor, reduced expression of cellular adhesion molecules such as P-selectin, inflammatory markers such as CD40L and platelet-leukocyte interactions (28–32). Adenosine diphosphate (ADP)'s role in amplifying platelet activation through other agonists is also inhibited (32, 33). Factors that reduce the amount of active metabolite formed may therefore increase post-treatment platelet reactivity (contribute to clopidogrel resistance) and lead to increased adverse outcomes.

### Genetic polymorphisms and clopidogrel

Polymorphisms of genes involved in the absorption (ABCB1), metabolism (CYP2C9, CYP2C19, CYP3A4/5) and action of clopidogrel at the P2Y12 platelet receptor (P2RY12, ITGB3) have been studied extensively. However, conflicting evidence regarding effect on clopidogrel's pharmacodynamic and pharmacokinetic effects (35, 51–52) has led to ongoing debate on the clinical significance of these genetic variations. Given the paucity of studies assessing clinical outcomes and conflicting biochemical studies, further study is required particularly through larger preferably prospective cohort studies.

### Table 1: CYP2C19 allele combinations and their corresponding phenotypes.

<table>
<thead>
<tr>
<th>Phenotype (metabolism)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra-metaboliser</td>
<td>Homozygous *17</td>
</tr>
<tr>
<td></td>
<td>17/*1a</td>
</tr>
<tr>
<td>Extensive-metaboliser</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Intermediate-metaboliser</td>
<td>*1 paired with *2/*3 or rarer reduced function allele</td>
</tr>
<tr>
<td>Poor-metaboliser</td>
<td>Combination of alleles except *17 and *1</td>
</tr>
</tbody>
</table>

*aThere is speculation regarding whether the combination. 17/*1 should be classified as ultra or extensive metaboliser phenotype (37).*

### Table 2: Polymorphisms without clear association with adverse clinical outcomes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Mechanism of impaired clopidogrel activity</th>
<th>Findings</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>Codes for cytochrome CYP2C9 involved in hepatic conversion of 2-Oxo-clopidogrel to its active metabolite R-130964</td>
<td>Reduced function leads to reduced conversion from clopidogrel to R-130964</td>
<td>*3 allele associated with an increased risk of subacute stent thrombosis in a case study (50) but not in Mega et al.’s study possibly due to lesser events (35). Conflicting evidence regarding effect on clopidogrel’s pharmacodynamic and pharmacokinetic effects (35, 51–52)</td>
<td>Given the paucity of studies assessing clinical outcomes and conflicting biochemical studies, further study is required particularly through larger preferably prospective cohort studies</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>Codes for Cytochrome CYP3A4/5 involved in hepatic conversion of 2-Oxo-clopidogrel to R-130964 active metabolite</td>
<td>Reduced function leads to reduced conversion from clopidogrel to R-130964. CYP3A4 has a higher prevalence amongst Caucasians compared to CYP3A5 which has a higher prevalence amongst African Americans.</td>
<td>Most studies have not found an association between CYP3A4 polymorphisms and reduced active metabolite concentrations of clopidogrel or increased adverse outcomes (53–54), only one study has found a positive association with biochemical activity only and this may have been due to CYP2C19 allele variations within the group (not tested) (55). CYP3A5 polymorphisms have only been associated with increased platelet reactivity post-clopidogrel in the presence of CYP3A4 substrates/ inhibitors but no association with adverse clinical outcomes has been found (47, 56).</td>
<td>Lack of clinical significance suggests effect on clopidogrel’s pharmacodynamic action is not enough to lead to adverse outcomes</td>
</tr>
<tr>
<td>P2RY12</td>
<td>Codes for the P2Y12 receptor</td>
<td>Increased function leading to increased platelet activity in response to ADP resulting in higher baseline and post-clopidogrel reactivity</td>
<td>Conflicting results regarding relationship between polymorphisms and increased post-clopidogrel platelet reactivity but no association found with increased adverse outcomes (32, 47, 57)</td>
<td></td>
</tr>
<tr>
<td>ITGB3</td>
<td>Codes for GP IIb/IIIa receptor</td>
<td>PIA2 polymorphism associated with increased activity of receptor</td>
<td>Increased baseline platelet reactivity and reduced response to clopidogrel in patients with polymorphism – conflicting data regarding whether clinically relevant however prospective data suggest no correlation (47, 58–60)</td>
<td></td>
</tr>
</tbody>
</table>
identified as causes of reduced response to clopidogrel. However, there is uncertainty regarding which polymorphisms are clinically significant and the role of genetic testing in clinical practice.

There are over 20 alleles currently identified (34) for CYP2C19 of which combinations have been categorised into ultra, extensive, intermediate and poor metaboliser phenotypes (see Table 1) (35). The allele *1 represents the normal-functioning wild type whilst *17 denotes a gain-of-function allele. Importantly, the *17 allele has been shown to increase the risk of bleeding (36) and has a prevalence ranging from approximately 15–30% (37, 38) in Caucasians.

The most common alleles associated with poor metabolism are the *2 and *3 alleles. Mega et al. reported carriage of *2 allele in 95% of patients with a poor metaboliser phenotype (35). Recently, one meta-analysis and three analyses of large randomised studies found conflicting results relating to the clinical significance of loss of function CYP2C19 alleles (39–42). Mega et al.’s meta-analysis found that when data was pooled, patients heterozygous and homozygous for loss of function alleles were at greater risk of cardiovascular disease and myocardial infarction (MI) (40). The authors mentioned that when pooled, over 90% of patients had PCI. In contrast, Pare et al.’s analysis of both CURE and ACTIVE-A studies did not find any association between increased adverse outcomes and CYP2C19 loss-of-function alleles (39). This may be due to the comparison of loss-of-function allele carriers taking clopidogrel to placebo, which showed that carriers still had improved clinical outcomes compared to placebo. Also, greater proportions of patients in the CURE and ACTIVE-A studies were treated without PCI where the quantitative benefit of clopidogrel therapy in reducing adverse events is comparatively less (43–45). Studies by both Wallentin et al. and Mega et al. analysing results from the PLATO genetic sub-study supported these results finding that patients with the TT genotype had a higher incidence of ischaemic events when taking clopidogrel but not ticagrelor (42). However, this conflicts with previous data which suggests that the C3435T allele was associated with reduced active metabolite concentrations and adverse clinical outcomes only in the presence of CYP2C19 loss-of-function polymorphisms (46, 47).

Current evidence suggests that CYP3A4/5, P2RY12 and ITGB3 receptor polymorphisms are not clinically relevant, however further information is required for CYP2C9 polymorphisms (see Table 2). Finally, whilst platelet adrenergic receptor activity may be associated with clopidogrel resistance (48), a recent study did not find an association between platelet adrenergic receptor polymorphisms and high post-clopidogrel platelet reactivity (49).

Studies suggest CYP2C19 and ABCB1 polymorphisms may be clinically relevant. However, we are awaiting the results of current studies designed to determine whether there is a clinical benefit of genetic analysis guided management before it can be recommended routinely for patients receiving DAPT.

There are also several barriers to routine genotyping of patients requiring DAPT. Since clopidogrel resistance is multifactorial (see Fig. 1), a positive or negative genotype result may not explain a patient’s response to clopidogrel. Further, as point-of-care genetic testing is not commercially available and cannot be used to assist decision making in the acute care setting where risk of thrombotic events is greater (42). As genetic testing is not subsidised, the cost-effectiveness of testing when newer antiplatelet agents are available (that are not affected by these polymorphisms) (41, 42, 51, 61) needs to be considered.

Thus from currently available data we recommend undertaking genetic testing in patients with an episode of stent thrombosis to evaluate a possible cause for treatment failure. Further, genotyping may be appropriate in patients with planned PCI procedures where stent thrombosis would be catastrophic such as left main stenting. In these cases, identification of poor metabolisers may warrant use of newer antiplatelet agents such as prasugrel and ticagrelor. Finally, there may be a role for genetic testing in patients with major bleeding events (to determine presence of *17 allele). These patients may benefit from use of ticagrelor (when available) as the PLATO genetic sub-study demonstrated reduction in bleeding events compared to clopidogrel use in this group (42).

To add further complexity, platelet function tests may be used to guide clinical management as well. A recent study by Bonello et al. reported that dose adjustment based on platelet function testing may be more useful in improving outcomes than genetic analysis (62). In addition, platelet function testing can be used to evaluate...
changes in management such as dose increment or changeover onto newer antiplatelet agents (through serial measurement). The most recent consensus guidelines regarding platelet function testing suggest that platelet testing may be used in patients with an adverse event (i.e. stent thrombosis) or prior to a high-risk PCI procedure but not for routine use (4).

The ongoing TARGET-PCI study may assist in answering the question of whether genotyping and platelet function testing (VerifyNow) can be used to stratify patients requiring PCI that are more likely to have clopidogrel resistance and thus benefit from prasugrel therapy (63). Alternatively, RAPID GENE uses CYP2C19 genotyping to randomise *2 carriers to routine therapy or prasugrel and uses platelet function determined by VerifyNow as the primary outcome and MACE as the secondary outcome (64). This will test the ability of VerifyNow to predict adverse outcomes in this population. Alternatively, the GIANT study uses genotyping as an advisory tool only, leaving individual treatment decisions to the treating physicians (65). It also uses the VerifyNow assay to test patient compliance one year following initiation of treatment plan. Hence, this study aims to assess another use for platelet function testing.

These studies will help assess the clinical utility of CYP2C19 testing and the varied uses of the VerifyNow assay. However, combination of genetic and platelet function testing to guide clinical management cannot be recommended currently due to paucity of evidence and is not supported by current myocardial revascularisation guidelines (66). Future studies should also assess the clinical utility of ABCB1 genetic testing.

**Drug interaction with proton-pump inhibitors**

Proton-pump inhibitors (PPIs) are recommended as concomitant gastroprotective therapy in patients receiving DAPT (67). Of concern are the clinical implications of a drug interaction due to metabolism by common hepatic enzyme pathways (26, 68).

**Pharmacokinetics of PPIs and proposed mechanism of interaction**

PPIs are rapidly absorbed after oral administration (69). The CYP2C19, CYP3A4 and CYP2C9 cytochrome P450 enzymes play major roles in the metabolism of PPIs which act as both a substrate and an inhibitor. The proportional role of each enzyme in drug metabolism varies between different PPIs (see Fig. 3). Metabolism by CYP2C19 is particularly important for omeprazole, lansoprazole and esomeprazole (68–70). Lansoprazole and omeprazole are also potent inhibitors of CYP2C19 (68–70). Despite esomeprazole being a structural isomer of omeprazole it is metabolised to a greater extent through CYP3A4 and less through CYP2C19 (69–72). However, repeated dosing of esomeprazole may have inhibitory effects on activity of CYP2C19 more than a single dose due to slower elimination (68). Pantoprazole is metabolised by CYP2C19 and CYP3A4 but has a low affinity for these enzymes compared to other PPIs. Further, following metabolism through the cytochrome pathways, the metabolite formed undergoes phase II biotransformation through sulphate conjugation in the cytosol, independent of the above enzymes (68). The cytochrome independent second step is hypothesised to reduce the potential for interaction with other medications metabolised through the CYP450 enzyme pathways. Rabeprazole is mainly metabolised through cytochrome P450-independent pathways (68).

Competition for metabolism through CYP2C19 is a theoretical mechanism by which clopidogrel’s antiplatelet action can be attenuated by concurrent PPI use. Given this hypothesis and taking into account the strong affinity of omeprazole, lansoprazole and esomeprazole for the CYP2C19 enzyme, these PPIs may have a greater potential for interaction with clopidogrel compared with pantoprazole and rabeprazole (70).

**Studies assessing interaction between clopidogrel and PPIs**

Gilard et al. published a study in 2008 verifying the results of their previous observational study in 2006 (73) by demonstrating a statistically significant increase in platelet reactivity as determined by VASP assay in patients taking omeprazole and clopidogrel (74). This is a flow cytometry assay utilising whole blood dependant on P2Y12 receptor activity (75) that is standardised, reproducible, used to define poor response to clopidogrel, correlated with other platelet function tests (e.g. light transmittance aggregometry [LTA], multiple electrode aggregometry [MEA] and VerifyNow) and reported to predict clinical outcomes (2, 6, 12, 76–83).

Reduced biochemical response to clopidogrel (measured by LTA) was also seen in subjects taking lansoprazole in the 2008 randomised study by Small et al. (84). LTA is the gold standard measure of platelet function, it is sensitive to P2Y12 receptor blockade, has been used in assessing efficacy of clopidogrel and has been correlated with clinical outcomes (14, 85–88).

Follow-on studies using differing tests of platelet function confirmed biochemical attenuation of clopidogrel’s action with concomitant omeprazole therapy (89–92). Whether this interaction extended to all PPIs or only those metabolised primarily through the cytochrome CYP2C19 pathway was a new question that emerged.

**Follow-on studies assessing whether interaction applies to PPIs as a class**

Two observational studies failed to show an interaction between clopidogrel and both pantoprazole or esomeprazole, one using VASP and MEA and MEA alone, respectively, to test platelet function (89, 93). MEA is a point-of-care test based on impedance ag-
gregometry. It correlates well with other platelet assays (LTA, VASP, VerifyNow, PFA-100, Cone and Platelet Analyser), has been used to identify high platelet reactivity, predict clinical outcomes and has good sensitivity and reproducibility (94–100).

The studies by Sibbing et al. and Silber-Matula et al. were well designed, evaluated multiple PPIs and used validated tests of platelet function (89, 93). Cuis et al.’s randomised study provided additional support to these studies by demonstrating that omeprazole attenuated clopidogrel’s actions significantly however pantoprazole did not according to the VASP assay (90).

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The strength of Cuisset et al.’s study design provides reassurance that pantoprazole does not interact with clopidogrel at doses used for gastroprotection. The lesser interaction profile of pantoprazole given its pharmacokinetic properties also supports the pharmacodynamic findings. Thus, follow-on studies assessing whether the biochemical interaction extends to all PPIs have found important results suggesting the interaction is not a class effect. Hence, biochemical studies suggest that certain PPIs may be safer than omeprazole given lesser pharmacokinetic and pharmacodynamic interaction potentials (101).

Due to the concern of a clinically relevant interaction, alternative means of avoiding a biochemical interaction were sought. Biochemical studies assessed whether higher doses of clopidogrel (150 mg per day) or staggered dosing (separation of clopidogrel and PPI dose by 12 h) reduced or avoided the interaction. However, neither strategy is promising as Cuisset et al. demonstrated that use of 600 mg loading and 150 mg maintenance therapy for 30 days did not overcome an interaction with omeprazole (20 mg daily) (90). Further Ferreiro et al. investigated the effect on the interaction of separating the omeprazole and clopidogrel maintenance doses by 8–12 h but found no significant effect on the interaction with this regime compared to taking tablets at the same time (102). Instead, there is some evidence to suggest that the dose of PPI influences the magnitude of the interaction as an 80 mg daily dose of omeprazole resulted in higher post-clopidogrel platelet reactivity compared with a 40 or 20 mg dose (74, 102, 103).

### Studies assessing clinical outcome

Whilst many studies have assessed this interaction, comparatively few have been published as full papers. Of all full papers published, seven suggest that concomitant PPIs increase the risk of adverse events whilst seven do not (104–117).

Notable early studies by Ho et al. and Juurlink et al. reported an increased risk of adverse outcomes following PPI use (108, 109). This risk remained following multivariable logistic regression for changes in baseline characteristics and sensitivity analyses. Whilst these study designs cannot prove causation due to the observational nature of the studies, they are rigorous enough to warrant further investigation.

Rassen et al. also undertook a large multicentre study to assess this interaction (111). The study used high-density propensity score adjustment (HD-PS) which has been shown to improve accuracy (118). Prior to this adjustment technique, the results of the

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Unadjusted OR (CI)</th>
<th>Multivariable adjusted OR (CI)</th>
<th>High-density propensity score matching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juurlink et al. (109) Reinfarction and current PPI use</td>
<td>1.32 (1.08–1.62)</td>
<td>1.27 (1.03–1.57)</td>
<td>N/A</td>
</tr>
<tr>
<td>Ho et al. (108) Death or rehospitalisation for ACS</td>
<td>1.62 (1.45–1.8)</td>
<td>1.25 (1.11–1.41)</td>
<td>N/A</td>
</tr>
<tr>
<td>Rassen et al. (111) AMI or death</td>
<td>1.74 (1.44–2.1)</td>
<td>1.32 (1.08–1.61)</td>
<td>1.22 (0.99–1.51)</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; CI, confidence interval; OR, odds ratio; PPI, proton pump inhibitor.

Table 3: Results for primary endpoint before and after adjustment techniques for three observational studies.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Unadjusted OR (CI)</th>
<th>Propensity score matched OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ray et al. (112) AMI, stroke, CV death and sudden cardiac death</td>
<td>-</td>
<td>0.99 (0.82–1.19)</td>
</tr>
<tr>
<td>O’Donoghue et al. (110) CV death, non-fatal MI/Stroke</td>
<td>0.98 (0.84–1.14)</td>
<td>0.94 (0.8–1.11)</td>
</tr>
<tr>
<td>Tentzeris et al. (116) All-cause mortality</td>
<td>0.915 (0.42–1.99)</td>
<td>0.776 (0.34–1.761)</td>
</tr>
<tr>
<td>Stockl et al. (115) Rehospitalisation for AMI or PCI following AMI</td>
<td>1.94 (1.06–3.54)</td>
<td>1.93 (1.05–3.54)</td>
</tr>
<tr>
<td>Simon et al. (114) Stroke, MI, all cause mortality</td>
<td>-</td>
<td>1.24 (0.87–1.78)</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction; PCI, percutaneous coronary intervention; OR, odds ratio.

Table 4: Results for five studies utilising propensity score adjustment to evaluate clinical endpoints.
two above studies and Rassen et al.’s study after adjustment by multivariable logistic regression (which was conducted in all three studies) were very similar for primary endpoints (which were also comparable) (see Table 3). However, following adjustment for 400 potential confounding factors and differences between the study centres, the confidence interval for Rassen et al.’s primary endpoint extended just below significance (0.99–1.51) (111). The authors compared the results post multivariable logistic regression and HD-PS adjustment to suggest that the increased risk seen in the studies by Juurlink et al. and Ho et al. studies is not related to PPI use but due to residual confounding factors present in the cohort taking PPIs. Rassen et al.’s conclusion relied on the assumption that HD-PS matching was able to adjust for confounding that remains after multivariable adjustment. Whilst the validity of this technique has not been rigorously tested, the greater prevalence of comorbidities in patients taking PPIs became an alternate explanation for increased risk of adverse outcomes in this group.

In assessing whether the increased risk of adverse outcomes in patients taking PPIs was due to comorbidities or PPI use, three recent studies assessed the endpoint of stent thrombosis (106, 113, 116). All three studies did not identify an increased risk among patients taking PPIs; however, the small number of events in each group may limit interpretation of these results. Whilst Saroff et al.’s study found no increased risk of definite stent thrombosis (primary endpoint), the secondary endpoints of death and combined death/definite stent thrombosis were significantly higher in patients taking PPIs (after multivariable adjustment) (113). Furthermore, patients taking PPIs had greater comorbidities at baseline (age, previous MI, high body mass index, gastrointestinal disease, diabetes). From these results, the authors suggested that the increased risk of death was likely due to greater comorbidities and that multivariable adjustment was unable to correct for it. Hence, this may apply to the previous studies of Juurlink et al. and Ho et al. (108, 109) which mirrored this scenario but assessed endpoints more vulnerable to confounding such as major adverse cardiovascular events (MACE).

To manage the effects of confounding factors in observational studies, five recent studies undertook propensity score analysis to assess whether the increased risk of adverse outcomes with PPIs remained following stronger adjustment techniques. There were also higher proportions of pantoprazole use in most studies (110, 112, 115, 116). Four studies showed no association with adverse clinical outcomes for any PPI (110, 112, 114, 116) whilst one study found an increased risk (115) (see Table 4).

Tentzeris et al. and Ray et al.’s studies, which did not identify an association between PPI use and increased adverse outcomes (112, 116), proposed that the lack of interaction may be due to a reduced sample size taking omeprazole, suggesting pantoprazole (taken by majority of subjects) may not be associated with increased risk. Whilst the sample sizes taking omeprazole, lansoprazole and esomeprazole were small and possibly underpowered in three negative studies and pantoprazole underpowered in Simon et al.’s study, all did not find an increased risk with any specific PPI.

However, Stockel et al.’s study found an increased risk after propensity score adjustment for all PPIs including the subgroup of patients taking pantoprazole (results presented in Table 4).

Most recently, Simon et al.’s study noted that patients with two loss-of-function CYP2C19 alleles taking PPIs may be at increased risk of adverse events; however, this needs investigation with a larger sample size.

Four negative propensity score adjusted studies provide some evidence for lack of a clinically significant interaction and lack of an interaction in post-hoc analyses of the TRITON-TIMI 38 and PLATO randomised studies is also reassuring (110, 119). However, all observational studies have inherent weaknesses that limit their ability to provide a definitive answer. Therefore, there is a need for prospective data to address this issue.

Prospective data relating to interaction

In 2007, Bhatt et al. undertook the COGENT study to measure gastrointestinal and cardiovascular outcomes in patients receiving a new combination pill containing clopidogrel and omeprazole (104). It should be noted that the study was due to end once 143 gastrointestinal events occurred; however, a similar safety endpoint or sample size calculation was not undertaken for the cardiovascular endpoint. Before completion of recruitment and follow-up, the study ended prematurely due to loss of funding. Due to the abrupt cessation of the study, median follow up was 106 days, less than the planned 180 day follow-up. Analysis showed a significant reduction in gastrointestinal events with the combination of clopidogrel and omeprazole but no significant increase in cardiovascular events between treatment and placebo groups. However, the number of MACE (MI, stroke, death from cardiovascular causes) was small (total 43) and there was a higher number of events in the omeprazole group (23) vs. placebo group (20) (hazard ratio [HR] = 1.15, not statistically significant) (120).

We suggest that the study is significantly underpowered to evaluate a clinically meaningful interaction. With the sample size of the current study and the observed adverse cardiovascular events rate in the control group, an increased event rate of at least 100% would be needed in the PPI group for the difference in risk to reach statistical significance. Therefore, we believe the COGENT study does not exclude a potentially significant clinical interaction particularly in patients with acute coronary syndromes (which represented less than half of the sample size) where clopidogrel is most effective in reducing cardiovascular events (44, 121, 122).

As a result, future randomised studies completed per-protocol with power calculation to identify cardiovascular risk are needed to determine if this interaction affects clinical outcome.

Meta-analysis of studies assessing clinical outcome

A recent meta-analysis and systematic review by Siller-Matula et al. aimed to address whether the combination of clopidogrel and PPI increases the risk of adverse events by utilising the collective data from 25 studies with patient outcome data (123).
A statistically significant increased risk of both MACE and MI were seen with PPI use when data from the studies were combined. PPI use was associated with a 29% and 31% relative risk increase of MACE and MI, respectively. Furthermore, whilst trending but not significant, PPI use was associated with an almost two-fold increased risk of stent thrombosis. Interestingly, subgroup analysis for specific PPIs found that neither pantoprazole nor omeprazole use was associated with a significantly higher risk of MACE compared to no PPI. In addition, all-cause mortality was not affected by PPI use.

These results are similar to a previous meta-analysis by Kwok et al. where an association between PPIs and increased risk of MACE, MI but not all-cause mortality was found. Siller-Matula et al. suggest that this may be explained by reduced risk of death through avoidance of gastrointestinal bleed in the PPI group balanced by increased risk of cardiovascular death. Both authors note considerable heterogeneity between studies; however, Siller-Matula et al. found through sensitivity analysis that the direction of effect was unchanged regardless of whether the study was small or large, an abstract or full paper. Hence, even collectively, the evidence is insufficient to assist in clarifying whether a biochemical interaction between clopidogrel and PPIs translates to increased adverse outcomes.

**Other drug interactions with clopidogrel**

As CYP2C19 and CYP3A4 are major enzyme pathways of drug metabolism, several other medications are metabolised through this pathway and therefore the potential for other drug interactions exist. Propanolol and warfarin are both metabolised by CYP2C19 whilst some lipophilic statins, particularly atorvastatin, are metabolised by CYP3A4. The latter has been evaluated by studies assessing biochemical and clinical endpoints. Whilst initially there was concern regarding a clinically significant interaction, follow-on prospective studies did not identify a biochemical or clinically significant interaction (124–128). A significant biochemical interaction was reported between clopidogrel and phenprocoumon (129) where competition at CYP3A4 and CYP2C9 hepatic enzymes is the proposed mechanism of interaction. Further, calcium-channel blockers were seen to interact with clopidogrel potentially by inhibiting CYP3A4 enzyme activity (130, 131). Of note, Siller-Matula et al. reported an increased risk of adverse clinical outcomes due to the combination of calcium-channel blockers and clopidogrel (130). In contrast, studies have not identified an interaction between clopidogrel and theophylline (132), digoxin (133), rifampicin (134), erythromycin (134), troleandomycin (134) and donepezil (135).

**Newer antiplatelet agents**

The preliminary results of the GRAVITAS study demonstrated that double-dose clopidogrel in patients with clopidogrel resistance did not significantly reduce adverse outcomes over six months follow-up (136). The results increasingly suggest that newer antiplatelet agents may hold the key to effectively managing patients with clopidogrel resistance.

Prasugrel, the newest thienopyridine, has not been shown to interact with PPIs in initial biochemical or clinical studies (84, 110). Like clopidogrel, prasugrel is a prodrug and is metabolised through the hepatic cytochrome P450 enzyme system to its active metabolite which inhibits the P2Y12 receptor (137). However, as it is converted to its intermediate metabolite through intestinal enzyme pathways, only one hepatic enzyme step is required for active metabolite formation (26, 138). Therefore, at a mechanistic level, prasugrel has a lower reliance on CYP2C19 and a lower potential for interaction with PPIs. Additionally, antiplatelet actions of prasugrel are less vulnerable to loss-of-function polymorphisms involved in its absorption, metabolism and activity at the platelet (51). The differing pharmacokinetics between prasugrel and clopidogrel lead to increased percentage of oral dose converted to active metabolite and greater, faster and more consistent platelet inhibition amongst individuals (26, 110, 139, 140).

In comparison, ticagrelor differs from the thienopyridines due to its ability to reversibly antagonise the P2Y12 receptor. Further, as it is absorbed in active form and does not require hepatic metabolism, drug interactions or genetic polymorphisms involving CYP450 enzymes are not expected to interfere with ticagrelor’s antiplatelet actions (141). When compared to clopidogrel, it has a faster onset of action and earlier peak antiplatelet effect (141). Additionally, response to ticagrelor is more consistent amongst individuals and one study showed that clopidogrel non-responders achieved therapeutic platelet inhibition when switched to ticagrelor (142).

The pharmacokinetic properties of these agents suggest that neither prasugrel nor ticagrelor are likely to be affected by genetic polymorphisms or interact with PPIs. Hence, these medications are expected to provide an important solution to managing these two factors. The TARGET-PCI and RAPID-GENE study results will be useful in determining if change of treatment from clopidogrel (in patients more likely to have clopidogrel resistance) to prasugrel or ticagrelor will improve outcomes.

**Conclusion**

Current evidence indicates that polymorphisms of the CYP3A4/5, P2RY12 and ITGB3 are unlikely to be of clinical significance; however, further research is required for CYP2C9. In contrast, CYP2C19 and ABCB1 polymorphisms have been associated with increased adverse outcomes. Whilst genotyping for CYP2C19 and ABCB1 may be clinically useful in patients with an episode of stent thrombosis, major bleeding or planned high-risk stenting (left-main), it cannot be recommended for routine clinical practice based on current evidence. Ongoing studies may assist in answering this question in the near future.
Biochemical studies suggest that an interaction exists between omeprazole, lansoprazole and clopidogrel but not for esomeprazole and pantoprazole.

The observational studies assessing clinical outcomes provide conflicting results despite use of similar methodology and adjustment techniques. Hence, whilst an interaction between clopidogrel and omeprazole has been identified by all biochemical studies assessing these medications, some studies suggest there is increased clinical risk whilst others do not (110). Similarly, some clinical studies suggest that concomitant pantoprazole use is associated with increased risk of clinical outcomes despite lack of biochemical interaction. Given the disparity between pharmacokinetic, pharmacodynamic and clinical studies for individual PPIs and the lack of an adequately powered prospective clinical study, the presence and magnitude of a clinically relevant interaction between clopidogrel and PPIs is unknown.

Using the current level of evidence, including the recently published COGENT study, which cannot exclude a clinically significant interaction, we support the American Heart Association and European Society of Cardiology (66, 143) recommendations that only patients with a history of upper gastrointestinal bleeding or European Medicines Agency position statements suggesting use of DAPT. As specific PPIs are not recommended or cautioned against, we agree with the U.S. Food and Drug Administration and European Medicines Agency position statements suggesting use of pantoprazole instead of omeprazole as it may be the safest PPI to use from a biochemical perspective (144).

Ongoing studies assessing the utility of genetic and platelet function testing in improving response to clopidogrel are also much anticipated and newer antiplatelet agents are expected to play a key role in managing clopidogrel resistance.

Conflict of interest
None declared.

References
22. Savi P, Combalbert J, Gaich C, et al. The antiaggregating activity of clopidogrel is much anticipated and newer antiplatelet agents are expected to play a key role in managing clopidogrel resistance.

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