Implementing pharmacogenetics to personalize antiplatelet therapy after myocardial infarction

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Part I: Introduction

General introduction and thesis outline

Antiplatelet agents are prescribed to reduce blood platelet aggregation. This plays an important role in occlusion of coronary vessels. It is recommended that patients with an acute coronary syndrome (ACS) and patients undergoing percutaneous coronary intervention (PCI) are treated with dual antiplatelet therapy (DAPT) consisting of aspirin and a P2Y₁₂ inhibitor [1,2]. DAPT has proven to reduce atherothrombotic event rates such as stent thrombosis and recurrent myocardial infarction as compared to aspirin monotherapy in the first months and year(s) after an ACS or PCI [3]. The antithrombotic effect of aspirin is mediated by the irreversible inhibition of platelet cyclooxygenase-1, which leads to inhibition of Thromboxane A₂ synthesis. Thromboxane A₂ is part of the platelet activation pathway that leads to thrombus formation. Several different oral P2Y₁₂ inhibitors are prescribed, but they all work by binding to the P2Y₁₂ receptor. This prevents binding of adenosine diphosphate to the P2Y₁₂ receptor, which is an important mediator in platelet aggregation.

P2Y₁₂ inhibitors

Clopidogrel

Clopidogrel is a prodrug. It is converted into its active metabolite by cytochrome P450 isoenzymes. After metabolizing, it irreversibly binds to the P2Y₁₂ receptor. The most important enzyme in the two-step conversion of clopidogrel is the CYP2C19 enzyme. The CYP2C19 gene encodes this enzyme. Many different polymorphisms of the *CYP2C19* gene have been identified, but most are rare [4]. The *CYP2C19**1 allele is considered the normal function allele, while the *17 allele is the most common polymorphism. The *17 allele is considered to be a gain-of-function allele by some, because it would lead to increased platelet inhibition as compared to the *1 allele. However, data on this effect is conflicting and an effect on clinical outcomes has not been well-established [4-5]. The *2 is the most common allele leading to a reduced function (loss-of-function), while the *3 loss-of-function allele is quite common in some populations (mostly Asian) and rare in others (American) [4]. Therefore, these two polymorphisms are the most clinically relevant.

Approximately one-third of patients treated with clopidogrel have high on-treatment platelet reactivity when measured with platelet function tests [6]. This is associated with an increased risk of experiencing atherothrombotic events [6]. Carrying *CYP2C19* loss-of-function alleles is one of the most important risk factors for high on-treatment platelet reactivity [7-9]. This has prompted the United States Food and Drug Administration (FDA) to issue a boxed warning that in people carrying two loss-of-function alleles an alternative for clopidogrel should be considered [10]. Testing for the

presence of *CYP2C19* loss-of-function alleles could help identify those patients who should not be treated with clopidogrel, but with one of the other P2Y₁₂ inhibitors instead.

Prasugrel and ticagrelor

Prasugrel is a prodrug, which irreversibly binds to the P2Y₁₂ receptor, like clopidogrel, but its effect is not affected by *CYP2C19* polymorphisms [11]. Ticagrelor is a direct-acting P2Y₁₂ inhibitor and is therefore not dependent on *CYP2C19* metabolism [12]. Both drugs have demonstrated to be more potent platelet inhibitors and have a more consistent pharmacodynamic effect than clopidogrel [13-14].

Initially, after publication of the prospective Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial, clopidogrel became the recommended P2Y₁₂ inhibitor in addition to aspirin [3]. The introduction of prasugrel and ticagrelor a few years later led to even lower levels of platelet aggregation, thus preventing even more atherothrombotic events [13-14]. Due to the reduction in atherothrombotic events, both American and European guidelines recommend the use of ticagrelor and prasugrel instead of clopidogrel in patients with an ACS, unless there is a contraindication for these drugs. [1,2,15]. However, more intensified platelet inhibition does not only decrease the number of atherothrombotic events, but inevitably also leads to more bleeding events [13-14]. Bleeding is associated with a reduction in quality of life, extra healthcare costs and mortality [16]. Therefore, it is important to not only prevent atherothrombotic events, but bleeding events as well.

De-escalation of antiplatelet therapy

The highest risk of recurrent atherothrombotic events is seen early after an ACS, while the bleeding risk remains consistent during the period a P2Y₁₂ inhibitor is used [13-14]. This has prompted a theory that switching from the stronger P2Y₁₂ inhibitors ticagrelor or prasugrel to clopidogrel in the period after an ACS could reduce the overall bleeding risk, without increasing the thrombotic risk. This concept is called de-escalation. Three different strategies have been investigated to test this theory. A more detailed description is provided later in this thesis, but the three different strategies are unguided de-escalation (switching without using any test), platelet function testing-guided de-escalation and *CYP2C19* genotype-guided de-escalation [16].

Switching between different P2Y₁₂ inhibitors

Switching between P2Y₁₂ inhibitors is common in clinical practice, either as part of a conscious strategy, due to concomitant therapies, side effects or for socio-economic reasons [16,17]. Since the different P2Y₁₂ inhibitors have different pharmacological properties, concerns have been raised for drug-drug interactions (DDIs). A DDI is defined as a modification of the effect of a drug when administered with another drug. In the case of P2Y₁₂ inhibitors, a DDI could lead to inadequate platelet inhibition and an increased risk of atherothrombotic complications when switching. Based on pharmacodynamic and pharmacokinetic data from the different drugs, an expert consensus statement has been published to provide handles for clinicians on how to switch between P2Y₁₂ inhibitors [17]. When de-escalating from ticagrelor or prasugrel to clopidogrel, it is recommended to administer a loading dose of 600mg, 24 hours after the last dose of prasugrel or ticagrelor. When de-escalation is performed due to bleeding, instead of a loading dose, a maintenance dose of 75mg can be considered 24 hours after the last dose of ticagrelor or prasugrel. However, a paucity of studies investigating the effect of different strategies on clinical outcomes still exist.

Outline of the thesis

The focus of this thesis will be on *CYP2C19* genotype-guided de-escalation and results of the *CYP2C19* Genotype-Guided Antiplatelet Therapy in ST-Segment Elevation Myocardial Infarction Patients – Patient Outcome after Primary PCI (POPular Genetics) trial. **Chapter 1** starts with an overview of the current status of *CYP2C19* genotyping. **Chapter 2** focuses on the feasibility of using *CYP2C19* genetics testing in clinical practice. It describes the time it takes until genetic results are available and reports results on both a test performed in a laboratory and a point-of-care system. **Chapter 3** provides an overview of the different strategies that can be used to de-escalate antiplatelet therapy. It presents data from trials that have studied the different strategies, provides pros and cons of the different strategies and provides handles on when and how to de-escalate. In **Chapter 4** the clinical outcomes of the POPular Genetics trial are presented. It investigated the effect of a *CYP2C19* genotype-guided strategy compared to standard treatment with ticagrelor or prasugrel on atherothrombotic and bleeding events. **Chapter 5** is a sub-analysis of the POPular Genetics trial, which, instead of comparing the two different strategies, compared clopidogrel in noncarriers of *CYP2C19* loss-of-function alleles directly with ticagrelor and prasugrel. **Chapter 6** is a sub-analysis from both the POPular Genetics and POPular Age trials. It also

compares clopidogrel in noncarriers of *CYP2C19* loss-of-function alleles with ticagrelor and prasugrel in a subgroup of elderly patients with ACS. In **Chapter 7**, the cost-effectiveness of a *CYP2C19* genotype-guided strategy compared to standard treatment with ticagrelor and prasugrel is investigated. The final chapter, **Chapter 8**, describes outcomes of patients de-escalating to clopidogrel with and without administering a new loading dose.

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Genotype-guided treatment of oral P2Y₁₂ inhibitors: Where do we stand?

Daniel M.F. Claassens, Jurriën M. ten Berg
Pharmacogenomics 2020; 21:83-86

Introduction

Dual antiplatelet therapy, consisting of aspirin and a P2Y₁₂ inhibitor, remains the cornerstone of treatment in preventing recurrent thrombotic events such as stent thrombosis or spontaneous myocardial infarction (MI) after percutaneous coronary intervention (PCI) or acute coronary syndrome (ACS). In patients with ACS, the guidelines favour the stronger P2Y₁₂ inhibitors ticagrelor or prasugrel over weaker clopidogrel [1,2]. This recommendation is based on the Platelet Inhibition and Patient Outcomes (PLATO) trial which demonstrated ticagrelor was superior in reducing MI and death as compared with clopidogrel and on the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38, which demonstrated prasugrel was superior in reducing MI as compared with clopidogrel [3,4]. However, the improved efficacy of these stronger platelet inhibitors is counterbalanced by a higher bleeding risk.

Clopidogrel is a prodrug, which is transformed into its active metabolite by hepatic cytochrome P450 enzymes. The active metabolite irreversibly inhibits the P2Y₁₂ receptor on platelets, resulting in inhibition of platelet aggregation [5]. However, clopidogrel-treated patients demonstrate a substantial individual response variability [6]. The most important gene to cause reduced clopidogrel effectiveness is the *CYP2C19* gene [7–9]. Carriers of *CYP2C19* loss-of-function (LoF) alleles are at an increased risk of thrombotic events [10–12]. This has prompted the US FDA and the European Medicines Agency to add a boxed warning to the label, describing that clopidogrel might not be effective in poor metabolizers [13].

More than 30 gene alleles have been identified [14]. The *CYP2C19* *1 is the most prevalent (60–70% of the population) and is regarded as the allele causing normal activity. *CYP2C19**2 –*8 are LoF alleles. Of these, the *CYP2C19**2 (15–35%) and, depending on the population, *3 (0.02–9%) alleles are, by far, the most common LoF alleles [15]. The *CYP2C19**17 is regarded as a gain-of-function allele by many. Based on these assumptions, patients are divided into four different phenotype categories (Table 1) [16]. However, Lewis *et al.* demonstrated that *CYP2C19**17 is not independently associated with clopidogrel response [17]. Furthermore, data on the influence of the *CYP2C19**17 allele on clinical outcomes are inconsistent and its clinical relevance is therefore questionable [15].

Despite the better efficacy of the stronger P2Y₁₂ inhibitors, clopidogrel remains the most utilized platelet inhibitor of the three [15]. The reduced

risk of bleeding and reduced costs, especially in non-western countries, probably plays an important part in this. In addition to the introduction of the stronger P2Y₁₂ inhibitors, the introduction of newer generation stents have caused a dramatic decrease in thrombotic events, which has shifted attention to the prevention of bleeding complications [18]. This has spurred on research that tries to combine the improved efficacy of the stronger P2Y₁₂ inhibitors in patients that are unable to metabolize clopidogrel with the reduced risk of bleeding in patients that react properly to clopidogrel [19–21]. This can be achieved in two ways, either by using proof from platelet function testing or from genetic testing. Furthermore, it is possible to either choose a strategy in which these tests are used to escalate a strategy from clopidogrel to the stronger P2Y₁₂ inhibitors or a de-escalation strategy from the stronger P2Y₁₂ inhibitors to clopidogrel [21].

Table 1. *CYP2C19* phenotypes based on genotypes

Phenotype	Genotypes	Examples of diplotypes
(Ultra)rapid metabolizer¥	Carriers of one or two gain-of-function alleles	*1/*17, *17/*17
Extensive metabolizer	Non-carrier of loss-of-function alleles	*1/*1
Intermediate metabolizer	Carriers of one loss-of-function allele	*1/*2, *1/*3
Poor metabolizer	Carriers of two loss-of-function alleles	*2/*2, *2/*3, *3/*3 or combinations of two other LoF alleles (*2-*8)

Table 1. ¥ Data on the existence of a gain-of-function allele are conflicting. LoF: Loss-of-function

Feasibility

There have been many observational studies and randomized trials investigating the use of platelet function testing. However, there has never been a randomized trial which demonstrated a benefit in using platelet function testing over standard treatment. Still, the latest European Society of Cardiology guidelines on myocardial revascularization give a class IIB recommendation to use platelet function testing to guide de-escalation of P2Y₁₂ inhibitor therapy in ACS patients [22]. However, there are some distinct advantages of genotyping over platelet function testing when it comes to feasibility. First, a point-of-care system like the Spartan Cube (Spartan Bioscience, Ontario, Canada) only requires a buccal swab, which is patient friendly and easy to use and returns a result in one hour [19]. Second, genetic testing can be performed at any time, either prior to elective PCI or in the Cath lab in ST-segment elevation MI patients since it does not require stable platelet reactivity measurements to provide a reliable result [23]. Third, compared with the strategy used in for instance the Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment for Acute Coronary Syndromes trial, there is no need to return to the hospital for measuring platelet reactivity. At last, only one switch of P2Y₁₂ inhibitors is necessary instead of two when patients are unresponsive to clopidogrel and have to be switched back to the potent platelet inhibitors [21].

Escalation using CYP2C19 genotype-guided treatment

Though the concept of CYP2C19 genotype-guided treatment has been around for some time, larger randomized trials evaluating the concept have been missing until recently. Results of the Reassessment of Anti-Platelet Therapy Using an Individualized Strategy Based on Genetic Evaluation trial, which included 187 patients, were already published in 2012. It demonstrated that one week after initiation of antiplatelet therapy there were significantly less patients with high on-treatment platelet reactivity in the genotype-guided group than in the standard treatment group, in which all patients received clopidogrel [24]. Since then, multiple smaller and mostly single centre trials have been performed [25]. A meta-analysis from Kheiri *et al.* showed that there was no significant difference in major adverse cardiac events and bleeding events between genotype-guided treatment and standard treatment [25]. They, however, found a significantly lower rate of MI in the genotype-guided group (Hazard ratio [HR] = 0.44; 95% CI 0.28–0.70). However, this meta-analysis only included 2371 patients and there was a lot of heterogeneity in study population and in the definitions used in the different trials. The trials in

the meta-analysis investigated escalation of antiplatelet therapy from clopidogrel to ticagrelor or prasugrel, though these platelet inhibitors were also prescribed in the standard treatment arm in a significant portion of the patients. Currently, there are two larger ongoing randomized trials also investigating escalation of antiplatelet therapy after PCI. In the Tailored Antiplatelet Therapy Following PCI (NCT 01742117), 5300 patients undergoing PCI for stable coronary artery disease and ACS are randomized to standard treatment with clopidogrel or to genetic testing of the *CYP2C19**2, *3 and *17 alleles. Carriers of the LoF alleles are treated with ticagrelor, while noncarriers receive clopidogrel. Results of this trial are expected in the first half of 2019. A second trial, conducted in Saudi Arabia (NCT 01823185), is expected to include between 1500 and 2000 patients with STEMI. Patients in the standard treatment arm receive clopidogrel, while in the genotype-guided arm the *CYP2C19**2 allele is tested. Carriers receive ticagrelor or prasugrel while noncarriers receive clopidogrel. Based on the meta-analysis, there might be an advantage in using genotyping to escalate treatment, but we have to wait for the results of these larger trials before we can draw more definitive conclusions.

De-escalation using CYP2C19 genotype-guided treatment

Though clopidogrel is still the most widely used P2Y₁₂ inhibitor, the use of the potent P2Y₁₂ inhibitors is preferred in ACS patients [1,2]. Based on this assumption the recently published CYP2C19 Genotype-Guided Antiplatelet Therapy in ST-Segment Elevation Myocardial Infarction Patients – Patient Outcome after Primary PCI (POPular Genetics) trial was conducted. Patients in the standard treatment arm received ticagrelor or prasugrel, while in the genotype-guided arm the *CYP2C19**2 and *3 alleles were assessed. Carriers were also treated with ticagrelor or prasugrel, while noncarriers received clopidogrel [19]. The trial included 2488 STEMI patients undergoing primary PCI with more than 90% of the patients in the standard treatment arm receiving ticagrelor or prasugrel and more than 60% of the patients in the genotype-guided arm receiving clopidogrel. It concluded that a genotype-guided strategy was noninferior for net clinical benefit consisting of all-cause death, recurrent MI, definite ST, stroke and PLATO major bleeding (HR = 0.87; 95% CI: 0.62–1.21, $P_{\text{non-inferiority}} < 0.001$), while it was superior in reducing combined PLATO major and minor bleeding (HR = 0.78; 95% CI: 0.61–0.98; $p = 0.04$). This reduction was mainly driven by a reduction in minor bleedings (HR = 0.72; 95% CI: 0.55–0.94), with no difference in major bleeding (HR = 0.97; 95% CI: 0.58–1.63).

Conclusion

Currently, the guidelines still recommend a one-size-fits-all approach using the more potent platelet inhibitors ticagrelor and prasugrel. However, the POPular Genetics trial demonstrated that a personalized approach using genetic testing to de-escalate to clopidogrel benefits patient outcomes and is feasible in clinical practice in a high-risk STEMI population. In the near future, other trials will provide an answer to the question whether a strategy using genotyping to escalate antiplatelet therapy will also benefit patients.

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De-escalation of antiplatelet treatment in patients with myocardial infarction who underwent percutaneous coronary intervention: a review of the current literature

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Abstract

In acute coronary syndrome (ACS) patients undergoing percutaneous coronary intervention (PCI), treatment with the P2Y₁₂ inhibitors ticagrelor or prasugrel is recommended over clopidogrel due to a better efficacy, albeit having more bleeding complication. These higher bleeding rates have provoked trials investigating de-escalation from ticagrelor or prasugrel to clopidogrel in the hope of reducing bleeding without increasing thrombotic event rates. In this review, we sought to present an overview of the major trials investigating several different options for de-escalation; unguided, platelet function testing- and genotype-guided. Based on these results, and on other established literature sources, such as guidelines and expert consensus papers, we provide an overview to help decide when and how to de-escalate antiplatelet therapy in ACS patients undergoing PCI.

Introduction

Patients with myocardial infarction and percutaneous coronary intervention (PCI) require dual antiplatelet therapy (DAPT) consisting of aspirin and a P2Y₁₂ inhibitor for at least 6 to 12 months [1,2]. In patients with myocardial infarction, potent platelet inhibition with ticagrelor or prasugrel instead of clopidogrel has been preferred by the major guidelines in the past decade. These recommendations followed after results of the Platelet Inhibition and Patient Outcomes (PLATO) and the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38) trials, demonstrated a reduction in thrombotic events in patients using ticagrelor and prasugrel compared to clopidogrel, and the U.S. Food and Drug Administration (FDA) added a boxed warning regarding the reduced effectiveness of clopidogrel in poor metabolizers [3,4]. However, the increased efficacy of the potent P2Y₁₂ inhibitors is hampered by a higher bleeding risk.

Clopidogrel is a prodrug. The active metabolite irreversibly binds to the P2Y₁₂ receptor on platelets leading to reduced platelet activation [5]. Patients on clopidogrel demonstrate a wide variability in platelet reactivity and approximately 30% of the patients have an inadequate reduction in platelet reactivity measured using platelet function testing [6]. The most important enzyme in the activation process is encoded by the CYP2C19 gene. This gene has many different alleles, some of which are considered loss-of-function alleles and can be present in more than 30% of the population [7]. Patients carrying loss-of-function alleles generally show higher residual platelet reactivity and are at an increased risk for thrombotic events [8,9].

De-escalation of antithrombotic therapy

The higher risk of bleeding in patients on potent P2Y₁₂ inhibitors remains present in the chronic treatment phase, while the greatest benefit of the potent drugs are seen early, when the risk of recurrent thrombotic events is highest [10,11]. De-escalation is the process of switching from the potent P2Y₁₂ inhibitors prasugrel or ticagrelor to weaker clopidogrel. Despite a lack of evidence supporting de-escalation, de-escalation is common in clinical practice and occurs in up to 30% of patients with myocardial infarction [12–14]. This is triggered by both clinical factors (e.g., side effects like dyspnoea or (minor) bleedings) and socioeconomic factors (e.g., higher costs of ticagrelor and prasugrel treatment) [12–14]. For instance, in the Comparison of Prasugrel and Ticagrelor in the Treatment

of Acute Myocardial Infarction (PRAGUE-18) trial, more than one-third of patients de-escalated to clopidogrel for economic reasons [12], while in the POPular AGE trial (including acute coronary syndrome (ACS) patients of 70 years and older), treatment adherence in the ticagrelor and prasugrel group was just 53% during the one year follow-up, mainly due to side effects and a perceived high bleeding risks [13].

This has prompted many observational studies investigating the effects of de-escalation of antiplatelet therapy [15], but in the last few years different randomized controlled trials have been published as well [16–18]. These trials investigated several different methods of de-escalation; unguided de-escalation, platelet function testing (PFT)-guided de-escalation and genotype-guided de-escalation. For this review, we focus on randomized controlled trials that investigate de-escalation from ticagrelor or prasugrel to clopidogrel. Results are summarized in Table 1.

Unguided de-escalation

The Timing of Platelet Inhibition After Acute Coronary Syndrome (TOPIC) trial investigated unguided de-escalation from a potent platelet inhibitor to clopidogrel 1 month after an ACS [16]. It included 646 patients, 323 randomized to de-escalation of DAPT, and 323 to continuing potent platelet inhibition. It found a significant reduction in bleeding academic research consortium (BARC) 2 and higher bleedings (4.0% vs. 14.9% for de-escalated and potent DAPT, respectively, HR 0.30, 95% confidence interval (CI) 0.18–0.50), with no difference in thrombotic events, consisting of cardiovascular death, unplanned revascularization and stroke (9.3% vs. 11.5%, HR 0.80, 95% CI 0.50–1.29). A pre-specified sub analysis of the TOPIC trial assessed the effect of on-treatment platelet reactivity (on prasugrel or ticagrelor) on clinical outcomes [19]. It found that de-escalation was superior regardless of initial platelet reactivity, but that patients classified as low on-treatment platelet reactivity had the highest risk of experiencing a clinical event (either bleeding or thrombotic) and benefited the most from de-escalation. Though these results seem very promising, several important limitations have to be considered. First, the trial had a small sample size. Second, there was no detailed reporting and external event adjudication on key endpoints and third, a difference in event rates, both thrombotic and bleeding, in favour of de-escalation was already seen prior to actual de-escalation.

The recently published HOST-REDUCE-POLYTECH-ACS was a randomized trial that investigated a different kind of de-escalation. It included 2338 East Asian patients with ACS and PCI who were treated with prasugrel 10mg daily for one month [20]. After one month, patients were randomized to either 5mg daily for 11 months or continued 10 mg daily for the rest of the year. It found the reduced dose to be superior for a combined bleeding and ischemic outcome composed of all-cause death, myocardial infarction, stent thrombosis, repeat vascularization, stroke, or bleeding academic research consortium (BARC) grade 2 bleeding or higher (7.2% vs. 10.1%, hazard ratio (HR) 0.70, 95% CI 0.52–0.92, $p = 0.012$) [20]. This was driven by a difference in BARC 2 bleeding, with no difference in ischemic outcomes. These results are promising, but there are some important limitations, mostly caused by exclusion of patient groups who cannot be treated with 10mg of prasugrel (history of transient ischemic attack/stroke, Age ≥ 75 years and weight <60 kg). The mean age was only 59 years and due to the weight restriction and the low average weight of Korean women, there were very few women ($<10\%$). Furthermore, most ischemic events were repeat revascularization, with all other ischemic endpoints having an incidence of $<1\%$. In addition, the mean body weight was 72 kg and therefore it is unsure if in other populations with much higher mean body weight, such as American and European populations, the same effect is reached. Similar to this trial, a randomized trial investigating de-escalation from a high dose to a low dose ticagrelor (90mg vs. 60mg) after 1 week until 1 year is ongoing (NCT04255602).

A meta-analysis from Angiolillo et al. pooled observational studies, which studied de-escalation of treatment from ticagrelor to clopidogrel for various reasons [15]. It found a rate of major adverse cardiac events (MACE) (defined as cardiovascular death, myocardial infarction and stroke) of 2%, a rate of cardiovascular death of 2% and of major bleeding of 1%. Contrary to these results are the results from the Switching from Clopidogrel to New Oral Antiplatelet Agents during Percutaneous Coronary Intervention (SCOPE). It found much higher rates of MACE, mostly driven by higher rates of myocardial infarction and transient ischemic attack/stroke in patients that de-escalated to clopidogrel [21]. However, patients de-escalating were significantly older and more often had a history of transient ischemic attacks and stroke. In addition, the de-escalation group was very small and it is unclear what the timing of events was relative to the timing of de-escalation.

Table 1. Overview of clinical trials

Trial	TOPIC	TROPICAL-ACS	POPular Genetics
Study size	n = 646	n = 2610	n = 2488
Population	ACS + PCI (40% STEMI)	(N)STEMI + PCI (55% STEMI)	STEMI + primary PCI (100% STEMI)
Timing of de-escalation	1 month after ACS	7 days after discharge	1–3 days after primary PCI
Method of de-escalation	Unguided	PFT-guided	Genotype-guided
Study design	Single-centre, randomized, open-label trial of unguided de-escalation vs. standard treatment	Randomized, open-label, non-inferiority trial of PFT-guided de-escalation vs. standard treatment	Randomized, open-label, non-inferiority trial of genotype-guided de-escalation vs. standard treatment
Control arm	Ticagrelor/prasugrel for 12 months	Prasugrel for 12 months	Ticagrelor/prasugrel for 12 months
Experimental arm	1 month of ticagrelor/prasugrel followed by 11 months of clopidogrel	PFT-guided de-escalation with 1 week prasugrel followed by 1 week clopidogrel, then depending on PFT results clopidogrel or prasugrel from day 14 to 12 months	CYP2C19 genotyping immediately after primary PCI. Non-carriers of LoF alleles switched to clopidogrel as soon as possible, carriers continued ticagrelor/prasugrel for 12 months
Primary endpoint	1-yr incidence of cardiovascular death, unplanned hospitalization leading to urgent coronary revascularization, stroke or BARC ≥ 2 bleeding	1-yr incidence of cardiovascular death, myocardial infarction, stroke or BARC ≥ 2 bleeding	1-yr incidence of all-cause death, myocardial infarction, definite stent thrombosis, stroke or PLATO major bleeding

Trial	TOPIC	TROPICAL-ACS	POPular Genetics
Key safety endpoint	BARC ≥2 bleeding	BARC ≥2 bleeding	PLATO major and minor bleeding
Key findings	Primary net clinical benefit endpoint (13.4% in de-escalation vs. 26.3% in control group; $p < 0.01$; HR: 0.48, 95% CI: 0.34–0.68) Thrombotic event rates of 9.3% in de-escalation vs. 11.5% in control group; $p = 0.36$ Bleeding event rates of 4.0% in de-escalation vs. 14.9% in control group; HR 0.30, 95% CI: 0.18–0.50; $p < 0.01$	Primary net clinical benefit endpoint (7.3% in de-escalation vs. 9.0% in control group; $P_{\text{noninf}} < 0.001$; HR: 0.81, 95% CI: 0.62–1.06) Thrombotic event rates of 2.5% in de-escalation vs. 3.2% in control group; HR 0.77, 95% CI: 0.48–1.21; $P_{\text{noninf}} = 0.01$ Bleeding event rates of 4.9% in de-escalation vs. 6.1% in control group; HR 0.83, 95% CI: 0.59–1.13; $p = 0.23$	Primary net clinical benefit endpoint (5.1% in de-escalation vs. 5.9% in control group; $P_{\text{noninf}} < 0.001$; HR: 0.87, 95% CI: 0.62–1.21) Thrombotic event rates of 2.7% in de-escalation vs. 3.3% in control group; HR 0.83, 95% CI: 0.53–1.31 Bleeding event rates of 9.8% in de-escalation vs. 12.5% in control group; HR 0.78, 95% CI: 0.61–0.98; $p = 0.04$
Funding	Investigator initiated trial. Funded by Hôpitaux de la Timone	Investigator initiated trial, Funded by Roche Diagnostics. Eli Lilly & Daiichi Sankyo company supported prasugrel purchase and drug delivery	Investigator initiated trial. Funded by Netherlands Organization for Health Research and Development. Spartan bioscience provided genotyping equipment for free

Table 1. Major randomized clinical trials investigating de-escalation of P2Y₁₂ inhibitor treatment in patients with ACS. ACS = acute coronary syndrome, BARC = bleeding academic research consortium, CI = confidence interval, HR = hazard ratio, noninf = non-inferiority, LoF = loss-of-function, NSTEMI = non-ST-elevation myocardial infarction, PCI = percutaneous coronary intervention, PFT = platelet function testing, PLATO = Platelet Inhibition and Patient Outcomes, STEMI = ST-elevation myocardial infarction.

Platelet function testing-guided de-escalation

There have been many trials investigating the use of PFT to guide antithrombotic therapy. However, almost all investigated escalation to stronger or more intensified antiplatelet regimes when high platelet reactivity was found [22–24]. An exception to this was the Testing Responsiveness To Platelet Inhibition On Chronic Antiplatelet Treatment For Acute Coronary Syndromes (TROPICAL-ACS) trial, which included 2610 patients and investigated de-escalation from prasugrel to clopidogrel 7 days after hospital discharge in ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) patients (all-comers ACS cohort) who underwent PCI [17]. After 1 week of clopidogrel use, PFT was performed. Patients demonstrating high platelet reactivity switched back to prasugrel, while the other patients remained on clopidogrel until 12 months after myocardial infarction. Patients in the control arm were treated with prasugrel for 12 months.

The PFT guided group was non-inferior regarding the primary net clinical benefit outcome consisting of cardiovascular death, myocardial infarction, stroke or BARC grade 2 bleeding or higher (7.3% vs. 9.0% in the PFT-guided and standard treatment group respectively, $P_{\text{non-inferiority}} < 0.001$ HR 0.81, 95%CI 0.62–1.06) [17]. The PFT guided group was also non-inferior compared to the prasugrel treated group regarding the thrombotic outcome, defined as cardiovascular death, myocardial infarction, and stroke (2.5% vs. 3.2% in the de-escalation and prasugrel group respectively, HR 0.77, 95%CI 0.48–1.21, $P_{\text{non-inferiority}} = 0.012$). Of note, the trial showed numerically lower bleeding events in the guided de-escalation arm (4.9% vs. 6.0% BARC 2 or higher bleeding for the de-escalation and prasugrel group, respectively, HR 0.82, 95%CI 0.59–1.13), but this difference failed to reach a level of statistical significance. A pre-specified sub-analysis of the trial assessed the impact of age clinical outcomes following PFT guided de-escalation [25]. It found that PFT guided de-escalation was associated with a significant reduction in the primary outcome in patients aged 70 and younger (HR 0.70, 95%CI 0.51–0.96, $p = 0.03$), while there was no difference in elderly patients (HR 1.17, 95%CI 0.69–2.01, $p = 0.56$). This effect was mainly driven by a reduction in bleeding events in younger patients, while it must be noted that the elderly group contained only 370 patients. The TROPICAL-ACS trial also had an important limitation; the non-inferiority margin was 30%, though in a post-hoc analysis non-inferiority for the primary endpoint was maintained with a non-inferiority margin of 10%. In addition, the PFT guided group had both numerically lower thrombotic and bleeding event rates than the standard treatment

group. Further limitations included, the sole use prasugrel in the control group and not ticagrelor, excluding patients with a history of stroke, the open-label design and the loss-to-follow-up of 4% in both arms.

In TROPICAL ACS, the Multiplate analyser was used to determine platelet inhibition [17]. Correlation between different PFTs is not very good and it is therefore unsure if results can be extrapolated to other PFTs than the Multiplate [6,26]. However, the guidelines do not endorse a specific PFT and thus leave the option on what PFT to use open.

Genotype-guided de-escalation

The last option to guide P2Y₁₂ inhibitor therapy is to use CYP2C19 genetic testing. Similar to PFT, most trials and observational studies investigated escalation of therapy, including the recently published Tailored Antiplatelet Therapy Following PCI (TAILOR-PCI) [27,28]. In the primary analysis of this trial, which compared clopidogrel with ticagrelor in patients with loss-of-function alleles only, ticagrelor treatment led to a 34% reduction in ischemic events, though it missed statistical significance (HR 0.66, 95%CI 0.43–1.02, $p = 0.06$). This, because the trial was powered to detect a very ambitious 50% reduction in ischemic events [28]. Still, this difference is much larger than the 16% difference seen in PLATO [3].

A trial that investigated genotype-guided de-escalation was the CYP2C19 Genotype-Guided Antiplatelet Therapy in STEMI Patients–Patient Outcome after Primary PCI (POPular Genetics) trial. It investigated de-escalation from potent P2Y₁₂ inhibitors to clopidogrel within 1 to 3 days after primary PCI in 2488 patients with ST-elevation myocardial infarction [18]. In patients randomized to the genotype-guided strategy the presence of the CYP2C19*2 and *3 allele was determined as soon as possible after primary PCI. Non-carriers of these alleles were de-escalated to clopidogrel, while carriers remained on potent platelet inhibitors. Median time from primary PCI to de-escalation was approximately 1.5 days. Patients in the control group received standard treatment with either ticagrelor or prasugrel for 12 months. The trial found that the genotype-guided group was non-inferior to the standard-treatment group regarding net clinical benefit, defined as all-cause death, myocardial infarction, definite stent thrombosis, stroke, and PLATO major bleeding (5.1% vs. 5.9% for genotype-guided and standard treatment respectively, $P_{\text{non-inferiority}} < 0.001$, HR 0.87, 95%CI 0.62–1.21). It was also non-inferior regarding the thrombotic outcome, defined as cardiovascular death, myocardial infarction, definite stent thrombosis, and stroke (2.7% vs. 3.3% for genotype-guided and standard treatment respectively, HR 0.83, 95% CI

0.53–1.31). Furthermore it found that a genotype-guided strategy was superior in reducing combined PLATO major and minor bleedings (9.8% vs. 12.5% for genotype-guided and standard treatment respectively, HR 0.78, 95% CI 0.61–0.98), which was mainly driven by a reduction in PLATO minor bleedings. Therefore, the genotype-guided therapy proved to be beneficial by reducing bleeding events, while not increasing thrombotic events. An important limitation of the POPular Genetics trial was the much lower than anticipated event rate. Since the non-inferiority margin was fixed (2%), the relative margin was much greater than expected. However, similar to the PFT guided group in the TROPICAL-ACS trial [17], patients in the genotype-guided group had numerically less thrombotic and bleeding events than patients in the standard treatment group. Other limitations included the open-label design and that more, though rare, *CYP2C19* loss-of-function alleles exist which were not tested in the trial.

Summary

Based on the currently available research, the European Society of Cardiology (ESC) guidelines provide a class IIb recommendation to use unguided, PFT, or genotyping to guide antithrombotic treatment in a subgroup of ACS patients deemed unsuitable for potent platelet inhibition [29]. An expert consensus paper concerning platelet function and genetic testing to guide P2Y₁₂ inhibitor treatment in PCI patients was published last year, prior to the results of the POPular Genetics trial. This consensus paper gives some suggestions as to when de-escalation in patients with myocardial infarction could be considered and when to escalate P2Y₁₂ inhibitor therapy (Figure 1). In general, de-escalation should be considered in patients with a high bleeding risk (Table 2). This includes prior major bleeding, prior haemorrhagic stroke, anaemia, and clinically significant bleeding on dual-antithrombotic therapy [30]. Bleeding risk scores might also be used to help decision making, though this has never been tested in a clinical trial. Furthermore, socio-economic reasons could be a factor in deciding to de-escalate therapy. If opted to de-escalate antithrombotic therapy in a patient, choosing either PFT or genetic testing both have their advantages and disadvantages (Table 3). Ultimately deciding on what strategy to use will depend on the availability of the different tests and assays, the experience and logistics in the hospital and on the country and healthcare system the hospital is located in.

Figure 1. Strategies for DAPT after PCI

Figure 1. Strategies for dual antiplatelet therapy after PCI. The majority of patients undergoing percutaneous coronary intervention (PCI) should be treated with guideline recommended dual antiplatelet therapy (DAPT) (clopidogrel in elective PCI and ticagrelor or prasugrel in patients with acute coronary syndrome (ACS)). In elective PCI patients, an escalation strategy can be considered in some situations, when the thrombotic risk is higher than the bleeding risk. In ACS patients, de-escalation can be considered when the bleeding risk is higher than the thrombotic risk or for socio-economic considerations.

Table 2. Variables favouring de-escalation

Prior Major Bleeding
Anaemia
Clinically significant bleeding on potent P2Y ₁₂ inhibitors
High bleeding risk defined by bleeding risk scores
Socio-economic factors favouring the lower costs of clopidogrel
Side effects on prasugrel and ticagrelor, especially dyspnoea on ticagrelor
Need for triple treatment due to new onset atrial fibrillation or left ventricular thrombus after myocardial infarction

Table 2. Variables that could be considered for favouring de-escalation of dual antiplatelet therapy.

Table 3. Platelet function testing versus genotyping

	Platelet function testing	Genotyping
Availability of different assays	Yes	Yes
Availability of point-of-care systems	Yes	Yes
Inter-assay variability	Yes	No
Variability of results over time	Yes	No
Association with thrombotic events	Yes	Yes
Association with bleeding events	Yes	Yes
Availability of clinical trial data on guided therapy	Yes	Yes
Feasibility in clinical practice	Yes	Yes
Results influenced by extra patient factors	Yes	No
Direct measure of response to therapy	Yes	No
Assessment of influence of both genetic and non-genetic factors on platelet function	Yes	No
Need to be performed while on treatment	Yes	No
Modified and adapted with permission from Sibbing et al. [30]		

Table 3. Advantages and disadvantages of platelet function and genetic testing.

How to de-escalate antiplatelet therapy

After deciding to de-escalate to clopidogrel, it is possible to do this either by giving a loading dose or not. There have not been any randomized trials powered for clinical outcomes investigating this, but an expert consensus paper by Angiolillo et al. gives a recommendation based on pharmacodynamics studies. Whether or not giving a loading dose is based on the timing of de-escalation and what P2Y₁₂ inhibitor is used prior to de-escalation (Figure 2) [31]. When de-escalating in the early phase (<30 days from the index event) a 600mg loading dose of clopidogrel should be administered 24 h after the last dose of prasugrel or ticagrelor. In a sub-analysis of the POPular Genetics trial, this method seemed save with no bleeding and thrombotic events in 172 patients who switched to clopidogrel within seven days after STEMI [32]. When de-escalating from ticagrelor to clopidogrel in the late phase (>30 days from the index event) a 600mg loading dose 24 h after the last dose of ticagrelor is recommended, while it is not recommended to use a new loading dose when switching from prasugrel to clopidogrel in the late phase. If de-escalating due to bleeding or bleeding concerns, a 75mg clopidogrel dose could be considered instead of a loading dose irrespective of the phase or initial P2Y₁₂ inhibitor [31].

Conclusion and future perspective

With a decline in ischemic events in the last decade, focus has shifted more and more towards preventing bleeding complications. In recent years, we have seen a growing body of evidence supporting de-escalation of antithrombotic therapy in patients with ACS who underwent PCI. Current guidelines offer the option to de-escalate in a subset of ACS patients who are not deemed suitable for potent platelet inhibitors. No new randomized data are expected in the near future. However, cost-effectiveness analyses and meta-analyses, including data from the latest randomized trials, might help expand the current recommendation. Subgroup analyses from currently published trials have not consistently identified patient groups that might benefit more from de-escalation. Therefore, for as long as guidelines do not give a higher recommendation to use de-escalation in a broader population, it will be up to the clinician to assess whether a patient could benefit or not.

Figure 2. Recommended de-escalation strategy

Figure 2. Recommended clopidogrel doses when de-escalating. When de-escalating from ticagrelor or prasugrel to clopidogrel in the early phase (≤30 days after the index event), a loading dose of 600mg should be administered 24 h after the last dose of the potent P2Y₁₂ inhibitor. In the late phase (>30 days after the index event), a loading dose of 600mg should only be administered from ticagrelor to clopidogrel, while a maintenance dose of 75mg should be administered when de-escalating from prasugrel to clopidogrel. If de-escalating due to bleeding or bleeding concerns, a 75mg clopidogrel dose could be considered instead of a loading dose irrespective of the phase or initial P2Y₁₂ inhibitor.

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Part II: Outcomes of using CYP2C19 genetic testing in clinical practice

3

Feasibility and implementation of CYP2C19 genotyping in patients using antiplatelet therapy

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Abstract

Aim

A tailored antiplatelet strategy based on *CYP2C19* genotype may reduce atherothrombotic and bleeding events. We describe our experience with *CYP2C19* genotyping, using on-site TaqMan or Spartan genotyping or shipment to a central laboratory.

Methodology

Data from two ongoing projects were used: Popular Risk Score project (non-urgent percutaneous coronary intervention patients) and the Popular Genetics study (ST-segment elevation myocardial infarction patients). For both projects, the time to genotyping result was calculated.

Results

In the Popular Risk Score project ($n = 2556$), median time from blood collection to genotyping result was 4:04 h. In the Popular Genetics study ($n = 1038$), median time from randomization to genotyping result was 2:24 h.

Conclusion

CYP2C19 genotyping is feasible in everyday clinical practice, both in the acute and non-acute settings.

Introduction

Clopidogrel is the P2Y₁₂ inhibitor of choice in patients after planned percutaneous coronary intervention (PCI). It is also still widely used in patients with acute coronary syndrome (ACS), although the European Society of Cardiology guidelines recommend the more potent P2Y₁₂ inhibitors prasugrel and ticagrelor in those patients [1,2]. The higher efficacy with regard to the prevention of atherothrombotic events when using prasugrel or ticagrelor, however, comes at the cost of an increased bleeding risk and higher drug costs [3,4].

Clopidogrel is subject to a wide interpatient variation in drug response, influenced by genetic factors, clinical factors and drug–drug interactions (i.e., calcium channel blockers, certain proton pump inhibitors such as omeprazole and esomeprazole or St John's Wort) [5–7]. Clopidogrel is a prodrug that needs to be activated by hepatic CYP450 enzymes to become effective [8]. Patients who are carrier of one or more *CYP2C19* loss-of-function alleles have a lower active metabolite level [9–12], higher level of platelet reactivity and an increased risk for recurrent atherothrombotic events, in particular in the patients with the highest thrombotic risk [13–16].

In contrast, ticagrelor does not need biotransformation to become effective, and, although CYP2C19 is involved in prasugrel metabolism as well, the *CYP2C19* genotype does not have a clinically relevant influence on prasugrel efficacy [17,18]. Non-functional *CYP2C19* polymorphisms are no rare phenomena, while for Caucasian patients, the allele frequency of *CYP2C19**2 (*rs4244285*, 681G>A) is approximately 15% and approximately 0.4% for *CYP2C19**3 (*rs4986893*, 636G>A). In Asian patients, those numbers are even higher (respectively, 29–35% and 2.4–8.9%) [19].

The finding of a diminished clopidogrel efficacy in patients carrying a *CYP2C19* loss-of-function allele has led to the addition of a black-box warning to the clopidogrel label by the US FDA, who warned physicians that an alternative antiplatelet drug should be considered in *CYP2C19**2 homozygote (poor-metabolizer) patients [20]. Furthermore, the EMA gave a warning for a diminished effect in CYP2C19 poor-metabolizer patients [21]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline as well as the Dutch Pharmacogenetics Working Group guideline state that an alternative antiplatelet agent for clopidogrel should be considered in ACS and PCI patients who are intermediate or poor metabolizer for CYP2C19 [19,22].

Randomized trials testing personalized treatment strategies based on *CYP2C19* genotype in patients treated with P2Y₁₂ inhibitors are ongoing, such as the Popular Genetics study in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI [23], and the Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response after Percutaneous Coronary Intervention (TAILOR-PCI) study in PCI patients (ClinicalTrials.gov NCT01742117). Those personalized treatment strategies are based on the hypothesis that clopidogrel efficacy is comparable to ticagrelor or prasugrel in the subgroup of normal metabolizer (*1/*1) patients, while the bleeding risk might actually be lower [4,11,16,24]. Therefore, treating CYP2C19 normal metabolizer ACS patients with clopidogrel, although not recommended by current non-STEMI and STEMI guidelines, might reduce bleeding risk and drug costs, without an increase in thrombotic events [1,2]. However, to make these personalized medicine strategies a success, *CYP2C19* genotyping has to be feasible in everyday clinical practice. In this paper, the feasibility of *CYP2C19* genotyping is studied, using the data of two projects in which *CYP2C19* genotyping is used to tailor antiplatelet treatment.

Methods

Popular risk score project

The Popular Risk Score project was a single-centre prospective registry that included consecutive patients in whom non-urgent PCI with stent implantation was performed. A risk score was calculated in all patients, consisting of platelet reactivity testing using the VerifyNow P2Y₁₂ assay (Accriva Diagnostics, CA, USA), *CYP2C19**2 and *3 genotyping using the TaqMan StepOnePlus assay, and clinical variables (diabetes, adjoined stent length >30 mm and left ventricular ejection fraction <30%). The risk score and associated platelet function and genetic testing were performed as part of routine clinical care. Patients with a high-risk score were treated with prasugrel for 1 year, while patients with a low risk score were treated with clopidogrel for 1 year [25].

Implementation

In all patients planned for PCI, one ethylenediaminetetraacetic acid (EDTA) tube with blood was collected using venepuncture before the procedure to perform *CYP2C19* genotyping. When a blood sample could not be obtained before PCI, a blood sample was collected during PCI from the arterial catheter or after PCI using venepuncture (in a small proportion of patients). The technicians of the pharmacogenetics laboratory collected the samples on a routine basis three-times a day (at 8:00, 10:00 and 14:00

h) and performed genotyping for *CYP2C19**2 and *3. Genotyping results were reported in the hospital electronic patient file. After the PCI, the medical physician responsible for the Cath lab reviewed all results and decided, based on the calculated Popular Risk Score, if the antiplatelet therapy had to be changed.

Validation

*CYP2C19**2 and *3 genotyping was performed by using a real-time PCR technique with TaqMan Genotyping MasterMix and TaqMan Drug Metabolism Genotyping assay performed on the StepOnePlus (ThermoFisher, MA, USA). The assays were validated by the pharmacogenetics laboratory before applying the test in routine patient care. As part of the validation process, selected samples with different genotypes based on the results of the TaqMan assay were also sequenced. Genotypes obtained by both methods were equal.

POPular Genetics study

The POPular Genetics study (ClinicalTrials.gov, NCT01761786) is an ongoing international multicentre, open label, randomized clinical trial consisting of patients with STEMI in whom primary PCI was performed. The rationale and design of this study have been published earlier [23]. In short, patients were randomized to a control group, treated with ticagrelor or prasugrel for 1 year, or an intervention group in which *CYP2C19**2 and *3 genotyping was performed. Normal metabolizer patients (*1/*1) were treated with clopidogrel, while patients carrying one or two loss-of-function alleles (*2 or *3) were treated with prasugrel or ticagrelor. *CYP2C19**17 was not tested. Different *CYP2C19* genotyping strategies were used in different study sites: on-site testing using the TaqMan StepOnePlus assay, on-site genotyping using the Spartan RX Point-of-Care (POC) device or shipment to a central laboratory using postal or courier service for TaqMan StepOnePlus genotyping. In the central laboratory (which is the same laboratory in which genotyping is performed for the Popular Risk Score project), *CYP2C19* genotyping is routinely performed three-times daily on working days (Monday to Friday) and one time daily on Sunday. No routine testing was performed on Saturday.

Implementation

An EDTA blood sample was collected from all study patients and sent to the central laboratory. Genotyping for *CYP2C19**2 and *3 was performed in patients randomized to the genotyping arm, after written informed consent was obtained. During the course of this study, a Spartan RX genotyping device was made available by the company in seven centres for use in this study. A buccal swab was collected and directly analysed in the

genotyping device in these centres. In one centre, the Spartan RX genotyping was performed by laboratory personnel; in the other centres Spartan RX genotyping was performed by a local study investigator or a study nurse. Users were trained by the Spartan company personnel in person or via a video conference session. The device could be placed in the clinical chemistry laboratory, catheterization laboratory or coronary care unit. From six centres without on-site genotyping capability, blood samples were shipped to the study's central laboratory for TaqMan genotyping. Results from central laboratory testing were reported in the local electronic patient file and sent to the referring hospital by email by the coordinating study investigator. In one centre, on-site TaqMan genotyping was used. This centre was also the central laboratory site. Over time, centres could change their genotyping strategy – that is, when a Spartan RX device came available. All genotyping results were sent to the patient's general practitioner, treating cardiologist and local pharmacy.

Validation

Because central laboratory testing was performed in the same laboratory where the Popular Risk Score genotyping was performed, validation was equal to the method described above. For confirmation purposes, 199 patients tested with the Spartan RX device were also genotyped using TaqMan genotyping on the blood sample sent to the central laboratory.

Statistical analysis

Statistical analyses were performed using SPSS software (v24.0, IBM, USA). For patients in the Popular Risk Score project, the time between blood collection and reporting of the genotyping test result was calculated. For patients in the Popular Genetics study, the time between randomization and reporting of the genotyping test result was calculated. The time between blood collection and test result was not considered the most relevant measure to study the feasibility of the different genotyping methods, because of the design of the study: genotyping could only be performed after informed consent was obtained and randomization was performed.

Results

Popular risk score

Between July 2010 and July 2016, 2556 patients were genotyped for the purpose of calculating the Popular Risk Score. Those patients were on average 65 years of age (± 10.7 years) and 74.3% of the patients were male. Distribution of *CYP2C19* genotype is shown in Figure 1A. Median time between blood collection and genotyping result was 4:04 h ([interquartile

range (IQR) 2:27, 16:57], range 1:16–96:51 h) (Figure 2). For 49.1% of patients, the *CYP2C19* genotyping result was made available to the treating medical physician within 4 h after PCI, in 71.9% at the same day PCI was performed, in 94.5% within 24 h and in 96.9% within 48 h after PCI. In almost all patients in whom genotyping result took more than 48 h, the blood sample was collected at Friday afternoon, after the last genotyping round. In those patients, genotyping results were available the following Monday morning. In the Popular Risk Score cohort, 2199 patients (86.0%) received coronary stent implantation. In those patients, data of P2Y₁₂ inhibitor use following the PCI were available in 1449 patients (65.9%). In total, 991 patients (68.4%) had a low risk score and were advised to use clopidogrel. This advice was followed in 988 patients (99.7%); one patient had an allergy for clopidogrel, one patients received coronary artery bypass grafting after which the P2Y₁₂ inhibitor was stopped, and for one patient it was not clear why the risk score advice was not followed. In total, 456 patients (31.5%) were advised to use prasugrel or ticagrelor, which was followed in 405 patients (88.8%); three patients received clopidogrel because of a high bleeding risk, two because of concomitant use of acenocoumarol, two patients had a contraindication for prasugrel (previous stroke) and in 44 patients, it was not clear why the risk score advice was not followed.

POPular Genetics study

All patients included between July 2014 (study initiation) and 8th December 2017 in the POPular Genetics study, and randomized to the genotyping arm, were selected. This selection contained 1283 patients. Because it is an ongoing trial, data were not yet entered in the electronic case record form for a part of the patient cohort. The electronic case record form was complete with data describing the method of genotyping and the time between randomization and genotyping result in 1038 patients (80.9%) from nine study sites. These patients were on average 61.9 years of age (± 11.2 years) and 74.3% of the patients were male. On-site TaqMan genotyping was used by one study site (487 patients), on-site Spartan RX genotyping by seven study sites (411 patients) and shipment to the central laboratory was used by six study sites (140 patients). Distribution of *CYP2C19* genotype is shown in Figure 1B. The median time between randomization and genotyping result was 2:24 h ([IQR 1:46, 12:53], range 0:30–13:32 h); 2:04 h (1:44, 3:58) for in-hospital TaqMan genotyping, 2:16 h (1:38, 4:29) for Spartan RX genotyping and 52:32 h (28:16, 80:13) for central laboratory testing (Figure 2 & Supplementary Materials). For all patients in the database tested with the Spartan RX device, the test results were inconclusive in 39 patients (8.0%). This was solved by

shipment to the central laboratory for TaqMan genotyping (30 patients), or repeated Spartan RX testing (two patients). In seven patients, no further genotyping was performed; in five patients ticagrelor was continued, one patient was switched to clopidogrel because a vitamin K antagonist was started and the discharge medication of the remaining patient was unknown. In one patient, the Spartan RX result did not match the TaqMan result, which was most likely due to contamination of the Spartan RX sample. Repeated testing of the same patient gave results matching the central laboratory result.

Figure 1. CYP2C19 allele frequency

Figure 1. (A) CYP2C19 allele frequency in the Popular Risk Score project; (B) CYP2C19 allele frequency in the POPular Genetics study.

Figure 2. Time to genotyping results

Figure 2. Time between blood collection (A) or randomization (B) and CYP2C19 genotyping results, for each genotyping method. (A) Popular Risk Score project; (B) POPular Genetics study. CL: Central laboratory.

Discussion

In this analysis, we evaluated the feasibility of different strategies of CYP2C19 genotyping, used to tailor antiplatelet therapy in patients admitted with STEMI or undergoing a planned PCI. We conclude that it is feasible to have genotyping results available within 24–48 h in the majority of patients, using TaqMan StepOnePlus genotyping or the Spartan RX POC device. As expected, shipment to a central laboratory takes more time than on-site genotyping. The use of the Spartan RX POC device has advantages over TaqMan genotyping. First, results can be available within 1 h after collection of a buccal swab. This makes it possible for patients undergoing planned PCI to have genotyping results available before same day discharge. Furthermore, in the acute setting of myocardial infarction antiplatelet therapy might be adjusted shortly after hospital arrival, even during weekend-, evening- or night-time hours. Second, the use of a buccal swab is more patient friendly compared with venepuncture for an additional blood sample. Third, although the test must be performed by trained personnel, the POC device can be situated outside the laboratory – in example, in the Cath lab or on the CCU. Another disadvantage for

TaqMan genotyping is that laboratory personnel performing the test have to be trained to a higher level and a certified laboratory has to be available.

There are also disadvantages related to POC testing. First, although confirmation studies show a good correlation between Spartan RX and laboratory-based genotyping [26,27], in one patient the Spartan test result did not match with TaqMan genotyping result, and in 8% of patients in our cohort the test result was inconclusive on the first attempt. Although this problem might be solved at least for some part with more extensive training, laboratory based testing has to be available for backup. Second, in high-volume centres with a well-organized laboratory for genotyping, batch-wise testing using, in other words, TaqMan might be cost-efficient in comparison to POC genotyping. This should be analysed in more detail. Third, when TaqMan genotyping is used, more SNP's and patients can be tested simultaneously, while the Spartan RX is limited to *CYP2C19**2, *3 and *17, for one patient at a time per genotyping device. Fourth, for low-risk patients - in other words, patients planned for elective PCI - it might not be necessary to have genotyping results available the same day and genotyping could actually be performed days or weeks in advance, in which batch wise laboratory-based testing can be a good alternative to POC testing.

CYP2C19 genotyping has additional costs. Current list prices for the SPARTAN RX system at time of publication are US\$200 (€162) per test kit, including testing for *2, *3 and *17, and US\$15,000 (€12,173) for the SPARTAN RX POC device. The costs for laboratory-based testing are estimated at €80 per patient, including reagent and personnel costs, but excluding equipment. The costs of laboratory based testing, however, may vary substantially per country and per laboratory site, in other words, with the number of tests performed. Nevertheless, a tailored antiplatelet strategy might be cost-effective, based on the much lower drug costs of generic clopidogrel (€14.40 per year in The Netherlands) compared with prasugrel and ticagrelor (€722 and €877 per year, respectively) [28]. This difference might be even bigger if a tailored approach would be able to reduce clinical events or give a gain in quality-adjusted life-years. In an observational study comparing 32 *CYP2C19* poor-metabolizer patients treated with clopidogrel compared with 41 poor-metabolizer patients treated with prasugrel in The Netherlands, a significant lower number of events was seen in the prasugrel-treated patient group [29]. Non-randomized trials showed that personalized antiplatelet treatment based on *CYP2C19* genotype might be able to reduce cardiovascular event rates and bleeding events [16,30]. Furthermore, several simulation studies showed superiority

in cost-effectiveness for a *CYP2C19* genotype guided strategy compared with the use of prasugrel or ticagrelor, in both ACS and PCI patients [31–33]. In the ongoing Popular Genetics study, cost-effectiveness will be studied as a co-primary end point [23].

The putative gain-of-function allele *CYP2C19**17 has been associated with a lower platelet reactivity and an increased risk of bleeding [34,35]. In both the Popular Risk Score project and the Popular Genetics study, the *17 allele was not tested because it would not have had consequences for the treatment advice in those studies. Patients with *1/*1, *1/*17 or *17/*17 genotype would all be considered good responders and treated with clopidogrel, while patients with *2/*17 are classified as intermediate metabolizers and therefore treated with prasugrel or ticagrelor [34].

With genotyping becoming part of routine care, it is important to realize that the results might affect other treatments than just antiplatelet therapy, as it is involved in the metabolism of selective serotonin reuptake inhibitors, tricyclic antidepressants, proton pump inhibitors and voriconazole [22,36–38]. Therefore, it is important that the genotyping results are reported in the electronic patient file and that protocols that describe how to handle possible genotype–drug interactions, made in collaboration with the clinical pharmacist, are available. Genotyping results should also be shared with the local pharmacy and the general practitioner [22].

Limitations

The aim of this paper was to study the feasibility of different *CYP2C19* genotyping strategies in everyday practice, not to compare the time it takes to run the tests itself. When using the Spartan RX POC device, the time between collection of the buccal swab and test result will be around 1 h, while DNA extraction and TaqMan genotyping will take less than 2 h in total, when a blood sample is available.

Conclusion

It is feasible to perform *CYP2C19* genotyping within 24–48 h using on-site TaqMan genotyping or the Spartan RX POC device, making personalized medicine in antiplatelet therapy, based on pharmacogenetic data, feasible for everyday practice.

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Supplementary appendix

Table 1: Time between randomization and genotyping result per centre (POPular Genetics study)

			< 2	< 24	< 48	>= 48	
			hours	hours	hours	hours	
1	Shipment to CL	Count		4	3		7
		%		57.1%	42.9%		100.0%
	Total	Count		4	3		7
		%		57.1%	42.9%		100.0%
2	On-site TaqMan	Count	366	110	10	1	487
		%	75.2%	22.6%	2.1%	0.2%	100.0%
	Spartan	Count	76	6	0	0	82
		%	92.7%	7.3%	0.0%	0.0%	100.0%
	Total	Count	442	116	10	1	569
		%	77.7%	20.4%	1.8%	0.2%	100.0%
3	Shipment to CL	Count	0	3	11	3	17
		%	0.0%	17.6%	64.7%	17.6%	100.0%
	Spartan	Count	156	52	4	3	215
		%	72.6%	24.2%	1.9%	1.4%	100.0%
	Total	Count	156	55	15	6	232
		%	67.2%	23.7%	6.5%	2.6%	100.0%
4	Shipment to CL	Count	3	3	11	2	19
		%	15.8%	15.8%	57.9%	10.5%	100.0%
	Total	Count	3	3	11	2	19
		%	15.8%	15.8%	57.9%	10.5%	100.0%
5	Spartan	Count	6	9	3	2	20
		%	30.0%	45.0%	15.0%	10.0%	100.0%
	Total	Count	6	9	3	2	20
		%	30.0%	45.0%	15.0%	10.0%	100.0%
6	Shipment to CL	Count	0		3	4	7
		%	0.0%		42.9%	57.1%	100.0%
	Spartan	Count	8		1	0	9

	Total	%	88.9%		11.1%	0.0%	100.0%
		Count	8		4	4	16
		%	50.0%		25.0%	25.0%	100.0%
7	Spartan	Count	26				26
		%	100.0%				100.0%
	Total	Count	26				26
		%	100.0%				100.0%
8	Shipment to CL	Count	0	5	5	1	11
		%	0.0%	45.5%	45.5%	9.1%	100.0%
	Spartan	Count	10	2	0	0	12
		%	83.3%	16.7%	0.0%	0.0%	100.0%
	Total	Count	10	7	5	1	23
		%	43.5%	30.4%	21.7%	4.3%	100.0%
9	Shipment to CL	Count	0	0	15	64	79
		%	0.0%	0.0%	19.0%	81.0%	100.0%
	Spartan	Count	12	21	9	5	47
		%	25.5%	44.7%	19.1%	10.6%	100.0%
	Total	Count	12	21	24	69	126
		%	9.5%	16.7%	19.0%	54.8%	100.0%
Total	On-site TaqMan	Count	366	110	10	1	487
		%	75.2%	22.6%	2.1%	0.2%	100.0%
	Shipment to CL	Count	3	15	48	74	140
		%	2.1%	10.7%	34.3%	52.9%	100.0%
	Spartan	Count	294	90	17	10	411
		%	71.5%	21.9%	4.1%	2.4%	100.0%
	Total	Count	663	215	75	85	1038
		%	63.9%	20.7%	7.2%	8.2%	100.0%

Abbreviations: CL=central laboratory

A genotype-guided strategy for oral P2Y₁₂ inhibitors in primary PCI

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Abstract

Background

It is unknown whether patients undergoing primary percutaneous coronary intervention (PCI) benefit from genotype-guided selection of oral P2Y₁₂ inhibitors.

Methods

We conducted a randomized, open-label, assessor-blinded trial in which patients undergoing primary PCI with stent implantation were assigned in a 1:1 ratio to receive either a P2Y₁₂ inhibitor on the basis of early *CYP2C19* genetic testing (genotype-guided group) or standard treatment with either ticagrelor or prasugrel (standard-treatment group) for 12 months. In the genotype-guided group, carriers of *CYP2C19**2 or *CYP2C19**3 loss-of-function alleles received ticagrelor or prasugrel, and noncarriers received clopidogrel. The two primary outcomes were net adverse clinical events — defined as death from any cause, myocardial infarction, definite stent thrombosis, stroke, or major bleeding defined according to Platelet Inhibition and Patient Outcomes (PLATO) criteria — at 12 months (primary combined outcome; tested for noninferiority, with a noninferiority margin of 2 percentage points for the absolute difference) and PLATO major or minor bleeding at 12 months (primary bleeding outcome).

Results

For the primary analysis, 2488 patients were included: 1242 in the genotype-guided group and 1246 in the standard-treatment group. The primary combined outcome occurred in 63 patients (5.1%) in the genotype-guided group and in 73 patients (5.9%) in the standard-treatment group (absolute difference, −0.7 percentage points; 95% confidence interval [CI], −2.0 to 0.7; $P < 0.001$ for noninferiority). The primary bleeding outcome occurred in 122 patients (9.8%) in the genotype-guided group and in 156 patients (12.5%) in the standard-treatment group (hazard ratio, 0.78; 95% CI, 0.61 to 0.98; $P = 0.04$).

Conclusion

In patients undergoing primary PCI, a *CYP2C19* genotype-guided strategy for selection of oral P2Y₁₂ inhibitor therapy was noninferior to standard treatment with ticagrelor or prasugrel at 12 months with respect to thrombotic events and resulted in a lower incidence of bleeding.

Introduction

In patients with ST-segment elevation myocardial infarction (STEMI), the preferred method of reperfusion is primary percutaneous coronary intervention (PCI) with stent implantation. In these patients, dual antiplatelet therapy, consisting of aspirin and a P2Y₁₂ inhibitor, is essential to prevent recurrent thrombotic events such as stent thrombosis [1]. Current guidelines [1,2] favour the more potent platelet inhibitors ticagrelor and prasugrel over clopidogrel because these drugs are more effective for the prevention of thrombotic events [3,4]. However, this greater efficacy comes with a higher risk of bleeding [3,4].

Clopidogrel is a prodrug, transformed into its active metabolite by hepatic cytochrome P450 enzymes. The active metabolite irreversibly inhibits the P2Y₁₂ receptor on platelets, which results in inhibition of platelet aggregation. However, approximately 30% of white patients have an inadequate response to clopidogrel as measured with platelet-function tests [5]. Part of this variation in response can be explained by genetic variations, such as the *CYP2C19**2 and *CYP2C19**3 loss-of-function alleles [6–9]. In patients without these loss-of-function alleles, clopidogrel has shown similar efficacy to that of ticagrelor and prasugrel [10–12]. We therefore conducted the *CYP2C19* Genotype-Guided Antiplatelet Therapy in ST-Segment Elevation Myocardial Infarction Patients — Patient Outcome after Primary PCI (POPular Genetics) trial to determine whether a *CYP2C19* genotype-guided strategy for selection of oral P2Y₁₂ inhibitors can reduce bleeding risk without increasing thrombotic risk in patients with STEMI undergoing primary PCI with stent implantation.

Methods

Trial design

The POPular Genetics trial was an investigator-initiated, randomized, open-label, assessor-blinded trial performed at 10 European sites (8 in the Netherlands, 1 in Belgium, and 1 in Italy). It was sponsored by the Netherlands Organization for Health Research and Development, and Spartan Bioscience provided the Spartan RX point-of-care system and the reagents for free. Neither entity had any role in the design or execution of the trial or in the analysis of the data. Details of the design have been published previously [13].

The trial was approved by the appropriate ethics committees and national authorities in each country. The trial was registered after the enrolment of the first 18 patients, as described in the Supplementary Appendix

(available with the full text of this article at NEJM.org). An independent data and safety monitoring board monitored the trial and had full access to all data. An independent clinical-event committee whose members were unaware of the trial-group assignments adjudicated all outcomes.

Trial monitoring was executed by an external service provider (Research Drive, Norg, the Netherlands). The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol, available at NEJM.org.

Major protocol revision

The initial plan was for the trial to compare a genotype-guided strategy for selecting oral P2Y₁₂ inhibitors with a standard-treatment strategy for which clopidogrel was recommended. However, the 2011 European Society of Cardiology (ESC) guideline for patients with acute coronary syndrome without ST-segment elevation recommended use of ticagrelor or prasugrel over clopidogrel.¹⁴ In anticipation that the same recommendation would be made for patients with ST-segment elevation, we changed the treatment in the standard-treatment group from clopidogrel to ticagrelor or prasugrel. Because we were now testing guided de-escalation of therapy instead of guided escalation of therapy, there were major changes in the primary outcomes and hypotheses of the trial. These changes are summarized in the Supplementary Appendix under “Major Protocol Revision May 2012.”

Trial population

Patients were eligible for enrolment if they had signs or symptoms of STEMI lasting 30 minutes to 12 hours, underwent primary PCI with stent implantation, and were 21 years of age or older. Exclusion criteria are provided in Table S1 in the Supplementary Appendix. Because patients were asked to participate in the trial during the acute phase of myocardial infarction, many provided only oral informed consent before randomization. Such patients were eligible for the final analysis only if written informed consent was subsequently obtained.

Pre-trial treatment

Pre-trial antithrombotic treatment was administered according to local protocol. This usually consisted of a loading dose of aspirin, a P2Y₁₂ inhibitor, and heparin. Other antithrombotic periprocedural therapies were chosen at the discretion of the treating physician, as was the P2Y₁₂ inhibitor administered before randomization.

Randomization and trial procedures

Using an Internet-based randomization procedure with computer-generated block randomization for each site, we randomly assigned patients in a 1:1 ratio to a *CYP2C19* genotype-guided strategy or to standard treatment with ticagrelor or prasugrel (Fig. S1 in the Supplementary Appendix). Patients were enrolled during or up to 48 hours after primary PCI.

In patients assigned to the genotype-guided strategy, *CYP2C19* genotyping was performed with the use of the TaqMan StepOnePlus assay at a central laboratory (St. Antonius Hospital, Nieuwegein, the Netherlands) or with an on-site point-of-care Spartan RX device (Spartan Bioscience). Use of the Spartan device began during the course of the trial; not all hospitals started using it simultaneously. Genetic testing for the *CYP2C19**2 and *CYP2C19**3 loss-of-function alleles was performed as soon as possible after randomization. Details of the logistics of these tests in this trial have been published previously [15]. Carriers of a loss-of-function *CYP2C19* allele were treated with ticagrelor or prasugrel, whereas noncarriers (*CYP2C19**1/*1) received clopidogrel according to the label instructions. If patients switched between P2Y₁₂ inhibitors, the use of a loading dose was at the discretion of the treating physician. Either ticagrelor or prasugrel was prescribed to each patient in the standard-treatment group, according to local protocol.

All patients were treated with a P2Y₁₂ inhibitor for at least 1 year after primary PCI. Patients were asked to fill out a questionnaire at 1 month, 6 months, and 12 months after primary PCI. Follow-up data were collected with the use of the hospital electronic health record. Questionnaires were used as an additional source of information. If necessary, the general practitioner, the patient's pharmacist, or the patient was contacted.

Outcomes

There were two primary outcomes. The first was the combined outcome of net adverse clinical events, which included death from any cause, myocardial infarction (defined according to the Third Universal Definition of Myocardial Infarction) [16], definite stent thrombosis (defined according to the Academic Research Consortium) [17], stroke (defined as a new neurologic deficit ending in death or lasting >24 hours, not due to another readily identifiable cause such as trauma), or major bleeding defined according to Platelet Inhibition and Patient Outcomes (PLATO) criteria (including major bleeding related to coronary-artery bypass grafting (CABG) as well as non-CABG-related major bleeding [18]), at 12 months (primary combined outcome). The second was PLATO major

bleeding (CABG-related and non-CABG-related) or minor bleeding at 12 months (primary bleeding outcome). A complete list of secondary thrombotic and bleeding outcomes is provided in Tables S2 and S3 in the Supplementary Appendix.

Statistical analysis

The incidence of the primary combined outcome was assumed to be 18.8% in the standard-treatment group and 16.9% in the genotype-guided group. These estimates were based on unpublished data from the Central Holland Acute Myocardial Infarction (CHAIR) study, the Ongoing Tirofiban in Myocardial Infarction Evaluation (On-TIME) 2 trial, and sub-analyses of the PLATO trial and the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38 [10,11,19]. Using a power of 80% and an alpha level of 0.05 and setting the noninferiority threshold for the absolute difference between the two groups in the incidence of the primary combined outcome at 2 percentage points, we calculated that 2474 patients would be needed to show noninferiority. For the primary bleeding outcome, the incidence was assumed to be 14.5% in the genotype-guided group and 18.9% in the standard-treatment group. Fewer patients were needed to show superiority for this outcome. Accounting for withdrawals and for approximately 200 patients enrolled before the May 2012 protocol amendment, we planned to include 2700 patients.

The end of the primary PCI procedure was defined as time 0, irrespective of when randomization took place. Owing to the logistics of obtaining informed consent, obtaining genotyping results, and (if necessary) switching the P2Y₁₂ inhibitor, outcome events during the first 24 hours after primary PCI were excluded from the primary analysis. The primary analysis followed the intention-to-treat principle and included all patients enrolled after the May 2012 protocol amendment. In addition, a per-protocol analysis was performed. The primary combined outcome was first assessed in an analysis of the noninferiority of the *CYP2C19* genotype-guided strategy to standard treatment. If noninferiority was confirmed, superiority was assessed. For the primary bleeding outcome, the *CYP2C19* genotype-guided strategy was assumed to be superior to standard treatment. No adjustment for alpha error was performed to account for the assessment of two primary outcomes, because both analyses were required to show significance at a P value of less than 0.05 in order to assert a beneficial effect of the genotype-guided strategy. Both primary outcomes were also assessed in 26 prespecified subgroups. Two

Figure 1. Randomization and follow-up.

Figure 1. Patients who underwent randomization despite screening errors or who were not treated according to the protocol were also included in the intention-to-treat analysis. Vital status was known for nearly all patients (99.9%), and data for three patients (0.1%) were censored because their vital status was unknown.

sensitivity analyses were planned. The first included events during the 24 hours after primary PCI, and the second analysis involved stratification according to centre.

Time-to-event curves were constructed with the use of the Kaplan–Meier method. Differences between the survival curves were compared with the use of the log-rank test, and Cox proportional-hazards models were used to calculate hazard ratios with 95% confidence intervals. Confidence intervals for secondary outcomes have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. P values were two-sided, and values of less than 0.05 were considered to indicate statistical significance. Data were analysed with R software, version 3.6.0 (R Foundation for Statistical Computing).

Results

Participants and follow-up

From June 2011 through April 2018, a total of 2751 patients were randomly assigned to a treatment group (Fig. 1). A screening log was kept at only one centre; the records of those patients screened are shown in Table S4 in the Supplementary Appendix. Patients who underwent duplicate randomization in error, those who withdrew consent, and those who were enrolled before the protocol amendment of May 2012 were excluded from all analyses. Therefore, 2488 patients entered the intention-to-treat analysis (1242 in the genotype-guided group and 1246 in the standard-treatment group), of whom 3 were lost to follow-up. The database was locked on May 28, 2019, after all trial participants had completed at least 12 months of follow-up.

Table 1. (see next page) Baseline Characteristics of the Patients and Initial Antithrombotic Therapy.* Plus–minus values are means ±SD. PCI denotes percutaneous coronary intervention. † The body-mass index is the weight in kilograms divided by the square of the height in meters. Data were missing for 66 patients in the genotype-guided group and 77 in the standard-treatment group. ‡ The creatinine clearance was calculated with the use of the Chronic Kidney Disease Epidemiology Collaboration formula.

Table 1. Baseline characteristics of the patients and initial antithrombotic therapy.*

Characteristic	Genotype-guided group (N = 1242)	Standard-treatment group (N = 1246)
Baseline characteristics		
Age – yr	61.9±11.1	61.4±11.5
Age ≥75 yr — no. (%)	188 (15.1)	175 (14.0)
Female sex – no. (%)	317 (25.5)	309 (24.8)
Body-mass index †	27.5±6.67	27.0±4.27
Creatinine clearance <60 ml/min/1.73 m2 at baseline – no. (%)	121 (9.8)	109 (8.8)
‡		
Cardiovascular risk factors		
Current smoker - no. (%)	562 (45.8)	565 (45.8)
Diabetes mellitus — no. (%)	150 (12.1)	138 (11.1)
Arterial hypertension — no. (%)	521 (41.9)	511 (41.0)
Hyperlipidaemia — no.	260/ (21.0)	255 (20.5)
Family history of coronary artery disease — no./total no. (%)	475 (40.6)	467 (39.5)
Other medical history — no. (%)		
Previous PCI with stenting	99 (8.0)	91 (7.3)
Previous coronary-artery bypass grafting	12 (1.0)	22 (1.8)
Previous myocardial infarction	97 (7.8)	87 (7.0)
History of coronary artery disease	133 (10.7)	118 (9.5)
Peripheral arterial disease	39 (3.1)	34 (2.7)
History of bleeding	30 (2.4)	23 (1.8)
Aspirin use before primary PCI — no. (%)	1232 (99.4)	1238 (99.4)
P2Y ₁₂ inhibitor use before primary PCI — no. (%)	1198 (96.8)	1190 (96.0)
Initial antithrombotic therapy after randomization and genotyping — no./total no. (%)		
P2Y₁₂ inhibitor		
Any	1236 (99.8)	1237 (99.8)
Clopidogrel	751 (60.6)	87 (7.0)
Prasugrel	13 (1.0)	28 (2.3)
Ticagrelor	472 (38.1)	1122 (90.5)
None	3 (0.2)	3 (0.2)
Aspirin	1211 (97.7)	1208 (97.4)
Oral anticoagulation	51 (4.1)	54 (4.4)

The two groups were balanced with regard to all baseline characteristics and non-trial-related medications and procedures (Table 1, and Tables S5 and S6 in the Supplementary Appendix). Table S7 in the Supplementary Appendix provides an overview of the genotype results, and Table S8 provides an overview of the intervals from symptom onset to the time that a patient was receiving the recommended therapy. In the genotype-guided group, 60.6% of the patients received clopidogrel and 39.1% received ticagrelor or prasugrel after their genetic results were available (Table 1). In the standard-treatment group, 92.7% of the patients were treated with ticagrelor or prasugrel and 7.0% with clopidogrel.

The percentage of patients who adhered to the recommended trial drug during the entire duration of follow-up, as assessed by the investigators, was 84.5% in the genotype-guided group and 82.0% in the standard-treatment group. Tables S9A, B, and C in the Supplementary Appendix provide an overview of how many patients switched or discontinued a P2Y₁₂ inhibitor and for what reasons, and Table S10 provides an overview of P2Y₁₂ inhibitor use during follow-up.

Primary outcomes

At 12 months, death from any cause, myocardial infarction, definite stent thrombosis, stroke, or PLATO major bleeding (the primary combined outcome) occurred in 63 patients (5.1%) in the genotype-guided group and in 73 patients (5.9%) in the standard-treatment group. The genotype-guided strategy met the prespecified criterion for noninferiority with respect to net adverse clinical events (absolute difference in incidence, -0.7 percentage points; 95% confidence interval [CI], -2.0 to 0.7; $P < 0.001$ for noninferiority) (Fig. 2A and Table 2). The analysis for superiority did not support superiority of the genotype-guided strategy (hazard ratio, 0.87; 95% CI, 0.62 to 1.21; $P = 0.40$). The primary bleeding outcome (PLATO major or minor bleeding) was significantly less common in the genotype-guided group than in the standard-treatment group (122 events [9.8%] vs. 156 [12.5%]; hazard ratio, 0.78; 95% CI, 0.61 to 0.98; $P = 0.04$) (Fig. 2B and Table 3). The results of the per-protocol analysis and the sensitivity analyses were consistent with those of the primary analyses (Tables S11 and S12 in the Supplementary Appendix).

Secondary outcomes

There were no significant differences between the two groups for the combined thrombotic outcome consisting of death from vascular causes, myocardial infarction, definite stent thrombosis, or stroke (2.7% in the genotype-guided group and 3.3% in the standard-treatment group; hazard ratio, 0.83; 95% CI, 0.53 to 1.31) or for any of the other secondary

thrombotic outcomes (Table 2, and Fig. S2 in the Supplementary Appendix). Furthermore, there was no difference in the incidence of PLATO major bleeding between the genotype-guided group and the standard-treatment group (2.3% in both groups; hazard ratio, 0.97; 95% CI, 0.58 to 1.63). The between-group difference in the primary bleeding outcome was driven by a lower incidence of PLATO minor bleeding in the genotype-guided group (7.6% vs. 10.5%; hazard ratio, 0.72; 95% CI, 0.55 to 0.94) (Fig. S3 in the Supplementary Appendix). Table 3 shows a full list of bleeding outcomes.

Analyses of the primary outcomes were performed in prespecified subgroups. The results were generally consistent with those in the whole cohort (Figs. S4 and S5 in the Supplementary Appendix).

Discussion

In this trial, we investigated the possible clinical benefit of *CYP2C19* genotype-guided antiplatelet therapy in patients with STEMI undergoing primary PCI. There are two key findings from this trial. First, the use of a genotype-guided strategy, in which patients without a *CYP2C19* loss-of-function allele received clopidogrel, was not associated with a higher risk of combined death from any cause, myocardial infarction, definite stent thrombosis, stroke, or major bleeding 12 months after primary PCI than standard treatment with the more potent P2Y₁₂ inhibitors ticagrelor and prasugrel. Second, the use of clopidogrel in the genotype-guided group resulted in a lower risk of (mostly minor) bleeding than standard treatment.

Since the trial was designed, the incidence of thrombotic events after acute coronary syndromes has decreased considerably, particularly because of the use of newer-generation stents [20]. Other recent trials with similar populations, such as the Dual Antiplatelet Therapy after Drug-Eluting Stent Implantation in ST-Elevation Myocardial Infarction (DAPT-STEMI) trial and the Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment for Acute Coronary Syndromes (TROPICAL-ACS) trial, have shown a similar incidence of thrombotic events [21,22]. With the lower-than-anticipated incidence of the primary combined outcome in our trial, the prespecified noninferiority margin was wider relative to the incidence than originally expected. The upper boundary of the 95% confidence interval for the primary combined outcome was 21% higher than the incidence in the standard-treatment group. However, the observed upper boundary of the 95% confidence interval in the per-protocol analysis (11% higher than the incidence in the standard-treatment group) (Table S11 in the Supplementary Appendix) gives stronger support to the

Figure 2. Incidence curves for the primary outcomes.

Figure 2. Panel A (previous page) shows the cumulative incidence of the primary combined thrombotic and bleeding outcome, consisting of death from any cause, myocardial infarction, definite stent thrombosis, stroke or major bleeding defined according to Platelet Inhibition and Patient Outcomes (PLATO) criteria. Panel B shows the primary bleeding outcome of PLATO major and minor bleeding. The inset in each panel shows the same data on an enlarged y-axis. PCI denotes percutaneous coronary intervention.

Table 2. Primary combined outcome and secondary thrombotic outcomes.*

Outcome	Genotype-Guided Group (N=1242)	Standard-Treatment Group (N=1246)	Absolute Difference (95% CI)†	Hazard Ratio (95% CI)	P Value
	no. of patients (%)	percentage points			
Primary combined outcome‡					
Noninferiority analysis	63 (5.1)	73 (5.9)	-0.7 (-2.0 to 0.7)		<0.001§
Superiority analysis	63 (5.1)	73 (5.9)		0.87 (0.62 to 1.21)	0.40
Secondary thrombotic outcomes					
Combined ischemic outcome of death from vascular causes, myocardial infarction, definite stent thrombosis, or stroke					
Noninferiority analysis	34 (2.7)	41 (3.3)	-0.3 (-1.4 to 0.8)		
Superiority analysis	34 (2.7)	41 (3.3)		0.83 (0.53 to 1.31)	
Combined ischemic outcome of death from vascular causes, acute coronary syndrome, definite stent thrombosis, stroke, or urgent TVR	57 (4.6)	59 (4.7)		0.97 (0.67 to 1.40)	
Death from any cause	19 (1.5)	19 (1.5)		1.00 (0.53 to 1.89)	
Death from vascular causes	9 (0.7)	10 (0.8)		0.90 (0.37 to 2.22)	
Acute coronary syndrome	43 (3.5)	43 (3.5)		1.00 (0.66 to 1.53)	

Myocardial infarction	19 (1.5)	26 (2.1)		0.73 (0.41 to 1.32)	
Stroke	8 (0.6)	11 (0.9)		0.73 (0.29 to 1.82)	
Ischemic	7 (0.6)	10 (0.8)		0.70 (0.27 to 1.85)	
Haemorrhagic	1 (0.1)	1 (0.1)		1.00 (0.06 to 16.1)	
Definite stent thrombosis	2 (0.2)	3 (0.2)		0.67 (0.11 to 4.01)	
Definite, probable, or possible stent thrombosis	9 (0.7)	11 (0.9)		0.73 (0.29 to 1.81)	
Urgent TVR	15 (1.2)	15 (1.2)		1.00 (0.49 to 2.05)	

Table 2. * All outcomes were confirmed by an independent adjudication committee. The 95% confidence intervals have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. TVR denotes target-vessel revascularization.

† Integrated differences were used to calculate the absolute differences at 12 months for the two Kaplan–Meier curves. Therefore, these values differ from the absolute difference in the cumulative incidence at 12 months.

‡ The primary combined outcome was death from any cause, myocardial infarction, definite stent thrombosis, stroke, or major bleeding defined according to Platelet Inhibition and Patient Outcomes criteria.

§ The P value was not adjusted to account for the assessment of two primary outcomes.

Table 3. Primary and secondary bleeding outcomes.*

Outcome	Genotype-guided group (N=1242)	Standard-treatment group (N=1246)	Hazard Ratio (95% CI)	P value
	no. of patients (%)			
Primary bleeding outcome: PLATO major or minor bleeding	122 (9.8)	156 (12.5)	0.78 (0.61-0.98)	0.04 †
Major or minor bleeding according to other criteria				
BARC type 2-5 bleeding	126 (10.1)	163 (13.1)	0.77 (0.61-0.97)	
TIMI major or minor bleeding	30 (2.4)	30 (2.4)	1.00 (0.61-1.67)	
Major bleeding, including CABG-related bleeding				
PLATO major bleeding	28 (2.3)	29 (2.3)	0.97 (0.58-1.63)	
BARC type 3-5 bleeding	31 (2.5)	29 (2.3)	1.08 (0.65-1.78)	
TIMI major bleeding	15 (1.2)	16 (1.3)	0.94 (0.47-1.90)	
Bleeding resulting in red-cell transfusion	19 (1.5)	18 (1.4)	1.06 (0.56-2.02)	
PLATO life-threatening or fatal bleeding	16 (1.3)	16 (1.3)	1.00 (0.50-2.01)	
Fatal bleeding	2 (0.2)	1 (0.1)	2.01 (0.18-22.1)	
Intracranial bleeding	6 (0.5)	4 (0.3)	1.51 (0.43-5.34)	
Intracranial fatal bleeding	0	0		
Non-CABG-related major bleeding				
Non-CABG-related PLATO major bleeding	25 (2.0)	23 (1.8)	1.09 (0.62-1.92)	

BARC type 3 or 5 bleeding	28 (2.3)	23 (1.8)	1.23 (0.71-2.13)
Non-CABG-related TIMI major bleeding	15 (1.2)	13 (1.0)	1.16 (0.55-2.44)
Non-CABG-related major or minor bleeding			
PLATO major or minor bleeding	119 (9.6)	152 (12.2)	0.78 (0.61-0.99)
BARC type 2, 3, or 5 bleeding	123 (9.9)	159 (12.8)	0.77 (0.61-0.97)
Non-CABG-related TIMI major or minor bleeding	27 (2.2)	25 (2.0)	1.09 (0.63-1.87)
Minor bleeding			
PLATO minor bleeding	95 (7.6)	131 (10.5)	0.72 (0.55-0.94)
BARC type 2 bleeding	96 (7.7)	138 (11.1)	0.69 (0.53-0.89)
TIMI minor bleeding	18 (1.4)	20 (1.6)	0.90 (0.48-1.71)

Table 3.* All events were confirmed by an independent adjudication committee. The 95% confidence intervals have not been adjusted for multiple comparisons, and therefore inferences drawn from these intervals may not be reproducible. On the Bleeding Academic Research Consortium (BARC) scale for bleeding, type 2 indicates any overt, actionable sign of bleeding; type 3 bleeding with a decrease in the haemoglobin level of 3 gram or more per decilitre, any transfusion, cardiac tamponade, or intracranial or ocular involvement; type 4 coronary-artery bypass grafting (CABG)-related bleeding; and type 5 fatal bleeding. PLATO denotes Platelet Inhibition and Patient Outcomes, and TIMI Thrombolysis in Myocardial Infarction.

† The P value was not adjusted to account for the assessment of two primary outcomes.

conclusion that genotype-guided P2Y₁₂ treatment is noninferior to standard treatment for the occurrence of thrombotic events. The results for both the thrombotic and bleeding outcomes of the trial are in line with data from observational studies and smaller trials investigating a genotype-guided strategy [13,23-26].

Guided de-escalation was also investigated in the TROPICAL-ACS trial, which included 2610 patients with biomarker-positive acute coronary syndromes undergoing PCI. It showed that guided de-escalation of P2Y₁₂ inhibitor therapy (switching to clopidogrel) with the use of platelet-function testing was noninferior to prasugrel for the prevention of thrombotic events [22]. However, the incidence of bleeding was not significantly lower in the de-escalation group than in the prasugrel group. Furthermore, this method of guiding therapy requires patients to switch between P2Y₁₂ inhibitors multiple times if they have high platelet reactivity during treatment with clopidogrel, and it requires patients to revisit the clinic to perform platelet function testing. On the basis of the TROPICAL-ACS trial, the latest ESC guidelines give a class IIb recommendation to use platelet-function testing for guided de-escalation, especially in patients who are not deemed to be candidates for 12 months of potent antiplatelet therapy [27].

Trials such as TROPICAL-ACS usually included Bleeding Academic Research Consortium (BARC) type 2 bleeding (any overt, actionable sign of bleeding) in their definition of net clinical benefit [22,28]. We chose to incorporate only major bleeding, which has an effect on morbidity and mortality similar to that of thrombotic outcomes [29]. Furthermore, although earlier trials consistently showed a lower incidence of minor bleeding with clopidogrel than with prasugrel or ticagrelor, this effect was not seen for major bleeding [3,4,22,28]. Our data confirm this finding. Therefore, guided de-escalation will mostly help to reduce minor bleeding. Nevertheless, minor bleeding is of clinical relevance, since it leads to medical intervention and influences treatment adherence and health care cost [30-32].

Limitations of our trial include the open-label design, the lower-than-anticipated incidence of the primary combined outcome, and the fact that genetic variation is not the only factor contributing to high platelet reactivity. Moreover, there are more polymorphisms of the *CYP2C19* gene, although most are very rare [13], which may be associated with increased thrombotic or bleeding risk. However, data on these other alleles are conflicting [33]. Therefore, our strategy based solely on the *CYP2C19* genotype may not be the most useful strategy for some patients.

In conclusion, in patients with STEMI undergoing primary PCI, a *CYP2C19* genotype-guided strategy for selection of oral P2Y₁₂ inhibitor therapy was noninferior to standard treatment with ticagrelor or prasugrel at 12 months with respect to thrombotic events and resulted in a lower incidence of bleeding.

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Supplementary appendix

Major protocol revision May 2012

When the trial started in July 2011, clopidogrel was the recommended P2Y₁₂ inhibitor. Patients in the standard-treatment group therefore received clopidogrel and patients in the genotype-guided group received either clopidogrel, or, if they carried *CYP2C19* *2 or *3 loss-of-function alleles, they received more potent antithrombotic therapy with prasugrel (See Protocol Version 3, available online at NEJM.org). At the time, we hypothesised that a genotype-guided strategy would be superior to standard treatment with clopidogrel with regard to a primary outcome consisting of only thrombotic events (all-cause death, myocardial infarction, stent thrombosis, urgent target-vessel revascularization and stroke). We also wanted to assess the safety of this strategy in terms of non-CABG related major bleeding.

In August 2011, the European Society of Cardiology published a guideline for patients with acute coronary syndromes without ST-segment elevation, which was later published in the European Heart Journal in December 2011 [1]. In this guideline the use of ticagrelor or prasugrel was recommended over clopidogrel in these patients. We anticipated that these changes would also follow in the guideline for patients with ST-segment elevation, which was published a year later, in 2012. In anticipation of this change we amended the protocol.

In the amended protocol, dated 16 February 2012, the standard-treatment group received either ticagrelor or prasugrel, as recommended by the guidelines (See Protocol Version 5, available online at NEJM.org). No changes were made to the genotype-guided group. Enrolment of patients under the amended protocol began on May 12, 2012.

Because of this change, patients in the genotype-guided group who did not carry *CYP2C19* *2 or *3 loss-of-function alleles now received less potent antithrombotic therapy than patients in the standard-treatment group. This led to changes in the hypotheses and the two primary outcomes. We now hypothesised that the genotype-guided group would be non-inferior for the primary outcome, which included both thrombotic outcomes and PLATO major bleeding. We did not expect a difference in non-CABG related major bleeding between these treatment arms anymore, but we did expect there to be an effect on all bleedings. Therefore, we hypothesised that the genotype-guided group would be superior in reducing combined PLATO major and minor bleeding.

Supplementary Figure S1. Study design and groups

The figure shows the standard-treatment group and the genotype-guided group. Patients were randomly assigned (1:1) during or shortly after primary PCI. In the standard-treatment group, the protocol mandated that patients were treated with either prasugrel or ticagrelor for at least 1 year. In the genotype-guided group, *CYP2C19* genetic testing was performed as soon as possible. Carriers of *CYP2C19**2 or *3 loss-of-function alleles were treated with ticagrelor or prasugrel, while noncarriers (*1/*1) were treated with clopidogrel for at least 1 year. LoF: Loss-of-function, PCI: Percutaneous Coronary Intervention, R*: Randomization.

Supplementary Figure S2. Event rate curve for the secondary outcome of vascular death, myocardial infarction, definite stent thrombosis and stroke

The panel shows the cumulative incidence of the secondary thrombotic outcome, consisting of death due to vascular causes, myocardial infarction, definite stent thrombosis and stroke (Absolute difference -0.3%; 95% CI, -1.4% to 0.8%; hazard ratio 0.83; 95% CI, 0.53 to 1.31). The inset shows the same data on an enlarged scale.

Supplementary Figure S3. Event rate curve for the secondary outcome of PLATO minor bleeding

The panel shows the cumulative incidence of PLATO minor bleeding (hazard ratio 0.72; 95% CI, 0.55 to 0.94). The inset shows the same data on an enlarged scale.

Supplementary Figure S4. Subgroup analyses for the combined primary thrombotic and bleeding outcome

Analyses for 26 pre-specified subgroups for the primary combined thrombotic and bleeding outcome. eGFR: estimated glomerular filtration rate in ml/min/1.73m2 calculated using the CKD-EPI formula; CAD: coronary artery disease consisting of a history of PCI, CABG, MI or known coronary stenosis >50%; CABG: Coronary artery bypass grafting surgery; MI: Myocardial infarction; PAD: Peripheral artery disease; PCI: percutaneous coronary intervention; PPI: Proton-pump inhibitor; TIA: Transient ischemic attack.

Supplementary Figure S5. Subgroup analyses for the combined primary bleeding outcome

Analyses for 26 pre-specified subgroups for the primary bleeding outcome. eGFR: estimated glomerular filtration rate in ml/min/1.73m2 calculated using the CKD-EPI formula; CAD: coronary artery disease consisting of a history of PCI, CABG, MI or known coronary stenosis >50%; CABG: Coronary artery bypass grafting surgery; MI: Myocardial infarction; PAD: Peripheral artery disease; PCI: percutaneous coronary intervention; PPI: Proton-pump inhibitor; TIA: Transient ischemic attack.

Supplementary Table S1. Exclusion criteria

<ul style="list-style-type: none">- Unable to provide informed consent or have a life expectancy of less than one year- Active malignancy causing an increased bleeding risk, at the investigator's opinion- Women who are known to be pregnant or who have given birth within the past 90 days or who are breastfeeding- Having received thrombolytic therapy within the previous 24 hours or oral anticoagulants during the previous 7 days- Severe renal function impairment needing dialysis- Confirmed or persistent severe hypertension (Systolic Blood Pressure (SBP) > 180 mmHg and/or Diastolic Blood Pressure >110 mmHg) at randomization- Contraindication to anticoagulation or an increased bleeding risk, at the investigator's opinion- Cardiogenic shock (SBP ≤ 80mmHg for >30 mins) or needing Intra-Aortic Balloon Pump- History of major surgery, severe trauma, fracture or organ biopsy within 90 days prior to randomization- Clinically significant out of range values for platelet count or haemoglobin at screening, at the investigator's opinion
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SBP: Systolic blood pressure.

Supplementary Table S2. Secondary thrombotic outcomes

<ul style="list-style-type: none">- Composite end points of :<ul style="list-style-type: none">o Vascular death, recurrent MI [2], definite stent thrombosis (ST) [3], strokeo Vascular death, readmission for acute coronary syndrome (ACS), definite ST, stroke, urgent target vessel revascularization (uTVR)- Individual efficacy outcome events:<ul style="list-style-type: none">o All deaths (and the following sub-categories separately)<ul style="list-style-type: none">▪ Vascular deatho Readmission ACS (and the following sub-categories separately)<ul style="list-style-type: none">▪ Recurrent MIo Stroke (and the following sub-categories separately)<ul style="list-style-type: none">▪ Ischemic stroke▪ Hemorrhagic strokeo Definite ST (and the following sub-categories separately)<ul style="list-style-type: none">▪ Any ST (definite/probable/possible)o uTVR by either percutaneous coronary intervention or coronary artery bypass surgery

ACS: Acute coronary syndrome, MI: Myocardial infarction, ST: stent thrombosis, uTVR: Urgent target vessel revascularization

Supplementary Table S3. Secondary bleeding outcomes

<div><div>○ Platelet Inhibition and Patient Outcome (PLATO) bleeding [4]</div><div><div>▪ PLATO major bleeding</div><div>▪ PLATO life threatening or fatal bleeding (and the following subcategories separately)<div><div>• Fatal bleeding</div><div>• Intracranial bleeding</div><div>• Fatal intracranial bleeding</div><div>• Nonfatal intracranial bleeding</div></div></div><div>▪ Non-CABG related PLATO major bleeding</div><div>▪ Non-CABG related PLATO major + minor bleeding</div><div>▪ PLATO minor bleeding</div></div></div> <tr><td><div><div>○ Bleeding Academic Research Consortium (BARC) bleeding [4]</div><div><div>▪ BARC 3/4/5</div><div>▪ BARC 3/5</div><div>▪ BARC 2/3/4/5</div><div>▪ BARC 2/3/5</div><div>▪ BARC 2</div></div></div></td></tr> <tr><td><div><div>○ Thrombolysis in Myocardial Infarction (TIMI) bleeding [4]</div><div><div>▪ TIMI major bleeding</div><div>▪ TIMI major + minor bleeding</div><div>▪ Non-CABG related TIMI major bleeding</div><div>▪ Non-CABG related TIMI major + minor bleeding</div><div>▪ TIMI minor bleeding</div></div></div></td></tr> <tr><td><div><div>○ Bleeding requiring Red Blood Cell transfusion</div></div></td></tr>	<div><div>○ Bleeding Academic Research Consortium (BARC) bleeding [4]</div><div><div>▪ BARC 3/4/5</div><div>▪ BARC 3/5</div><div>▪ BARC 2/3/4/5</div><div>▪ BARC 2/3/5</div><div>▪ BARC 2</div></div></div>	<div><div>○ Thrombolysis in Myocardial Infarction (TIMI) bleeding [4]</div><div><div>▪ TIMI major bleeding</div><div>▪ TIMI major + minor bleeding</div><div>▪ Non-CABG related TIMI major bleeding</div><div>▪ Non-CABG related TIMI major + minor bleeding</div><div>▪ TIMI minor bleeding</div></div></div>	<div><div>○ Bleeding requiring Red Blood Cell transfusion</div></div>
<div><div>○ Bleeding Academic Research Consortium (BARC) bleeding [4]</div><div><div>▪ BARC 3/4/5</div><div>▪ BARC 3/5</div><div>▪ BARC 2/3/4/5</div><div>▪ BARC 2/3/5</div><div>▪ BARC 2</div></div></div>			
<div><div>○ Thrombolysis in Myocardial Infarction (TIMI) bleeding [4]</div><div><div>▪ TIMI major bleeding</div><div>▪ TIMI major + minor bleeding</div><div>▪ Non-CABG related TIMI major bleeding</div><div>▪ Non-CABG related TIMI major + minor bleeding</div><div>▪ TIMI minor bleeding</div></div></div>			
<div><div>○ Bleeding requiring Red Blood Cell transfusion</div></div>			

BARC: Bleeding academic research consortium; CABG: Coronary artery bypass grafting surgery; PLATO: Platelet Inhibition and Patient Outcomes; TIMI: Thrombolysis in Myocardial Infarction.

Supplementary Table S4. Screenings log St. Antonius Hospital

2508 patients screened in St. Antonius Hospital
1257 randomized
1251 excluded
311 – did not meet inclusion criteria
122 Symptoms >12h
149 No stent
59 No ST-segment elevation myocardial infarction
940 – met an exclusion criterion
269 Not possible to obtain informed consent within 48h
91 Oral anticoagulation
129 Resuscitated
64 Cardiogenic shock or severe hypertension
37 Increased bleeding risk
30 Language barrier
84 Contraindication for aspirin or a P2Y ₁₂ inhibitor
8 Life expectancy of <1 year
5 Recent major surgery
114 Declined to participate
62 Participated in competing trial
6 Other/unknown reason
15 Stroke§

§ Stroke was an exclusion criterion before May 2012. After the major protocol amendment, this was no longer an exclusion criterion.

Supplementary Table S5. Angiographic and procedural characteristics

Table S5. Angiographic and procedural characteristics according to study group				
Characteristics	Genotype-guided (N=1242)	Missing	Standard treatment (N=1246)	Missing
Aspirin use before primary percutaneous coronary intervention	1232 (99.4)	2	1238 (99.4)	1
Received a loading dose	1190 (97.7)	24	1202 (98.2)	22
P2Y ₁₂ inhibitor use before primary percutaneous coronary intervention	1198 (96.8)	5	1190 (96.0)	6
Ticagrelor	1096 (88.6)		1083 (87.3)	
Prasugrel	14 (1.1)		14 (1.1)	
Clopidogrel	88 (7.1)		93 (7.5)	
Access site – no. (%)		26		32
Brachial	0		1 (0.1)	
Femoral	364 (29.3)		342 (27.4)	
Radial	852 (68.6)		871 (69.9)	
Number of diseased coronary vessels ≥50% – no. (%)		3		1
1	634 (51.2)		675 (54.2)	
2	417 (33.7)		376 (30.2)	
3	188 (15.1)		194 (15.6)	
Anticoagulant used during coronary intervention – no. (%)		1		1
Bivalirudin	56 (4.5)		52 (4.2)	
Low molecular weight heparin	2 (0.2)		3 (0.2)	
Unfractionated heparin	1176 (94.8)		1184 (95.0)	

Use of glycoprotein IIb/IIIa antagonist – no. (%)	533 (43.5)	16	532 (43.1)	13
Coronary vessels treated during index percutaneous coronary intervention – no. (%)		3		1
Left main	4 (0.3)		9 (0.73)	
Left anterior descending	525 (42.3)		515 (41.3)	
Ramus circumflex	212 (17.1)		233 (18.7)	
Right coronary artery	525 (42.3)		515 (41.3)	
Bypass graft	5 (0.4)		6 (0.5)	
Ostial lesion – no. (%)	76 (6.4)	59	65 (5.5)	62
Bifurcation lesion – no. (%)	214 (18.1)	59	239 (20.2)	61
Stent type – no. (%)		7		0
Bare metal stent	60 (4.8)		50 (4.0)	
Bioresorbable vascular scaffold	9 (0.7)		16 (1.3)	
Drug eluting stent	1167 (94.0)		1174 (94.2)	
Plain old balloon angioplasty	17 (1.4)		23 (1.9)	
Total stent length - mm	27.0±14.8	67	28.0±15.3	73

Plus-minus values are mean±SD.

Supplementary Table S6. Characteristics at discharge

Table S6. Characteristics at discharge according to study group				
Characteristics	Genotype -guided (N=1242)	Missing	Standard treatment (N=1246)	Missing
Left ventricular ejection fraction – no. (%)		231		211
Normal (≥50%)	578 (57.2)		601 (58.1)	
Moderately reduced (≥30% - <50%)	407 (40.3)		408 (39.4)	
Severely reduced (<30%)	26 (2.6)		26 (2.5)	
Other Medication at discharge – no. (%)				
Beta blocker	1110 (89.5)	0	1099 (88.6)	0
ACE inhibitor	942 (76.0)	0	966 (77.9)	0
Angiotensin-II- receptor blocker	119 (9.6)	0	109 (8.8)	0
Calcium-channel inhibitor	129 (10.4)	0	121 (9.8)	0
Proton-pump inhibitor	964 (77.7)	0	954 (76.9)	0
Statin treatment	1207 (97.3)	0	1200 (96.8)	0

Supplementary Table S7. Prevalence of genetic variants in the genotype-guided group

Genotype	Number of patients (%)
*1/*1	835 (67.2)
*1/*2	353 (28.4)
*1/*3	4 (0.3)
*2/*2	30 (2.4)
*2/*3	2 (0.2)
*3/*3	0
Not available	18 (1.4)

*1/*1: Extensive metabolizer;
*1/*2 and *1/*3: Intermediate metabolizer;
*2/*2, *2/*3 and *3/*3: Poor metabolizer.

Supplementary Table S8. Time intervals from symptom onset to initiation of assigned study drug

	Genotype-guided	Missing	Standard-treatment	Missing
Median symptom onset to balloon time in minutes (IQR)	150 (104 – 236)	61	149 (101 – 233)	68
Median first medical treatment to balloon time in minutes (IQR)	50 (35 – 71)	188	50 (35 – 72)	180
Median time from start of PCI until randomization in hours (IQR)	14.4 (3.7 – 23.6)	61	15.0 (4.1 – 22.9)	67
Median time from randomization to initiation of assigned study drug in hours (IQR)	TaqMan – patients at all centres§	23.3 (21.3 – 50.3)	21.6 (11.8 – 23.0)†	9
	TaqMan – patients not at St. Antonius hospital‡	70.5 (44.9 – 167.6)		
	Spartan Rx¶	21.4 (17.6 – 38.0)		

§ TaqMan was used in 582 patients. Of these, 363 switched P2Y₁₂ inhibitor based on their genetic result.

‡ TaqMan was used in 181 patients included from sites other than the St. Antonius Hospital where the central lab was located. Of these, 97 switched P2Y₁₂ inhibitor based on their genetic result.

¶ Spartan RX was used in 652 patients. Of these 404 switched P2Y₁₂ inhibitor based on genetic result.

† In the standard-treatment group 54 patients switched after randomization. Other patients were already on ticagrelor or prasugrel.

Supplementary Tables S9A, S9B & S9C. Discontinuing and switching P2Y₁₂ inhibitor therapy

Table 9A. Reasons for discontinuing or switching P2Y₁₂ inhibitors

	Genotype-guided (N)	Standard treatment (N)
Total	126	256
Side effects	60	123
CABG	13	27
OAC	19	54
Other	24	30
Unknown	10	22

One patient can have switched multiple times. CABG: Coronary Artery Bypass Grafting surgery; OAC: Oral Anticoagulants.

Table 9B. Overview of switches

Switches	Genotype-guided (N)	Standard treatment (N)
Ticagrelor to clopidogrel	65	199
Ticagrelor to prasugrel	13	22
Ticagrelor to no P2Y ₁₂ inhibitor	15	20
Clopidogrel to ticagrelor	16	4
Clopidogrel to prasugrel	2	0
Clopidogrel to no P2Y ₁₂ inhibitor	10	5
Prasugrel to ticagrelor	0	1
Prasugrel to clopidogrel	0	3
Prasugrel to no P2Y ₁₂ inhibitor	1	0
Other	4	2

Table 9C. Side Effects Causing Patients to Switch

	Ticagrelor	Prasugrel	Clopidogrel
Dyspnea	106	0	1
Bleeding	28	1	4
Gastro-intestinal bleeding	9	0	2
Itching or rash	10	2	3
Fatigue	13	0	1
Other/unknown	11	1	1

Some switches had more than one cause.

Supplementary Table S10. P2Y₁₂ inhibitor use during follow-up

	1 month after primary PCI		6 months after primary PCI		12 months after primary PCI	
P2Y ₁₂ inhibitor	Genotype-guided	Standard treatment	Genotype-guided	Standard treatment	Genotype-guided	Standard treatment
Ticagrelor – no (%)	464 (37.6)	1098 (89.0)	416 (33.9)	1001 (81.4)	395 (32.4)	968 (79.0)
Prasugrel – no. (%)	15 (1.2)	30 (2.4)	24 (2.0)	43 (3.5)	24 (2.0)	44 (3.6)
Clopidogrel – no (%)	753 (61.0)	101 (8.2)	780 (63.6)	174 (14.2)	777 (63.6)	190 (15.5)
None – no. (%)	3 (0.2)	5 (0.4)	6 (0.5)	11 (0.9)	25 (2.0)	23 (1.9)

PCI: Percutaneous coronary intervention.

Supplementary Table S11. Per-protocol analysis

Outcome	Genotype-guided (N=1153) No. of patients (%)	Standard treatment (N=1226)	Hazard ratio (95% CI)	P value
Primary outcomes				
Non-inferiority: Primary endpoint (all-cause death, MI, definite ST, stroke PLATO major bleeding)	55	70	Absolute difference: -1.3% (-2.8% to 0.2%)	
Primary endpoint (all-cause death, MI, definite ST, stroke PLATO major bleeding)	55 (4.8)	70 (5.7)	0.78 (0.55-1.11)	0.17
Co-primary endpoint (PLATO major & minor bleeding)	114 (9.9)	154 (12.6)	0.73 (0.57-0.92)	0.009

MI: Myocardial infarction; pPCI: primary percutaneous coronary intervention; PLATO: Platelet Inhibition and Patient Outcomes; ST: Stent thrombosis.

Supplementary Table S12. Sensitivity analyses

Outcome	Genotype-guided (N=1242) No. of patients (%)	Standard treatment (N=1246) No. of patients (%)	Hazard ratio (95% CI)	P value
Including events in the 24 hours after pPCI				
Non-inferiority: Primary endpoint (all-cause death, MI, definite ST, stroke PLATO major bleeding)	70	83	Absolute difference: -0.9% (-2.5% to 0.6%)	
Primary endpoint (all-cause death, MI, definite ST, stroke PLATO major bleeding)	70 (5.6)	83 (6.7)	0.84 (0.61-1.16)	0.29
Co-primary endpoint (PLATO major & minor bleeding)	151 (12.2)	190 (15.2)	0.78 (0.63-0.96)	0.02
Using stratification per center				
Primary endpoint (all-cause death, MI, definite ST, stroke PLATO major bleeding)	63 (5.1)	73 (5.9)	0.86 (0.62-1.21)	0.40
Co-primary endpoint (PLATO major & minor bleeding)	122 (9.8)	156 (12.5)	0.76 (0.60-0.97)	0.03

MI: Myocardial infarction; pPCI: primary percutaneous coronary intervention; PLATO: Platelet Inhibition and Patient Outcomes; ST: Stent thrombosis.

Supplementary appendix references

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5

Clonidogrel versus ticagrelor or prasugrel after primary percutaneous coronary intervention according to CYP2C19 genotype. A POPular genetics subanalysis

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Abstract

Background

Guidelines favour ticagrelor or prasugrel over clopidogrel in patients with myocardial infarction. However, the POPular Genetics trial (Patient Outcome After Primary Percutaneous Coronary Intervention [PCI]) showed that in patients with primary PCI, a *CYP2C19* genotype-guided strategy was associated with a lower bleeding risk without increasing thrombotic risk, compared with routine ticagrelor/prasugrel treatment. Nevertheless, optimal P2Y₁₂ inhibitor treatment in specific *CYP2C19* genetic subgroups is still a subject of debate.

Methods

A prespecified subanalysis of the POPular Genetics trial was performed, using patients in whom *CYP2C19**2, *3, and *17 genotypes was determined. Two different analyses were planned. The first assessed the effect of the *CYP2C19**17 allele in clopidogrel-treated patients. The second compared the effect of clopidogrel in noncarriers of a loss-of-function allele with ticagrelor/prasugrel-treated patients, irrespective of *CYP2C19* genotype. Main outcomes were a thrombotic outcome (cardiovascular death, myocardial infarction, stent thrombosis, and stroke) and a bleeding outcome (PLATO [Platelet Inhibition and Patient Outcomes] major and minor bleeding) after 12 months.

Results

A total of 2429 patients were used for analyses. In the first analysis, the *CYP2C19**17 polymorphism was not found to have a significant influence on thrombotic (adjusted hazard ratio, 0.95 [95% CI, 0.45–2.02]) or bleeding outcomes (adjusted hazard ratio, 0.74 [95% CI, 0.48–1.18]). In the second analysis, clopidogrel was associated with a lower number of bleeding events compared with ticagrelor/prasugrel (9.9% versus 11.7%, adjusted hazard ratio, 0.74 [95% CI, 0.56–0.96]), without a significant increase in thrombotic events (3.4% versus 2.5%, adjusted hazard ratio, 1.14 [95% CI, 0.68–1.90]).

Conclusions

In patients with primary PCI not carrying a *CYP2C19* loss-of-function allele, the use of clopidogrel compared with ticagrelor or prasugrel was associated with lower bleeding rates, without an increase in thrombotic events. No effect on clinical outcomes was found for the *CYP2C19**17 polymorphism.

Introduction

Dual antiplatelet therapy, consisting of aspirin and a P2Y₁₂ inhibitor, remains the cornerstone of antithrombotic treatment in patients with myocardial infarction. Guidelines favour ticagrelor and prasugrel because these showed a reduction in thrombotic events as compared with clopidogrel [1,2]. This higher efficacy, however, is hampered by a higher bleeding risk [3,4].

Clopidogrel is a prodrug, transformed into its active metabolite by hepatic cytochrome P450 enzymes in the liver. There is a large variability in antiplatelet effect in patients treated with clopidogrel, which in part can be explained by genetic variations in the *CYP2C19* gene [5]. Patients carrying a *CYP2C19**2 or *3 loss-of-function (LoF) allele are at higher risk for having high on-treatment platelet reactivity when treated with clopidogrel, which is associated with a higher risk of stent thrombosis and recurrent atherothrombotic events [6]. In patients carrying a *CYP2C19**17 gain-of-function allele, clopidogrel efficacy might be better, with possibly higher bleeding rates [7,8]. However, data on the influence of *CYP2C19**17 on clinical outcome are conflicting and mostly derived from observational studies [9]. Its clinical relevance is, therefore, not clear.

In the recently published POPular Genetics trial (Patient Outcome After Primary Percutaneous Coronary Intervention [PCI]), a *CYP2C19* genotype-guided antiplatelet strategy, using clopidogrel in patients without LoF allele and ticagrelor or prasugrel in patients carrying ≥ 1 LoF alleles, showed a reduction in bleeding risk (defined as PLATO [Platelet Inhibition and Patient Outcomes] major or minor bleeding) compared with standard treatment with ticagrelor or prasugrel, without an increase in thrombotic risk [10]. Moreover, in a subanalysis of the PLATO trial, ticagrelor was not superior to clopidogrel regarding the thrombotic outcome [11]. Since then, thrombotic event rates have declined considerably [12]. At the same time, the incidence of bleeding events, which are associated with a substantial morbidity and mortality risk, remained high [13]. Therefore, the question of which antiplatelet strategy is best to balance thrombotic and bleeding risk in patients with ST-segment-elevation myocardial infarction after primary PCI is still open, in particular for different subgroups of noncarriers of *CYP2C19* LoF alleles.

In this prespecified subanalysis of the POPular Genetics trial, we study the effect of the *CYP2C19**17 allele in clopidogrel-treated patients and the safety and efficacy of clopidogrel in patients without an LoF allele, compared with ticagrelor and prasugrel.

Figure 1. Flowchart of included patients

Figure 1. Flowchart of patients included in the different subgroup analyses. LoF indicates loss-of-function

Methods

Study design

The detailed design and results of the POPular Genetics trial have been reported previously [10,14]. In brief, POPular Genetics was an open-label, randomized, multicentre trial with 2488 patients with ST-segment-elevation myocardial infarction undergoing primary PCI. It compared a standard treatment with ticagrelor or prasugrel with a *CYP2C19* genotype-guided strategy, where patients received clopidogrel if they did not carry a *CYP2C19**2 or *3 LoF allele and ticagrelor or prasugrel if they were carrier of such a LoF allele. Treatment and follow-up duration were 12 months. An institutional review board approved the trial, and all participants provided informed consent.

Blood samples were collected from patients in both treatment groups. During the trial, *CYP2C19* genotyping was performed in the genotype-guided group, where the presence of the *CYP2C19**2 and *3 polymorphism was determined using the Spartan RX point-of-care system or the TaqMan StepOnePlus assay. After the trial was completed, genotyping was performed in all patients in the standard treatment arm, and both groups were tested for *CYP2C19**17. This was done by LGC Biosearch Technologies (Hoddesdon, United Kingdom) using a competitive allele-specific genotyping assay. For the analyses, an intention to treat analysis was performed in which patients were divided into a clopidogrel-treated or ticagrelor/prasugrel-treated group, based on the drug prescribed at discharge. Two different analyses were performed. The first analysis compared clopidogrel in *CYP2C19**1/*1 patients with clopidogrel in patients with a *1/*17 or *17/*17 genotype. No separate analysis for homozygous *CYP2C19**17 carriers was performed because of the low number of patients in this group (n=35). The second analysis compared clopidogrel-treated patients not carrying a *CYP2C19**2 or *3 LoF allele with ticagrelor- or prasugrel-treated patients irrespective of their *CYP2C19* metabolizer status. In addition, we performed 2 sensitivity analyses, one including only patients without LoF alleles in both the clopidogrel and ticagrelor- or prasugrel-treated group and one on-treatment analysis.

Table 1. (see next page) ACE: angiotensin-converting enzyme; ATII, angiotensin II; BMI, body mass index; *Creatinine clearance was calculated using the CKD-EPI formula

Table 1. Baseline characteristics of clopidogrel-treated patients according to *CYP2C1917 carrier status**

	<i>CYP2C19</i> *1/*1; N=420	<i>CYP2C19</i> *17 carriers; N=312	<i>P</i> value
Age, y, mean±SD	61.2±10.8	62.8±11.2	0.06
Female sex, n (%)	105 (25.0)	89 (28.5)	0.33
BMI, kg/m2, mean±SD	27.7±9.85	27.1±4.14	0.28
Creatinine clearance <60 mL/min/1.73m2 at baseline,* n (%)	39 (9.4)	29 (9.4)	1.0
History, n (%)			
Current smoker	199 (48.2)	126 (40.6)	0.05
Hypertension	174 (41.4)	127 (40.7)	0.90
Dyslipidaemia	83 (19.8)	63 (20.2)	0.96
Diabetes	43 (10.2)	32 (10.3)	1.0
Coronary artery disease	47 (11.2)	32 (10.3)	0.78
Peripheral arterial disease	17 (4.1)	11 (3.5)	0.87
Stroke	7 (1.7)	3 (1.0)	0.53
Bleeding	9 (2.1)	8 (2.6)	0.90
Discharge medication, n (%)			
Aspirin	404 (96.4)	302 (96.8)	0.94
P2Y ₁₂ inhibitor after discharge			
Clopidogrel	420 (100)	312 (100)	
Vitamin K antagonist	26 (6.2)	13 (4.2)	0.30
Novel anticoagulant	6 (1.4)	8 (2.6)	0.41
ACE inhibitor	320 (76.4)	227 (72.8)	0.30
ATII antagonist	42 (10.0)	33 (10.6)	0.90
Beta blocker	370 (88.3)	276 (88.5)	1.0
Statin	408 (97.4)	300 (96.2)	0.47
Proton pump inhibitor	332 (79.2)	247 (79.2)	1.0
Procedural characteristics			
Access site			0.54
Femoral	133 (31.7)	106 (34.2)	
Radial	286 (68.3)	204 (65.8)	
Multivessel disease	205 (48.8)	168 (53.8)	0.20
Stent			
None	10 (2.4)	4 (1.3)	0.42
Bare metal stent	21 (5.0)	20 (6.4)	0.51
Biovascular scaffold	4 (0.1)	3 (1.0)	1.0
Drug eluting stent	393 (93.6)	292 (93.6)	1.0
Ostial lesion	28 (6.7)	20 (6.5)	1.0
Bifurcation lesion	82 (19.6)	64 (20.6)	0.79
Total stent length, mm, mean±SD	27.6±14.0	26.6±13.3	0.33

Table 2. Baseline characteristics of clopidogrel in patients without *CYP2C192 or *3 LoF allele versus ticagrelor or prasugrel in all patients (irrespective of *CYP2C19* genotype).**

	Clopidogrel (no LoF allele); N=821	Ticagrelor and prasugrel; N=1608	<i>P</i> value
Age, y, mean±SD	62.0±10.9	61.3±11.3	0.14
Female sex, n (%)	224 (27.3)	388 (24.1)	0.10
BMI, kg/m2, mean±SD	27.5±7.66	27.2±4.22	0.32
Creatinine clearance <60 mL/(min·1.73 m2) at baseline,* n (%)	75 (9.2)	148 (9.2)	1.0
History, n (%)			
Current smoker	372 (45.9)	738 (46.3)	0.90
Hypertension	348 (42.4)	655 (40.7)	0.46
Dyslipidaemia	172 (21.0)	332 (20.7)	0.91
Diabetes	89 (10.8)	186 (11.6)	0.64
Coronary artery disease	82 (10.0)	157 (9.8)	0.92
Peripheral arterial disease	31 (3.8)	41 (2.6)	0.12
Stroke	10 (1.2)	20 (1.2)	1.0
Bleeding	19 (2.3)	33 (2.1)	0.78
Discharge medication, n (%)			
Aspirin	794 (96.8)	1578 (98.3)	0.03
P2Y ₁₂ inhibitor after discharge			
Clopidogrel	821 (100)	0 (0.0)	
Prasugrel 10mg	0 (0.0)	38 (2.4)	
Prasugrel 5mg	0 (0.0)	5 (0.3)	
Ticagrelor	0 (0.0)	1565 (97.3)	
Vitamin K antagonist	42 (5.1)	22 (1.4)	<0.001
Novel anticoagulant	14 (1.7)	3 (0.2)	<0.001
ACE inhibitor	618 (75.4)	1252 (78.0)	0.16
ATII antagonist	77 (9.4)	147 (9.2)	0.91
Beta blocker	726 (88.5)	1435 (89.4)	0.56
Statin	795 (97.0)	1560 (97.2)	0.83
Proton pump inhibitor	656 (80.0)	1217 (75.8)	0.02
Procedural characteristics			
Access site			0.23
Femoral	249 (31.1)	444 (28.2)	
Radial	552 (68.9)	1127 (71.7)	
Multivessel disease	402 (49.1)	739 (46.0)	0.17
Stent			

None	14 (1.7)	25 (1.6)	0.91
Bare metal stent	44 (5.4)	62 (3.9)	0.11
Biovascular scaffold	8 (1.0)	17 (1.1)	1.0
Drug eluting stent	766 (93.3)	1520 (94.5)	0.26
Ostial lesion	53 (6.8)	85 (5.6)	0.27
Bifurcation lesion	154 (19.8)	287 (18.7)	0.59
Total stent length, mm, mean±SD	27.3±13.7	28.8±17.8	0.07

Table 2. (see previous page) ACE: angiotensin-converting enzyme; ATII, angiotensin II; BMI, body mass index. *Creatinine clearance was calculated using the CKD-EPI formula.

Statistical analysis

This prespecified subanalysis was not prospectively powered and is based on the number of patients in the original trial of whom the CYP2C19 genetic profile was available. The outcomes were a thrombotic outcome consisting of cardiovascular death, myocardial infarction, stent thrombosis and stroke, a bleeding outcome consisting of PLATO major and minor bleeding, and the individual components of the thrombotic and bleeding outcome. The definitions were identical to those used in the POPular Genetics trial [10]. All outcomes were adjudicated by a blinded event committee.

Variables are presented as numbers (percentages) and mean±SD. Missing baseline variables were not imputed. P values were calculated using Student *t* test for continuous variables, χ^2 tests for categorical variables, and 1-way ANOVAs for variables with multiple categories. A P value below 0.05 was considered statistically significant. Kaplan-Meier curves were estimated and the log-rank test was used to calculate P values. If a patient was lost to follow-up, it was censored after the last known contact. Cox proportional hazard models were used to calculate hazard ratios (HR) and the 95% CI. The Efron approximation was used to handle ties. To adjust for possible confounders, all baseline characteristics with a P<0.10 were selected for univariate regression analysis. If there was a significant interaction (P<0.05) in the univariate analysis, they were selected for multivariable regression analysis. The final model included only those characteristics with a significant interaction in the multivariable analysis. Main effects were included in the model. All analyses were performed using R version 3.6.0.

Table 3. Outcomes in clopidogrel-treated patients according to CYP2C19*17 carrier status

Outcome, no. of patients (%)	CYP2C19 *1/*1 (N=420)	CYP2C19*17 carriers (N=312)	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)*	Adjusted P value
Thrombotic outcomes					
Cardiovascular death, MI, ST, and stroke	16 (3.8)	12 (3.8)	1.01 (0.48–2.13)	0.95 (0.45–2.02)	0.90
Cardiovascular death	1 (0.2)	3 (1.0)	4.04 (0.42–38.8)	...	0.23
Myocardial infarction	12 (2.9)	6 (1.9)	0.67 (0.25–1.79)	...	0.43
Stroke	3 (0.7)	3 (1.0)	1.34 (0.27–6.66)	...	0.72
ST	1 (0.2)	1 (0.3)	1.35 (0.08–21.6)	...	0.83
Bleeding outcomes					
PLATO major and minor bleeding	47 (11.2)	29 (9.3)	0.82 (0.52–1.31)	0.74 (0.48–1.18)	0.21
PLATO major	10 (2.4)	8 (2.6)	1.08 (0.43–2.74)	0.96 (0.38–2.43)	0.93
PLATO minor	37 (8.8)	22 (7.1)	0.79 (0.47–1.34)	0.73 (0.43–1.24)	0.24

Table 3. Outcomes in Clopidogrel-Treated Patients According to CYP2C19*17 Carrier Status. Only clopidogrel-treated patients with normal CYP2C19 metabolism were included for this analysis (i.e., *1/*1, *1/*17, and *17/*17). MI indicates myocardial infarction, PLATO, Platelet Inhibition and Patient Outcomes; and ST, stent thrombosis. *Significant variables are shown in Table II in the Data Supplement.

Results

Figure 1 shows a flow chart of the selection process of the subgroups. In the POPular Genetics cohort, *CYP2C19**2, *3, and *17 carrier status was available in 2100 patients, of which 1550 did not carry a LoF allele. In the remaining 388 patients, *CYP2C19* carrier status was unknown. Of these 388 patients, 329 were treated with ticagrelor or prasugrel and could therefore be included in the ticagrelor/prasugrel group of the second analysis. Of the 1550 patients not carrying a LoF allele, 821 were treated with clopidogrel and 729 with ticagrelor or prasugrel. Of the 821 clopidogrel-treated patients, 277 had a *1/*17 genotype, and 35 patients had a *17/*17 genotype. The baseline characteristics for the 2 analyses can be found in Tables 1 and 2. All groups were well balanced in baseline characteristics, except for the more common use of proton pump inhibitors and oral anticoagulation in the clopidogrel-treated patients as compared to the ticagrelor- or prasugrel-treated patients. Tables I and II in the Data Supplement show what variables were used in univariate and multivariate regression analysis and what variables were used in the final model.

Effect of the *CYP2C19**17 allele in clopidogrel-treated patients

For this analysis, 420 patients with a *CYP2C19**1/*1 genotype and 312 patients with a *CYP2C19**1/*17 or *17/*17 genotype were available. An overview of the outcomes is presented in Table 3. There were no significant differences between the patients with *CYP2C19**1/*1 genotype versus carriers of a *17 allele for the combined thrombotic outcome (3.8% versus 3.8%, adjusted hazard ratio, 0.95 [95% CI, 0.45–2.02], *P*=0.90; Figure 2A) and the combined bleeding outcome (11.2% versus 9.3%, adjusted hazard ratio, 0.74 [95% CI, 0.48–1.18], *P*=0.21; Figure 2B).

Clopidogrel in patients not carrying a *CYP2C19**2 or *3 allele versus ticagrelor or prasugrel irrespective of *CYP2C19* genotype

For this analysis, 821 clopidogrel and 1608 ticagrelor or prasugrel-treated patients were available. Table 4 shows an overview of the outcomes. There were no significant differences in the combined thrombotic outcome (3.4% versus 2.5%, adjusted hazard ratio, 1.14 [95% CI, 0.68–1.90], *P*=0.62; Figure 3A), whereas the combined bleeding outcome occurred significantly less frequently in clopidogrel-treated patients (9.9% versus 11.7%, HR, 0.74 [95% CI, 0.56–0.96], *P*=0.03; Figure 3B).

Figure 2. Outcomes on clopidogrel in *1/*1 patients vs *17 carriers

Figure 2. Outcomes on clopidogrel in *1/*1 patients vs *17 carriers. Kaplan-Meier curves for (A) the thrombotic outcome, defined as cardiovascular death, myocardial infarction, stroke, and (B) the bleeding outcome, defined as PLATO (Platelet Inhibition and Patient Outcomes) major and minor bleeding in clopidogrel-treated *1/*1 patients vs *17 carriers. *P* values are adjusted for baseline differences.

Table 4. Outcomes of clopidogrel in patients without CYP2C19*2 or *3 Loss-of-Function allele versus ticagrelor or prasugrel irrespective of CYP2C19 genotype

Outcome, no. of patients (%)	Clopidogrel (N=821)	Ticagrelor or prasugrel (N=1608)	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)*	Adjusted P value
Thrombotic outcomes					
Cardiovascular death, MI, ST, and stroke	28 (3.4)	41 (2.5)	1.35 (0.83–2.18)	1.14 (0.68–1.90)	0.62
Cardiovascular death	4 (0.5)	8 (0.5)	0.98 (0.29–3.25)	0.44 (0.11–1.84)	0.26
Myocardial infarction	18 (2.2)	25 (1.6)	1.42 (0.77–2.59)	1.47 (0.79–2.77)	0.23
Stroke	6 (0.7)	13 (0.8)	0.90 (0.34–2.38)	0.82 (0.29–2.34)	0.71
ST	2 (0.2)	3 (0.2)	1.30 (0.22–7.81)	...	0.77
Bleeding outcomes					
PLATO major and minor bleeding	81 (9.9)	188 (11.7)	0.84 (0.64–1.09)	0.74 (0.56–0.96)	0.03
PLATO major	19 (2.3)	33 (2.1)	1.13 (0.64–1.98)	1.00 (0.55–1.81)	1.0
PLATO minor	63 (7.7)	159 (9.9)	0.77 (0.57–1.03)	0.69 (0.51–0.93)	0.02

Table 4. Outcomes of clopidogrel in patients without CYP2C19*2 or *3 Loss-of-Function allele versus ticagrelor or prasugrel irrespective of CYP2C19 genotype. Analysis of clopidogrel-treated patients without loss-of-function allele vs ticagrelor- and prasugrel-treated patients, irrespective of CYP2C19 metabolizer status. MI indicates myocardial infarction, PLATO, Platelet Inhibition and Patient Outcomes; and ST, stent thrombosis.

* Significant variables are shown in Table II in the Data Supplement.

Sensitivity Analyses

Results for the two sensitivity analyses are presented in Table 5. The first analysis included 1550 patients without LoF allele, of which 821 patients were treated with clopidogrel and 729 with ticagrelor or prasugrel. The adjusted HR for the combined thrombotic outcome was 1.11 (95% CI, 0.61–2.03, P=0.72), whereas the adjusted HR for the combined bleeding outcome was 0.59 (95% CI, 0.44–0.80, P<0.001). The on-treatment analysis included the 821 clopidogrel-treated patients and 1613 ticagrelor- or prasugrel-treated patients. The adjusted HR for the combined thrombotic outcome was 0.98 (95% CI, 0.56–1.70, P=0.94), whereas the adjusted HR for the combined bleeding outcome was 0.68 (95% CI 0.52–0.90, P=0.008).

Discussion

In this prespecified subanalysis of the POPular Genetics trial, which included patients with ST-segment-elevation myocardial infarction who underwent primary PCI and compared clopidogrel-treated noncarriers of CYP2C19 LoF alleles with all ticagrelor- and prasugrel-treated patients, treatment with clopidogrel was associated with a significantly lower bleeding rate compared with treatment with ticagrelor or prasugrel. This finding held true in both the sensitivity analyses. The effect was primarily driven by a reduction of PLATO minor bleeding. There was no association found between clopidogrel use and a higher risk for the combined thrombotic outcome, although the number of patients with a recurrent myocardial infarction was numerically higher in clopidogrel-treated patients. These findings match the outcomes of the overall POPular Genetics trial [10]. Also in the PLATO genetic subanalysis, in which 3554 ticagrelor-treated patients without CYP2C19 LoF allele were compared with 3516 clopidogrel-treated patients without CYP2C19 LoF allele, similar to our second sensitivity analysis, the event rate for a combined thrombotic outcome consisting of cardiovascular death, myocardial infarction, and stroke was only numerically lower with the use of ticagrelor, not reaching statistical significance (8.8% versus 10.0%, HR, 0.86 [95% CI, 0.74–1.01], P=0.06) [11]. This in contrast to the overall PLATO trial, which showed clear benefit in reducing thrombotic risk with ticagrelor, compared with clopidogrel, at the cost of increased non-coronary artery bypass grafting surgery related major bleeding rates [3].

Figure 3. Outcomes of clopidogrel in patients without loss-of-function vs ticagrelor- or prasugrel-treated patients

Figure 3. Outcomes of clopidogrel in patients without loss-of-function vs ticagrelor- or prasugrel-treated patients. Kaplan-Meier curves for (A) the thrombotic outcome, defined as cardiovascular death, myocardial infarction, stent thrombosis, and stroke, and (B) the bleeding outcome, defined as PLATO (Platelet Inhibition and Patient Outcomes) major and minor bleeding in clopidogrel-treated patients without a loss-of-function allele vs ticagrelor- or prasugrel-treated patients. *P* values are adjusted for baseline differences.

Table 5. Outcomes of the sensitivity analyses

Outcome, no. of patients (%)	Clopidogrel	Ticagrelor or prasugrel	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)*	Adjusted <i>P</i> value
Clopidogrel (n=821) vs ticagrelor or prasugrel (n=729) in patients without LoF alleles					
Cardiovascular death, MI, ST, and stroke	28 (3.4)	21 (2.9)	1.19 (0.68–2.09)	1.11 (0.61–2.03)	0.72
PLATO major and minor bleeding	81 (9.9)	103 (14.1)	0.68 (0.51–0.91)	0.59 (0.44–0.80)	<0.001
Clopidogrel (n=821) vs ticagrelor or prasugrel (n=1613) on-treatment analysis					
Cardiovascular death, MI, ST, and stroke	23 (2.8)	39 (2.4)	1.13 (0.68–1.89)	0.98 (0.56–1.70)	0.94
PLATO major and minor bleeding	72 (8.8)	177 (11.0)	0.76 (0.57–0.99)	0.68 (0.52–0.91)	0.008

Table 5. Outcomes of the sensitivity analyses. Sensitivity analyses of clopidogrel-treated patients without loss-of-function alleles vs ticagrelor- and prasugrel-treated patients. LoF indicates loss-of-function; MI, myocardial infarction, PLATO, Platelet Inhibition and Patient Outcomes; and ST, stent thrombosis. *Significant variables are shown in Table II in the Data Supplement.

Nevertheless, the treatment effect in the PLATO subanalysis was almost similar to that of the main trial, so the lack of statistical significance could also have been due to a lack of power. With respect to the use of prasugrel, a genetic subanalysis of the TRITON-TIMI (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel-Thrombolysis in Myocardial Infarction) 38 trial did not provide a direct comparison between prasugrel and clopidogrel in different *CYP2C19* genetic subgroups but did conclude that clopidogrel-treated patients have significantly worse outcomes when carrying an LoF allele compared with noncarriers, which was not found in prasugrel-treated patients [15].

The primary analysis of the TAILOR-PCI trial (Tailored Antiplatelet Therapy Following PCI) included only patients who were carriers of *2 or *3 LoF alleles [16]. It compared 903 patients in a *CYP2C19* genotype-guided group, thus treated with ticagrelor, to 946 patients in a conventional therapy group, treated with clopidogrel, who were genotyped at the end of the follow-up period. The primary composite end point of cardiovascular death, myocardial infarction, stroke, stent thrombosis, and recurrent ischemia at 12 months, occurred less frequently in patients treated with ticagrelor but without reaching statistical significance (4% versus 5.9%, HR, 0.66 [95% CI, 0.43–1.02], *P*=0.056). Based on the results of these trials, one might conclude that in the absence of a *CYP2C19* LoF allele, the balance in net clinical benefit ends up equal for clopidogrel versus ticagrelor, albeit with a somewhat lower bleeding risk with clopidogrel and somewhat lower thrombotic risk with ticagrelor. In clinical practice, bleeding and thrombotic risk should be weighted to choose the optimal treatment strategy for the individual patient.

Furthermore, we studied the effect of the *CYP2C19**17 gain-of-function polymorphism in clopidogrel-treated patients. This allele has been associated with higher enzyme activity, increased platelet inhibition, and an increased risk for bleeding, which was also found in the genetics subanalysis of the PLATO trial [11,17]. The evidence, however, is conflicting. A meta-analysis performed by Li et al. [8] containing data from 11 studies, found the *CYP2C19**17 allele to be associated with less atherothrombotic events in patients treated with clopidogrel (based on six studies), whereas an increased risk for bleeding events was found (based on four studies). Comparable results were found in a meta-analysis performed by Zabalza et al. [18]. A meta-analysis performed by Bauer et al. [19], however, did not find any significant interaction for stent thrombosis or a composite of major adverse cardiac events related to the

*CYP2C19**17 allele, although bleeding risk was not assessed. In a study performed by Lewis et al [20], *CYP2C19**17 was in significant linkage disequilibrium with *CYP2C19**2 and was not found to be an independent predictor for pharmacokinetic and pharmacodynamic outcome in patients treated with clopidogrel. The effect found for the *17 allele was considered to be mostly, if not entirely, derived from non-independence with the *CYP2C19**2 variant. Our data support this hypothesis, although no significant differences in thrombotic or bleeding outcomes were found according to *CYP2C19**17 genotype. Therefore, our data do not support routine testing of *CYP2C19**17 to guide antiplatelet therapy in clinical practice.

Our findings are clinically relevant for different reasons. Although P2Y₁₂ inhibitors are primarily initiated to reduce thrombotic risk, the inevitable bleeding risk associated with the use of those agents is clearly associated with morbidity and mortality [13]. Therefore, the use of a tailored antiplatelet strategy capable of balancing the bleeding and thrombotic risks is expected to improve overall clinical outcome. Although trials studying the use of platelet function testing in tailoring antiplatelet therapy failed to show superiority compared with standard treatment and is, therefore, not routinely recommended, our results add to the growing body of evidence that *CYP2C19* genotype can be used to tailor antiplatelet therapy [10,21,22]. Recently published results from a large prospective multicentre study in France by Hulot et al. [23] also demonstrate that the availability of genetic results within days after ST-segment-elevation myocardial infarction can have a major impact on the prescription patterns of cardiologists. Additionally, because the use of ticagrelor and prasugrel is far more expensive compared with generic clopidogrel, a strategy in which two-thirds of patients can be treated with generic clopidogrel without losing the efficacy of the antiplatelet treatment is likely to be cost-effective [24]. A cost-effectiveness analysis was also one of the primary objectives of the POPular Genetics trial and is expected to be published in the near future. Finally, ticagrelor is associated with side effects like dyspnoea, which is a frequent reason to switch or even discontinue antiplatelet therapy [25], whereas prasugrel needs a dose adjustment for the elderly and low body weight patients and is contraindicated in patients with previous stroke or transient ischemic attack [4].

Some limitations to our analysis need to be mentioned. First, it is a subanalysis of a larger trial and therefore not powered for its primary outcome. In particular, in the analysis of *CYP2C19**17, the relatively low number of patients led us to the use of a dominant model (combining

patients with *1/*17 and *17/*17 genotype) instead of a recessive model (comparing *17 homozygous patients to *1/*1 and *1/*17 patients). A possible effect limited to patients homozygous for the *CYP2C19**17 allele might have been underestimated or missed. Second, we only tested for the *2 and *3 LoF alleles, since the prevalence of other LoF alleles is very small (<1% for all LoF alleles combined). Therefore, a handful of clopidogrel-treated patients might now be identified as not carrying a LoF allele, although, in fact, they do. Third, due to the low number of patients treated with prasugrel, we cannot draw any conclusions for this drug specifically. Although the recently published ISAR-REACT 5 trial (Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment 5) showed superiority of prasugrel over ticagrelor in patients with acute coronary syndrome, further research is needed to evaluate if our findings also apply to patients treated with prasugrel [26].

Conclusion

In patients after primary PCI not carrying a *CYP2C19* LoF allele, the use of clopidogrel compared with ticagrelor or prasugrel was associated with lower bleeding rates, without significant increase in thrombotic events. No effect on clinical outcome was found for the *CYP2C19**17 gain-of-function polymorphism.

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Supplementary appendix

Table I. Variables used in the *CYP2C19**17 univariate and multivariable regression analysis

Outcome	Significant variables in univariate model	Significant variables in multivariable model
Thrombotic outcomes		
Combined thrombotic outcome (Cardiovascular death, MI, definite ST, stroke)	Age	-
Death from vascular causes	-	-
Myocardial Infarction	-	-
Stroke	-	-
Definite Stent thrombosis	-	-
Bleeding outcomes		
PLATO major & minor bleeding	Age	-
PLATO major	Age	-
PLATO minor	Age	-

Table I: Variables used in univariate and multivariable regression for clopidogrel treated patients stratified according to *CYP2C19**17 carrier status*.
MI: Myocardial infarction, PLATO: platelet inhibition and patient outcome, ST: stent thrombosis.

* Age and smoker were selected for the model.

Table II. Variables used in the *CYP2C19* LoF noncarrier univariate and multivariable regression analysis.

Outcome	Significant variables in univariate model	Significant variables in multivariable model
Thrombotic outcomes		
Combined thrombotic outcome (Cardiovascular death, MI, definite ST, stroke)	Aspirin, vitamin K antagonist, NOAC at discharge	Vitamin K antagonist, NOAC at discharge
Death from vascular causes	Aspirin, vitamin K antagonist, NOAC at discharge	Vitamin K antagonist, NOAC at discharge
Myocardial Infarction	Stent length, NOAC at discharge	Stent length, NOAC at discharge
Stroke	Aspirin, vitamin K antagonist, NOAC at discharge	Vitamin K antagonist, NOAC at discharge
Definite Stent thrombosis	-	-
Bleeding outcomes		
PLATO major & minor bleeding	Sex, vitamin K antagonist, NOAC at discharge	Sex, vitamin K antagonist, NOAC at discharge
PLATO major	Aspirin, vitamin K antagonist, NOAC at discharge	Vitamin K antagonist, NOAC at discharge
PLATO minor	Sex, aspirin, vitamin K antagonist, NOAC at discharge	Vitamin K antagonist, NOAC at discharge

Table II: Variables used in univariate and multivariable regression analysis for clopidogrel in patients without *CYP2C19**2 or *3 LoF allele versus ticagrelor or prasugrel in all patients (irrespective of *CYP2C19* genotype)*.
LoF: loss-of-function, MI: Myocardial infarction, NOAC: Novel anticoagulant, PLATO: platelet inhibition and patient outcome, PPI: proton pump inhibitor, ST: Stent thrombosis.
* Sex, aspirin, PPI, vitamin K antagonist and NOAC at discharge were selected for the model.

6

Clonidogrel in noncarriers of CYP2C19 loss-of-function alleles versus ticagrelor in elderly patients with acute coronary syndrome: A pre-specified sub analysis from the POPular Genetics and POPular Age trials

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Abstract

Background

Patients with acute coronary syndrome (ACS) who are carrying *CYP2C19* loss-of-function alleles derive less benefit from clopidogrel treatment. Despite this, in elderly patients, clopidogrel might be preferred over more potent P2Y₁₂ inhibitors due to a lower bleeding risk. Whether *CYP2C19* genotype-guided antiplatelet treatment in the elderly could be of benefit has not been studied specifically.

Methods

Patients aged 70 years and older with known *CYP2C19**2 and *3 genotype were identified from the POPular Genetics and POPular Age trials. Noncarriers of loss-of-function alleles treated with clopidogrel were compared to patients, irrespective of *CYP2C19* genotype, treated with ticagrelor and to clopidogrel treated carriers of loss-of-function alleles. We assessed net clinical benefit (all-cause death, myocardial infarction, stroke and Platelet Inhibition and Patient Outcomes (PLATO) major bleeding), atherothrombotic outcomes (cardiovascular death, myocardial infarction, stroke) and bleeding outcomes (PLATO major and minor bleeding).

Results

A total of 991 patients were assessed. There was no significant difference in net clinical benefit (17.2% vs. 15.1%, adjusted hazard ratio (adjHR) 1.05, 95% confidence interval (CI) 0.77 – 1.44), atherothrombotic outcomes (9.7% vs. 9.2%, adjHR 1.00, 95% CI 0.66 – 1.50), and bleeding outcomes (17.7% vs. 19.8%, adjHR 0.80, 95% CI 0.62 – 1.12) between clopidogrel in noncarriers of loss-of-function alleles and ticagrelor respectively.

Conclusion

In ACS patients aged 70 years and older, there was no significant difference in net clinical benefit and atherothrombotic outcomes between noncarriers of a loss-of-function allele treated with clopidogrel and patients treated with ticagrelor. The bleeding rate was numerically; though not statistically significant, lower in patients using clopidogrel.

Introduction

In patients with acute coronary syndrome (ACS), dual antiplatelet therapy (DAPT) plays an essential role in preventing recurrent atherothrombotic events [1]. Although clopidogrel is still widely used in combination with aspirin, The Platelet Inhibition and Patient Outcomes (PLATO) [2] and Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel Thrombolysis in Myocardial Infarction (TRITON TIMI) 38 [3] trials demonstrated superiority of ticagrelor and prasugrel, respectively, over clopidogrel in preventing atherothrombotic events in patients with ACS. Therefore, current guidelines favour these more potent platelet inhibitors over clopidogrel [4,5]. However, patients treated with ticagrelor or prasugrel are at a higher bleeding risk compared to patients treated with clopidogrel [2,3], which is even more pronounced in elderly patients [6]. The Clopidogrel versus Ticagrelor or Prasugrel in Patients Aged 70 years or older with non-ST-elevation Acute Coronary Syndrome (POPular Age) trial showed that in elderly patients, DAPT with clopidogrel has similar results as DAPT with the more potent platelet inhibitors in terms of ischemic events, while bleeding events were lower [7].

Clopidogrel is a prodrug, which is converted to its active metabolite by CYP P450 hepatic enzymes [8]. Approximately 30% of the Caucasian population show an inadequate response to clopidogrel when measured with platelet function tests, which is associated with a worse clinical outcome [9]. At least part of this variation in drug efficacy can be explained by genetic variations, of which the *CYP2C19**2 and *3 loss-of-function alleles are the most important [10]. For ticagrelor and prasugrel, no relevant gene-drug interaction have been found [11,12]. Although the correlation of *CYP2C19* genotype and clinical outcome was not assessed in the POPular Age trial so far, it can be hypothesised that also in elderly patients *CYP2C19* genotype influences the balance between benefit and harm when the more potent P2Y₁₂ inhibitors are prescribed in comparison to clopidogrel.

A strategy, in which the antiplatelet treatment was chosen based on *CYP2C19* genotype, although in a cohort of patients with ST-segment elevation myocardial infarction (STEMI), was evaluated in the Genotype-Guided Antiplatelet Therapy in ST-Segment Elevation Myocardial Infarction Patients – Patient Outcome after Primary Percutaneous Coronary Intervention (POPular Genetics) trial [13]. In this trial, a genotype-guided strategy was found to be non-inferior compared to standard treatment with ticagrelor or prasugrel for a net clinical benefit

outcome and ischemic events, while bleeding events were lower. While both atherothrombotic and bleeding event rates are higher in elderly patients, and bleeding events have shown to be strongly correlated to a worse clinical outcome [14], the use of genetic testing may improve clinical decision-making in selecting patients who benefit most from clopidogrel versus prasugrel or ticagrelor treatment.

In the present analysis, containing ACS patients aged 70 years and older derived from the POPular Age and POPular Genetics trial cohorts, we compare the use of clopidogrel in noncarriers of *CYP2C19* loss-of-function alleles with ticagrelor, irrespective of *CYP2C19* genotype and we assess the effect of *CYP2C19* loss-of-function alleles in clopidogrel treated elderly patients.

Methods

Study design

The detailed design and results of the POPular Age and POPular Genetics trials have been published previously [15,16]. In brief, the POPular Genetics trial was an open label, assessor blinded, randomized controlled trial performed in 10 centres in the Netherlands, Belgium and Italy. Between 2012 and 2018, 2,488 patients with STEMI undergoing primary PCI aged 21 years and older were included. Patients were randomized within 48 hours of primary PCI to either a standard treatment arm (treatment with ticagrelor or prasugrel for one year), or to the genotype-guided arm (treatment adjustment after rapid *CYP2C19* genetic testing). In the genotype-guided arm, patients carrying a *CYP2C19**2 or *3 loss-of-function allele were treated with ticagrelor or prasugrel, while noncarriers (*1/*1) were treated with clopidogrel. The POPular Age trial was an open label, assessor blinded, randomized controlled trial performed in 12 centres in the Netherlands. Between 2013 and 2018, 1,002 patients with non-ST-segment elevation myocardial infarction (NSTEMI) and unstable angina aged 70 years and older were included. Within 72 hours after hospital admission, patients were randomized to either treatment with clopidogrel, or to treatment with ticagrelor or prasugrel. In both trials, follow-up duration was 12 months. An institutional review board at all study sites approved the trials and all patients provided written informed consent.

In both trials, blood samples were collected for genotyping purposes, although only the samples derived from patients in the genotyping arm of the POPular Genetics trial were genotyped prospectively. All other samples were genotyped after study completion for the *CYP2C19**2 and *3 loss-of-function alleles. In the POPular Age trial, blood samples were

not collected in all participating hospitals and are therefore not available for all patients. Genotyping was performed by LGC Biosearch Technologies (Hoddesdon, United Kingdom) using a KASP genotyping assay.

Statistical analysis

The current analysis was pre-specified in both trials, although this sub-analysis was not prospectively powered. Therefore, the number of patients is based on the available patients of 70 years and older in whom *CYP2C19* genotype was available in the study cohorts. The following outcome parameters were assessed: (1) a net clinical benefit outcome, consisting of all-cause death, myocardial infarction, stroke and PLATO major bleeding; (2) an atherothrombotic outcome, consisting of cardiovascular death, myocardial infarction and stroke; and (3) a bleeding outcome, consisting of PLATO major and minor bleeding. Other outcomes were the individual components of the composite outcomes. The outcome definitions were identical to the definitions used in both main trials, in which a blinded event committee adjudicated all adverse clinical events.

The current analysis compared noncarriers of the *CYP2C19**2 and *3 alleles treated with clopidogrel to patients treated with ticagrelor. A small number of prasugrel treated patients was therefore excluded. A modified intention-to-treat analysis was performed in which patients were divided in a clopidogrel or a ticagrelor treated group, based on the drug that was prescribed at discharge after the index hospital admission. Two additional analyses were performed. An on-treatment analysis, in which patients were censored if they discontinued or switched P2Y₁₂ inhibitor therapy and an analysis in which patients treated with oral anticoagulation were excluded. Additionally, outcomes in clopidogrel treated patients were assessed according to *CYP2C19* loss-of-function allele carrier status and we assessed outcomes in the subgroups of the POPular Age and POPular Genetics trial separately.

Variables are presented as number (percentages) and mean $\pm$ standard deviation, or median with an interquartile range. Missing data was not imputed. Time-to-event curves were constructed using the Kaplan-Meier method. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated using Cox proportional-hazard models. To adjust for possible confounders, all baseline characteristics with a p-value < 0.10 were selected for univariate regression analysis. If there was a significant interaction ($p < 0.05$) in the univariate analysis, they were selected for

Figure 1. Flowchart

Figure 1. Flowchart of patient selection for the different subgroup analyses. LoF: Loss-of-function

multivariable regression analysis. The final model included only those characteristics with a significant interaction in the multivariable analysis. P-values < 0.05 were considered statistically significant. Data were analysed using 'R' (version 3.6.0 or later).

Results

Figure 1 shows how the different subgroups were selected from the two trials. For 1,084 patients aged 70 years and older (548 patients from the POPular Genetics trial and 536 patients from the POPular Age trial) the *CYP2C19* genotype was available. Of those, 401 were noncarriers of loss-of-function alleles treated with clopidogrel, 82 were carriers of loss-of-function alleles treated with clopidogrel and 590 were treated with ticagrelor. Baseline characteristics are presented in Table 1. The mean age was 77 years. In noncarriers of loss-of-function alleles treated with clopidogrel, more female patients and a lower baseline haemoglobin level were observed, compared to patients treated with ticagrelor. Almost 10% of the elderly patients in this study received medical treatment only, without coronary angiography, even though it was suspected they had obstructive coronary artery disease. In 11% of patients who underwent coronary angiography, coronary artery disease was found, but there was no clear target lesion, while another 10% of patients who underwent coronary angiography, but not a PCI, either received coronary artery bypass surgery or they were deemed unsuitable for PCI, (e.g. due to the location or type of lesion and/or due to comorbidities). The sensitivity analysis excluding patients with oral anticoagulation included 345 clopidogrel and 530 ticagrelor treated patients. Baseline differences were similar to that of the main analysis. Between clopidogrel treated carriers and noncarriers of loss-of-function alleles there were many different baseline characteristics, like higher rates of dyslipidaemia, prior PCI and prior stroke, in addition to the more frequent diagnosis of NSTEMI and unstable angina at discharge in the *CYP2C19* loss-of-function allele carrier group and the less frequent use of drug eluting stents and PCI in this group.

In the POPular Genetics subgroup, 616 patients aged 70 years and older were available for analysis. Of those, 306 patients were in the genotype-guided cohort and 310 patients were in the standard treatment arm. Both groups were well balanced with regards to baseline and procedural characteristics. In the POPular Age subgroup, *CYP2C19* genotype was available in 536 patients. Of those, 199 patients were noncarriers of a *CYP2C19* loss-of-function allele treated with clopidogrel, and 265 patients were treated with ticagrelor. Groups were well balanced, except for more peripheral arterial disease in the ticagrelor treated patients.

Table 1. Baseline characteristics

Characteristics	Clopidogrel noncarrier N = 401	Ticagrelor N = 590	P value	Clopidogrel LoF allele carriers N = 82	P value
Age (years, mean ± SD)	77.0 ± 5.4	77.0 ±5.4	0.84	78.0 ± 5.1	0.09
Female sex	161 (40.1)	189 (32.0)	0.01	32 (39.0)	0.95
BMI (kg/m2, mean ± SD)	26.3 ± 3.98	26.5 ± 3.77	0.54	26.4 ± 3.55	0.87
Creatinine clearance (eGFR CKD-EPI) <60ml/minute/1.7 3m2	127 (31.8)	165 (28.2)	0.25	24 (29.3)	0.76
Hemoglobin at admission (mmol/l, mean ± SD)	8.40 ± 0.83)	8.54 ± 0.92)	0.02	8.51 ± 0.98	0.34
Medical history					
Current smoker	64 (16.1)	112 (19.2)	0.24	10 (12.3)	0.50
Hypertension	250 (62.3)	357 (60.5)	0.61	56 (69.1)	0.30
Dyslipidemia	143 (35.8)	234 (39.8)	0.22	41 (50.0)	0.02
Diabetes Mellitus	81 (20.2)	128 (21.7)	0.63	25 (30.5)	0.06
Myocardial infarction	73 (18.2)	107 (18.2)	1.0	21 (25.6)	0.16
PCI	52 (13.0)	95 (16.1)	0.22	19 (23.2)	0.03
CABG	40 (10.0)	56 (9.5)	0.89	13 (15.9)	0.17
Stroke	10 (2.5)	20 (3.4)	0.54	7 (8.5)	0.02
Peripheral arterial disease	27 (6.8)	54 (9.2)	0.21	8 (9.8)	0.47
Bleeding	16 (4.0)	20 (3.4)	0.74	5 (6.1)	0.38
CYP2C19 genotype					
Normal metabolizer	402 (100)	375 (63.6)		0 (0)	
Intermediate metabolizer	0 (0)	189 (32.0)		75 (91.5)	
Poor metabolizer	0 (0)	17 (2.9)		7 (8.5)	
At discharge					
Diagnosis			0.13		<0.00 1
Unstable angina	17 (4.3)	22 (3.8)		9 (11.1)	
NSTEMI	177 (44.4)	225 (38.4)		56 (69.1)	

STEMI	202 (50.6)	328 (56.0)		10 (12.3)	
Aspirin	349 (88.4)	529 (90.7)	0.27	70 (86.4)	0.76
P2Y ₁₂ inhibitor					
Clopidogrel	383 (100)	0 (0)		69 (100)	
Ticagrelor	0 (0)	554 (100)		0 (0)	
Oral anticoagulation	56 (14.0)	60 (10.2)	0.09	14 (17.1)	0.58
Beta blocker	304 (77.0)	451 (77.4)	0.95	62 (76.5)	1.00
Ace inhibitor	241 (61.0)	372 (63.8)	0.41	52 (64.2)	0.68
ARB	73 (18.5)	109 (18.7)	1.0	13 (16.0)	0.72
Statin	375 (93.8)	553 (94.2)	0.87	72 (87.8)	0.10
Procedural characteristics					
CAG performed	365 (91.0)	557 (94.4)	0.17	71 (86.6)	0.35
Femoral access site	125 (34.2)	184 (33.0)		29 (40.8)	
Radial access site	240 (65.8)	366 (65.7)		42 (59.2)	
Multivessel disease	213 (53.2)	330 (56.1)	0.41	41 (50.0)	0.68
POBA	10 (2.5)	9 (1.5)	0.39	2 (2.4)	1.00
Bare metal stent	12 (3.0)	20 (3.4)	0.87	0 (0)	0.23
Drug eluting stent	267 (66.6)	419 (71.0)	0.16	44 (53.7)	0.04

Table 1. Baseline characteristics of patients aged 70 years and older from both the POPular Age and POPular Genetics trial with acute coronary syndrome in whom the CYP2C19 genotype was known, stratified according to P2Y₁₂ inhibitor treatment and LoF carrier status.

All variables were expressed as number (%), unless specified. ACS: Acute coronary syndrome, ARB: Angiotensin II receptor blocker, BMI: Body mass index, CABG: Coronary artery bypass surgery, CAG: Coronary angiography, LoF: Loss-of-function, NSTEMI: Non-ST elevation myocardial infarction, PCI: Percutaneous coronary intervention, POBA: Plain old balloon angioplasty, STEMI: ST-elevation myocardial infarction.

Table 2. Main outcomes

Outcome	Clopidogrel noncarrier (N=401)	Ticagrelor (N=590)	Hazard ratio* (95% CI)	P value*	Clopidogrel LoF allele carrier (N=82)	Hazard ratio (95% CI)	P value
Net clinical benefit outcome							
All-cause death, MI, stroke, PLATO major bleeding	69 (17.2)	89 (15.1)	1.05 (0.77-1.44)	0.76	17 (20.7)	0.96 (0.56 – 1.63)	0.87
Thrombotic outcomes							
Combined outcome (cardiovascular death, MI, stroke)	39 (9.7)	54 (9.2)	1.00 (0.66-1.50)	0.98	11 (13.4)	0.83 (0.42 – 1.64)	0.60
Cardiovascular death	14 (3.5)	12 (2.0)	1.58 (0.73-3.42)	0.25	3 (3.7)	0.96 (0.28 – 3.35)	0.95
Stroke	5 (1.2)	16 (2.7)	0.44 (0.16-1.20)	0.11	3 (3.7)	0.34 (0.08 – 1.42)	0.14
Myocardial infarction	25 (6.2)	31 (5.3)	1.14 (0.67-1.93)	0.64	6 (7.3)	1.04 (0.43 – 2.56)	0.93
Stent thrombosis	1 (0.2)	1 (0.2)	1.48 (0.09-23.6)	0.78	0 (0)	-	-

Bleeding outcomes							
PLATO major & minor bleeding	71 (17.7)	117 (19.8)	0.83 (0.62-1.12)	0.23	18 (22.0)	0.80 (0.47 – 1.33)	0.39
PLATO major	28 (7.0)	37 (6.3)	1.04 (0.64-1.70)	0.88	8 (9.8)	0.71 (0.32-1.56)	0.39
PLATO minor	48 (12.0)	86 (14.6)	0.79 (0.56-1.13)	0.20	10 (12.2)	0.92 (0.47 – 1.83)	0.81

Table 2. The combined subgroup analysis of the POPular Genetics and POPular Age compares clopidogrel in noncarriers of *CYP2C19* LoF alleles with ticagrelor treated patients. Additionally, they were compared to clopidogrel treated carriers of LoF alleles. All patients are aged 70 years and older. LoF: Loss-of-function, MI: Myocardial infarction, PLATO: Platelet Inhibition and Patient Outcomes * Hazard ratio is adjusted for sex, baseline haemoglobin level and the use of oral anticoagulation at discharge (See Table S1 in the supplementary appendix for more details).

Figure 2. Cumulative event rates of the net clinical benefit outcome (defined as all-cause death, myocardial infarction, stroke and PLATO major bleeding; 2A), the thrombotic outcome (defined as cardiovascular death, myocardial infarction and stroke; 2B) and the bleeding outcome (defined as PLATO major and minor bleeding; 2C). ACS: acute coronary syndrome, PLATO: platelet inhibition and patient outcomes.

Results are presented in Table 2, Figure 2 and Figure 3. The variables included in the multivariable regression analysis to adjust for baseline differences are presented in Table S1 in the supplementary appendix. After adjustment for differences in baseline characteristics between the groups, there was no statistically significant difference in the net clinical benefit outcome (17.2% vs. 15.1%, adjusted hazard ratio (adjHR) 1.05, 95%CI, 0.77 – 1.44, $p = 0.76$) (Figure 2A), thrombotic outcome (9.7% vs. 9.2%, adjHR 1.00, 95%CI, 0.66 – 1.50, $p = 0.98$) (Figure 2B), and bleeding outcome (17.7% vs. 19.8%, adjHR 0.83, 95%CI, 0.62 – 1.12, $p = 0.23$) (Figure 2C), in noncarriers of loss-of-function alleles treated with clopidogrel compared to patients treated with ticagrelor irrespective of CYP2C19 genotype. Results for the on-treatment analysis and the analysis excluding patients treated with oral anticoagulation are presented in Figure 3 and in Table S2 in the supplementary appendix.

There were no significant differences between the clopidogrel treated noncarriers group and the small clopidogrel treated carriers of loss-of-function alleles group (Table 2) in the net clinical benefit outcome (17.2% vs. 20.7%, HR 0.96, 95% CI, 0.56 – 1.63, $p = 0.87$), thrombotic outcome (9.7% vs. 13.4%, HR 0.83, 95% CI, 0.42 – 1.64, $p = 0.60$) and bleeding outcome (17.7% vs. 22.0%, HR 0.80, 95% CI 0.47 – 1.33, $p = 0.39$).

Results for the subgroup analysis of the POPular Age and POPular Genetics trial cohorts are presented in Figure 3 and in Table S3 in the supplementary appendix. In both analyses, there are no significant differences in any of the outcomes.

Discussion

In this pre-specified sub analysis from the POPular Genetics and POPular Age trials, we compared the use of clopidogrel in noncarriers of CYP2C19 loss-of-function alleles with the use of ticagrelor in patients with ACS aged 70 years and older. No statistically significant differences were found for both atherothrombotic and bleeding outcomes. Nevertheless, the finding of a comparable atherothrombotic event rate (adjHR 1.00) combined with a numerically, though not statistically significant, lower number of bleeding events in the clopidogrel treated patients without *CYP2C19* loss-of-function allele (adjHR 0.83) is clinically relevant. Additionally, we identified numerically less

Figure 3. Outcomes of all analyses

Figure 3. A forest plot displaying all the main outcomes from the different sub analyses from the POPular Genetics and POPular Age trials. adjHR: adjusted Hazard ratio

atherothrombotic events and bleeding events in noncarriers of *CYP2C19* loss-of-function alleles treated with clopidogrel compared to carriers of loss-of-function alleles, though, in this very small subgroup with many baseline differences, these differences were not statistically significant. Therefore, any real conclusions cannot be made based on this data.

These results are in accordance with the elderly subgroup analysis of the TRITON-TIMI 38 trial (n = 1809), comparing prasugrel with clopidogrel, which found a lower bleeding risk in clopidogrel-treated patients of 75 years and older [3]. In contrast, the subgroup analysis of the PLATO trial, studying the use of ticagrelor in the elderly population (n = 2878), showed a benefit of ticagrelor in reducing ischemic events, without significantly increasing the risk of bleeding, although fatal bleeding and non-CABG related major bleeding were higher in the elderly treated with ticagrelor [17]. These results were confirmed in the Bremen STEMI registry, which included 1087 STEMI patients aged 75 years older treated with primary PCI, comparing ticagrelor to clopidogrel [18]. This benefit with ticagrelor was not found in the POPular Age trial (n = 1002), studying patients of 70 years or older with non-ST elevation ACS [7]. While thrombotic risk was not statistically different with the use of ticagrelor or clopidogrel, the bleeding rate was lower in the clopidogrel treated patients. These results are strengthened by the large (n = 14,005), nationwide SWEDEHEART registry including patients of 80 years and older with ACS [19]. Although the evidence is conflicting - which for some part might be due to differences in patient selection, selection of different age groups and development and improvement of drug-eluting stents, enhanced secondary prevention and improved intervention techniques in the past decade decreasing ischemic risk - it might be concluded that the more potent P2Y₁₂ inhibitors are not superior for all elderly patients with ACS regarding net clinical benefit outcomes, and clopidogrel is a reasonable alternative.

The POPular Genetics trial found lower bleeding rates without increasing atherothrombotic rates when using *CYP2C19* genetic testing compared to standard treatment with ticagrelor. These results also applied to the elderly subgroup, where no significant interaction was found. The results from the combined POPular Genetics and POPular Age subgroup analysis confirm the main results of the POPular Genetics trial in this elderly subgroup, with similar atherothrombotic and lower bleeding event rates in the clopidogrel group compared to the ticagrelor group. This was not statistically significant due to the limited sample size of this subgroup. The TAILOR PCI trial also investigated *CYP2C19* genetic testing and included patients who underwent PCI (82% because of ACS, 18% with stable coronary

artery disease) and randomized them to the control arm or genotype-guided therapy arm. In the control arm patients received clopidogrel, and in the genotype-guided arm patients received clopidogrel if they were noncarriers of the *CYP2C19**2 and *3 loss-of-function alleles or ticagrelor if they were carriers of these alleles [20]. A numeric reduction was found in thrombotic events in carriers of *CYP2C19* loss-of-function alleles when treated with ticagrelor as compared to clopidogrel, though this difference was not statistically significant ($p = 0.056$), which is in agreement with the results of this subanalysis [20]. However, there was also no significant benefit of the genotype-guided strategy in regard to the safety outcome, probably because they compared with clopidogrel in the control arm instead of ticagrelor. The elderly subgroup analysis of patients aged 75 years and older of the TAILOR PCI trial ($n = 261$) showed no significant interaction [20]. Combining the results of the TAILOR PCI with this subgroup analysis, specifying antiplatelet therapy by means of *CYP2C19* genotyping might be promising for improving clinical outcomes in the elderly ACS patient, however further research is needed.

To this day, clopidogrel remains the most widely used platelet inhibitor in elderly patients with ACS [21–23]. Although most evidence regarding elderly patients is based on subgroup analysis and is underpowered, it might be concluded that the more potent $P2Y_{12}$ inhibitors prasugrel and ticagrelor are not superior in all elderly patients with ACS regarding net clinical benefit outcomes, and clopidogrel could be a reasonable alternative. This effect might be the strongest in patients without *CYP2C19* loss-of-function allele, who are expected to have optimal clopidogrel efficacy. A *CYP2C19* genotype guided strategy might be promising for improving clinical outcome in the elderly ACS patient.

Several limitations to this study need to be mentioned. Although this is one of the largest analyses comparing clopidogrel with ticagrelor in elderly patients with ACS and known *CYP2C19* genotype, it was not powered to find statistical differences between both treatment strategies. In addition, both trials had an open-label design and the POPular Age trial was not designed to compare a genotype-guided strategy with standard treatment. Therefore, our results are hypothesis-generating and should be interpreted with caution. Furthermore, since our main analysis only included clopidogrel treated noncarriers of loss-of-function alleles, results cannot be extrapolated to all clopidogrel-treated patients. Lastly, since we compared our data to ticagrelor-treated patients only, it is unknown how outcomes compare to prasugrel-treated patients.

Conclusion

In patients with ACS aged 70 years and older, there were no significant differences in atherothrombotic or bleeding event rates between noncarriers of a *CYP2C19* loss-of-function allele treated with clopidogrel, and patients treated with ticagrelor irrespective of *CYP2C19* genotype. Nevertheless, the bleeding rate was numerically, though not statistically significant, lower in clopidogrel treated patients. Further research focusing on the value of *CYP2C19* genotyping in this specific and vulnerable group of patients might help to find an optimal antiplatelet treatment for the elderly.

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Supplementary appendix

Table S1. Variables selected for regression analysis

Outcome	Variables tested in univariate regression	Significant variables in univariate regression	Significant variables in multivariable regression model
	Net clinical benefit outcome		
All-cause death, MI, stroke, PLATO major bleeding	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
	Thrombotic outcomes		
Combined Outcome (Cardiovascular death, MI, stroke)	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
Cardiovascular death	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
Myocardial infarction	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
Stroke	Female sex, Hb, OAC	OAC	OAC
Stent thrombosis	Female sex, Hb, OAC	-	-
	Bleeding outcomes		
PLATO major & minor bleeding	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
PLATO major	Female sex, Hb, OAC	Hb, OAC	Hb, OAC
PLATO minor	Female sex, Hb, OAC	OAC	OAC

Table S1. Female sex, haemoglobin level at admission and the use of oral anticoagulation at discharge were selected for univariate and multivariable regression. In univariate regression, female sex was not a significant interaction variable for any of the outcomes. Hb: Haemoglobin, MI: Myocardial infarction, OAC: Oral anticoagulation, PLATO: Platelet Inhibition and Patient Outcomes

Table S2. Sensitivity analyses

Outcome	CYP2C19 *1/*1 treated clopidogrel (N=401)	Ticagrelor (N=590)	Unadjusted Hazard ratio (95% CI)	Adjusted Hazard ratio* (95% CI)	Adjusted P-value*
On-treatment analysis					
Net clinical benefit (all-cause death, MI, stroke, PLATO major bleeding)	65 (16.2)	75 (12.7)	1.19 (0.86 – 1.66)	1.06 (0.76 – 1.48)	0.74
Thrombotic outcome (cardiovascular death, MI, stroke)	34 (8.5)	47 (8.0)	1.01 (0.65-1.57)	0.92 (0.59-1.43)	0.71
Bleeding outcome (PLATO major & minor bleeding)	69 (17.2)	108 (18.3)	0.88 (0.65 – 1.18)	0.82 (0.61 – 1.11)	0.20
Excluding OAC patients	CYP2C19 *1/*1 treated clopidogrel (N=345)	Ticagrelor (N=530)	Unadjusted Hazard ratio (95% CI)	Adjusted Hazard ratio** (95% CI)	Adjusted P-value**
Net clinical benefit (all-cause death, MI, stroke, PLATO major bleeding)	55 (15.9)	66 (12.5)	1.31 (0.91 – 1.87)	1.15 (0.80 – 1.66)	0.44

Thrombotic outcome (cardiovascular death, MI, stroke)	29 (8.4)	41 (7.7)	1.10 (0.69 – 1.78)	1.04 (0.64 – 1.67)	0.89
Bleeding outcome (PLATO major & minor bleeding)	54 (15.7)	96 (18.1)	0.85 (0.61 – 1.20)	0.83 (0.60 – 1.16)	0.28

Table S2. (see previous page) Sensitivity analyses of clopidogrel treated noncarriers of loss-of-function alleles compared to ticagrelor-treated acute coronary syndrome patients aged 70 years and older. MI: Myocardial infarction, PLATO: Platelet Inhibition and Patient Outcomes

* Hazard ratio is adjusted for baseline haemoglobin level and the use of oral anticoagulation at discharge.

** Hazard ratio is adjusted for baseline haemoglobin level.

Table S3. (see next page) The POPular Age subgroup analysis compared clopidogrel-treated noncarriers of LoF alleles with ticagrelor/prasugrel-treated patients. The POPular Genetics subgroup analysis compared genotype-guided treatment with ticagrelor/prasugrel-treated patients aged 70 years and older. The net clinical benefit outcome was defined as all-cause death, myocardial infarction, stroke and PLATO major bleeding. The thrombotic outcome was defined as cardiovascular death, myocardial infarction and stroke. The bleeding outcome was defined as PLATO major and minor bleeding. LoF: loss-of-function, PLATO: Platelet Inhibition and Patient Outcomes

* Hazard ratio is adjusted for the more common occurrence of peripheral arterial disease in ticagrelor/prasugrel-treated patients in the POPular Age trial. Adjusted hazard ratio and P value are only presented if there was a significant interaction.

Table S3. Subgroup analysis of the POPular Age and POPular Genetics trial cohorts

Outcome	<i>CYP2C19</i> *1/*1 clopidogrel (N = 199)	Ticagrelor /prasugrel (N = 265)	Unadjusted Hazard ratio (95% CI)	Adjusted Hazard ratio* (95% CI)	(Adjusted) P value*
POPular Age sub analysis					
Net clinical benefit outcome	46 (23.1)	60 (22.6)	1.04 (0.71 – 1.52)	1.05 (0.71 – 1.55)	0.79
Thrombotic outcome	27 (13.6)	36 (13.6)	1.02 (0.62 – 1.68)	-	0.93
Bleeding outcome	35 (17.6)	62 (23.4)	0.73 (0.48 – 1.10)	0.74 (0.48 – 1.12)	0.16
POPular Genetics sub analysis of patients aged ≥70yr					
	Genotype-guided strategy (N = 306)	Ticagrelor /prasugrel (N = 310)			
Net clinical benefit outcome	31 (10.1)	29 (9.4)	1.09 (0.66 – 1.81)	-	0.73
Thrombotic outcome	15 (4.9)	20 (6.5)	0.76 (0.39 – 1.48)	-	0.42
Bleeding outcome	52 (17.0)	54 (17.4)	0.96 (0.66 – 1.41)	-	0.84

Cost-effectiveness of a CYP2C19 genotype-guided strategy in acute myocardial infarction patients: Results from the POPular Genetics trial

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Abstract

Objective

The POPular Genetics trial demonstrated that a *CYP2C19* genotype-guided P2Y₁₂ inhibitor strategy reduced bleeding rates compared to standard treatment with ticagrelor or prasugrel, without increasing thrombotic event rates after primary percutaneous coronary intervention (PCI). In this analysis, we aimed to evaluate the cost-effectiveness of a genotype-guided strategy compared to standard treatment with ticagrelor or prasugrel.

Methods

A 1-year decision tree based on the POPular Genetics trial in combination with a lifelong Markov model was developed to compare costs and quality-adjusted life years (QALYs) between a genotype-guided and a standard P2Y₁₂ inhibitor strategy in myocardial infarction patients undergoing primary PCI. The cost-effectiveness analysis was conducted from a Dutch healthcare system perspective. Within-trial survival and utility data were combined with lifetime projections to evaluate lifetime costs-effectiveness for a cohort of 1000 patients. Costs and utilities were discounted at 4% and 1.5% respectively according to Dutch guidelines for health-economic studies. Besides deterministic and probabilistic sensitivity analyses, several scenario analyses (different time horizons, different discount rates, equal prices for P2Y₁₂ inhibitors and equal distribution of thrombotic events between the two strategies) were conducted.

Results

Base-case analysis with a hypothetical cohort of 1000 subjects, demonstrated 8.98 QALYs gained and €725,550.69 cost-savings for the genotype-guided strategy (dominant). The deterministic and probabilistic sensitivity analysis confirmed the robustness of the model and the cost-effectiveness results. In scenario analyses, the genotype-guided strategy remained dominant.

Conclusion

In patients undergoing primary PCI, a *CYP2C19* genotype-guided strategy compared to standard treatment with ticagrelor or prasugrel resulted in QALYs gained and cost-savings.

Introduction

Patients with myocardial infarction (MI) and patients undergoing percutaneous coronary intervention (PCI) are treated with dual antiplatelet therapy consisting of aspirin and a P2Y₁₂ inhibitor. Guidelines favour ticagrelor and prasugrel over clopidogrel in MI patients due to a reduction in thrombotic events [1,2]. One of the reasons for the reduced effectiveness of clopidogrel compared to the other P2Y₁₂ inhibitors is that clopidogrel has to be converted into its active metabolite. The most important enzyme in this process is the CYP 450 2C19 enzyme. This enzyme is encoded by the *CYP2C19* gene, of which more than 30 different alleles have been identified [3]. Some of these alleles encode for an enzyme that is not functional. The *CYP2C19**2 and *3 alleles are the most common loss-of-function alleles and almost 1/3 of the people in western populations carry one or two of these alleles [4]. Several studies demonstrated that these patients have an increased risk of developing major adverse cardiac events [5]. This has prompted the Food and Drug administration (FDA) to add a boxed warning for clopidogrel, describing that the drug might not be effective in patients carrying two loss-of-function alleles [6]. Nevertheless, clopidogrel is still the most frequently prescribed P2Y₁₂ inhibitor, either due to fear of bleeding complications or other side effects when using the stronger P2Y₁₂ inhibitors or for economic reasons (e.g. lower costs of treatment) [7-9].

The Patient Outcomes after primary PCI (POPular) Genetics trial showed in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI, that a *CYP2C19* genotype-guided P2Y₁₂ inhibitor strategy was non-inferior for a net clinical benefit outcome while reducing bleeding outcomes as compared to standard treatment with ticagrelor and prasugrel [10]. Since genetic testing is associated with higher costs, while clopidogrel has a lower price than the other P2Y₁₂ inhibitors, the objective of this study was to assess the cost-effectiveness of the genotype-guided strategy compared to a standard treatment strategy with ticagrelor or prasugrel, within the context of the Dutch healthcare system.

Methods

Study design

Details on the methods and results of the POPular Genetics trial have been reported previously [10,11]. In brief, POPular Genetics was an open label randomized, multicentre trial with 2,488 STEMI patients undergoing primary PCI. It compared a standard treatment with the P2Y₁₂ inhibitors ticagrelor (90mg twice daily) or prasugrel (5 or 10mg once daily based on

the summary of product characteristics approved by the European Medicines Agency (EMA)) with a *CYP2C19* genotype-guided strategy, where patients without *CYP2C19**2 or *3 loss-of-function alleles were prescribed clopidogrel (75mg once daily), and ticagrelor or prasugrel (dosage identical to standard treatment) if they were carrier of such a loss-of-function allele. All patients also received guideline recommended aspirin. Treatment and follow-up duration were 12 months. At 1, 6 and 12 months all patients received a questionnaire either by mail or email. This questionnaire contained the EQ-5D-5L, which was used to calculate health utilities. The cost-effectiveness analysis was pre-specified as part of the trial protocol. The trial was approved by the appropriate ethics committees and national authorities. All patients provided informed consent.

Model overview

The current analysis was designed to calculate the cost-effectiveness of a *CYP2C19* genotype-guided strategy for STEMI patients undergoing primary PCI. A two-part decision-analytic model was developed, comprising of a 1-year decision tree to determine the initial distribution of the cohort over the Markov states (Figure 1A) and a Markov model to simulate life-long costs and effects (Figure 1B). In the 1-year decision tree, all patients could experience a minor or major bleeding independent of experiencing any of the other events. At the end of the 1-year decision tree period, patients would enter the respective Markov states depending on the experienced event (e.g. recurrent MI, recurrent stroke or death). The Markov-model structure is identical to previously published trials investigating antiplatelet therapy in similar populations [12], clinically validated and adjusted to allow for recurrence of stroke and MI. Four disease transient states were included reflecting the lifetime progression of individuals after STEMI, including MI, post-MI, stroke and post-stroke. Additionally, the model comprised two absorbing states, defined as cardiovascular (CV) death and non-CV death. A cohort of 1000 hypothetical subjects was used, to simulate the progression through the different disease states. Subjects could switch between disease states or remain in the same disease state, based on transition probabilities. In the base case analysis, a lifetime horizon was used with a cut-off at the age of 100.

Model assumptions

One of the assumptions underpinning the model was that patients in both groups were treated with aspirin monotherapy after the 1-year trial period was finished. Therefore, no difference in bleeding rates was expected in the Markov model. Furthermore, bleeding usually decreases quality of

Figure 1. Cost-effectiveness model

Figure 1. Model structure. **Figure 1A:** 1-year decision tree based on the POPular Genetics trial. **Figure 1B:** Long-term Markov model. MI: myocardial infarction.

life for only a short period of time. Hence, bleeding was not included as a separate health state in the Markov model. It was assumed that patients could not develop multiple events during the 1-year trial, which was in line with the Platelet Inhibition and Patient Outcomes (PLATO) trial cost-effectiveness analysis [12]. In addition, the assumption was made that

recurrent stroke or MI could only happen with a minimum interval of 1 year.

Population

The intention-to-treat population from the POPular Genetics trial was used for the current decision-analytic model (Table 1). The mean age of the trial population was 61 years old, 15% was 75 years and older, 25% was female and 10% had a prior history of coronary artery disease. In line with the mean age of the patients in the POPular Genetics trial cohort, all individuals were at the age of 61 at the start of the decision tree.

Model input parameters

Transition probabilities

All model inputs are presented in Table 2. Probabilities for all patients in the 1-year decision tree were based on the results from the POPular Genetics trial [10]. At the end of the decision tree, patients were allocated to their respective health state in the long-term Markov model. Starting from the second year, a Markov state-transition model with yearly cycles was used to simulate disease progression of patients over their lifetime. In each health state, patients could experience an MI, stroke or death in any year. Patients in the 'Post-MI' and 'Post-stroke' health states had a higher risk of subsequent events than patients in the 'No-event' health state. The transition probabilities of experiencing subsequent events were derived by multiplying the baseline probabilities by the relative risk factors (Table 2) [12]. For mortality, an age specific mortality rate was used based on the Dutch population lifetables. It was not possible to transition from 'post-stroke' to 'post-MI', since the MI health state had a lower risk and was less costly than the stroke health state.

Costs

Cost-effectiveness was estimated from the healthcare perspective, so only medical costs were included. Costs were inflated to 2020 using the consumer price index inflation from the Dutch Central Bureau of Statistics (Table 2). Costs were based on the Dutch healthcare system and obtained from literature or Dutch governmental agencies. Cost categories were treatment costs (genetic test and different antiplatelet drugs) and costs associated with the different events: minor bleeding, major bleeding, nonfatal MI, nonfatal stroke, post-MI, post-stroke and death. The cost associated with the use of aspirin and other medication was excluded from the analysis, since it was assumed to affect both treatments strategies equally. Costs were discounted using an annual rate of 4% in accordance with existing guidelines for conducting health-economic evaluations [23].

Health utilities

Health utilities, measured in quality-adjusted life years (QALYs), were dependent on the events experienced by patients (Table 2). Health utility estimates were either derived from the POPular Genetics trial (using the EQ-5D-5L questionnaire) or, in case data was not available from the trial, utility estimates were derived from literature focused on the Dutch healthcare system with similar populations. Based on previously published literature, bleeding led to a temporary disutility for the duration of the event during the first year following treatment. QALY's were discounted using an annual rate of 1.5% in accordance with Dutch guidelines for conducting health-economic evaluations [23].

Outcomes

The outcome measures to compare the two strategies in this study were costs, QALYs and the incremental cost-effectiveness ratios (ICERs) presented as cost per QALY gained.

Sensitivity and scenario analysis

The base-case analysis was based on the model inputs as shown in Table 2. To accommodate for the model uncertainty, univariate deterministic sensitivity analyses and probabilistic sensitivity analyses were conducted. For the sensitivity analysis, the estimated range of each parameter was based on the 95% CI in the studies. If 95% CI was not available, ranges were calculated with a standard error of the mean of 25%. For univariate deterministic sensitivity analyses, each of the parameters was varied one by one over the 95% CI, to examine the influence of individual parameters on the ICER. In the probabilistic sensitivity analysis, a Monte Carlo simulation was performed (1,000 iterations) by varying the parameters simultaneously over their 95% CI. The cost, probability and utility parameter distributions were varied with a Gamma-, Pert and Beta distribution, respectively. Outcomes of the sensitivity analysis are presented in a tornado-diagram and a cost-effectiveness plane. Four additional scenarios were conducted to capture the effect of the time horizon (1, 5, 10 and 25 years in the Markov model (scenario 1), adjustment of the discount rates to both costs and utilities at 4% (scenario 2), equal drug prices for all three drugs at €0.05/day, to mimic the availability of generic variants in the future (scenario 3) and equal distribution of the cohort over 'post-MI', 'post-stroke' and 'death' (both CV death and non-CV death) at the start of the Markov-model for both strategies to account for the uncertainty that a genotype-guided strategy does not result in numerically less stroke and MI as seen in the POPular Genetics trial (scenario 4).

Table 1. POPular Genetics trial population baseline characteristics

Characteristics	Genotype-guided (N=1242)	Standard treatment (N=1246)
Mean age - yr	61.9±11.1	61.4±11.5
Age ≥75 years – no. (%)	188 (15.1)	175 (14.0)
Female sex – no. (%)	317 (25.5)	309 (24.8)
Mean body-mass index ^a	27.5±6.67	27.0±4.27
Creatinine clearance <60ml/minute at baseline ^b – no. (%)	121 (9.8)	109 (8.8)
Medical history – no. (%)		
Current smoker	562 (45.8)	565 (45.8)
Diabetes Mellitus	150 (12.1)	138 (11.1)
Arterial hypertension	521 (42.0)	511 (41.0)
Hyperlipidaemia	260 (20.9)	255 (20.5)
History of coronary artery disease	133 (10.7)	118 (9.5)
Peripheral arterial disease	39 (3.1)	34 (2.7)
History of bleeding	30 (2.4)	23 (1.9)
Angiographic and procedural characteristics		
Number of diseased coronary vessels ≥50% – no. (%)		
1	634 (51.2)	675 (54.2)
2	417 (33.7)	376 (30.2)
3	188 (15.1)	194 (15.6)
Ostial lesion – no. (%)	76 (6.4)	65 (5.5)
Bifurcation lesion – no. (%)	214 (18.1)	239 (20.2)
Stent type – no. (%)		
Bare metal stent	60 (4.8)	50 (4.0)
Bioresorbable vascular scaffold	9 (0.7)	16 (1.3)
Drug eluting stent	1167 (94.0)	1174 (94.2)
Plain old balloon angioplasty	17 (1.4)	23 (1.9)
Total stent length - mm	27.0±14.8	28.0±15.3
Medication at discharge – no. (%)		
Aspirin	1211 (97.7)	1208 (97.4)
ADP receptor antagonist	1237 (99.8)	1237 (99.8)
Clopidogrel	688 (55.5)	91 (7.3)
Prasugrel	15 (1.2)	27 (2.2)
Ticagrelor	534 (43.1)	1119 (90.2)
(Novel) oral anticoagulation	14 (1.1)	9 (0.7)

Table 1. Baseline characteristics of the POPular Genetics trial population.
a: BMI is the weight in kilograms divided by the square of the height in meters.
b: The creatinine clearance was calculated using the CKD-EPI formula.

Table 2. Model input parameters

Parameters	Base-case value	Range	Distribution	Source
Probabilities (decision tree)				
Genotype-guided strategy				
Minor bleeding	0.0765	NA	NA	Trial
Major bleeding	0.0225	NA	NA	Trial
Myocardial infarction	0.0153	NA	NA	Trial
Stroke	0.0064	NA	NA	Trial
Non-CV Death	0.0080	NA	NA	Trial
CV death	0.0070	NA	NA	Trial
Standard treatment				
Minor bleeding	0.1051	NA	NA	Trial
Major bleeding	0.0233	NA	NA	Trial
Myocardial infarction	0.0209	NA	NA	Trial
Stroke	0.0088	NA	NA	Trial
Non-CV death	0.0070	NA	NA	Trial
CV death	0.0080	NA	NA	Trial
Probabilities (Markov model)^a				
Annual risk from 'No-event' to 'MI'	0.019	0.01-0.05	PERT	12
Annual risk from 'No-event' to 'Stroke'	0.003	0.001-0.02	PERT	12
Annual risk from 'No-event' to 'Non-CV death'	Age specific mortality rate		PERT	13
Increased risk of a subsequent event after having an event	2	1-4	PERT	14
Increased risk of death in 'No-event'	2	1.5-2.5	PERT	12
Increased risk of death in 'Non-fatal MI'	6	4.5-7.5	PERT	12

Increased risk of death in 'post MI'	3	2.25-3.75	PERT	12
Increased risk of death in 'Non-fatal stroke'	7.43	5.57-9.29	PERT	12
Increased risk of death in 'post stroke'	3	2.25-3.75	PERT	12
Costs (euro's)^b				
Genotyping	150.00	76.50-223.50	Gamma	Trial
1 year clopidogrel treatment	18.25	9.31-27.19	Gamma	15
1 year ticagrelor treatment	795.70	405.81-1,158.59	Gamma	16
1 year prasugrel treatment	602.25	307.15-897.35	Gamma	17
Minor bleeding	310.76	189.02-433.57	Gamma	18
Major bleeding	5,422.78	2,765.62-8,079.95	Gamma	19
Myocardial infarction	5,550.95	2,830.99-8,270.99	Gamma	20
Post-MI	2,536.81	2,367.22-2,667.82	Gamma	21
Stroke	28,233.36	18,378.83-38,806.05	Gamma	21
Post-stroke	11,551.15	7,724.37-14,596.40	Gamma	21
CV death	3,223.09	1,842.21-4,982.85	Gamma	21
Utilities^c				
No event	0.88	0.87-0.89	Beta	Trial
Myocardial infarction	0.80	0.71-0.90	Beta	Trial
Post-MI	0.81	0.72-0.90	Beta	Trial
Stroke	0.59	0.30-0.88	Beta	21

Post-stroke	0.74	0.71-0.77	Beta	22
Death	0	NA	Beta	
Minor bleeding (disutility 2 days)	0.06	0.03-0.09	Beta	20
Major bleeding (disutility 14 days)	0.14	0.07-0.21	Beta	20

Table 2. (See previous two pages) Cost-effectiveness model input parameters. CI: confidence interval, CV:

cardiovascular, NA: not applicable, MI: myocardial infarction
a: Range indicating min/max as provided by paper. If min/max was unavailable, ranges where calculated with 25% of the base-case value.
b: Range is based on 95% CI. If 95% CI was unavailable, ranges were calculated with standard error of 25% of the mean
c: Range is based on 95% CI

Results

Base-case analysis

For a cohort of 1000 patients undergoing primary PCI based on the POPular Genetics trial, the genotype-guided strategy resulted in a gain in QALYs of 8.98 while saving €725,550.69 (0.009 QALYs gained and €725.56 saved per patient) (Table 3). Cost-saving results for the genotype-guided strategy indicate that this strategy dominates current standard treatment without genotyping. In Figure 2, results of the univariate deterministic sensitivity analysis are displayed in a tornado diagram. The results demonstrate that when varying the different model inputs over their confidence intervals, the genotype-guided strategy remains cost-saving. Results of the 1000 iterations of the probabilistic sensitivity analysis were plotted on a cost-effectiveness plane (Figure 3). The genotype-guided strategy was cost-saving in each iteration of the Monte Carlo simulation, while it increased QALYs in almost all iterations.

Scenario analyses

In Table 3 results are presented for the varying time horizons (scenario 1), adjustment in discount rates (scenario 2), equal prices for all P2Y₁₂ inhibitors (scenario 3) and an equal distribution of patients amongst health states between the two strategies (scenario 4). In all different scenarios, the genotype-guided strategy remained cost saving and improved QALY's.

Discussion

In this prospectively designed cost-effectiveness analysis, based on the POPular Genetics trial data, a *CYP2C19* genotype-guided strategy was associated with an increase in QALYs and was cost-saving (dominant) as compared to standard treatment with ticagrelor or prasugrel in STEMI patients undergoing primary PCI. The robustness of this finding was confirmed by various additional sensitivity and scenario analyses. In the Netherlands, the willingness to pay threshold varies between €20.000 to €80.000 depending on the intervention, while the willingness to pay threshold in other European countries varies as well, but is generally of the same magnitude [24]. Our results are well below the lower end of this range. In the United States, a guideline on health economic analyses was published by the American College of Cardiology (ACC)/American Heart Association (AHA) [25]. According to this guideline, our findings suggest a high value of a genotype-guided strategy as compared to a standard treatment strategy with ticagrelor or prasugrel, since it improved QALYs while reducing costs.

The cost-effective results showing that the genotype-based strategy is dominant, were primarily driven by two differences in the first year. The first determining variable is the price for generic clopidogrel which is lower (€0.05/day) compared to the patented ticagrelor and prasugrel (€2.18/day and €1.65/day, respectively) [15-17]. The difference in costs between clopidogrel and the other P2Y₁₂ inhibitors reflects the potential additional cost of performing a genetic test that was estimated to be €150.00 for a point-of-care test. Since both ticagrelor and prasugrel are expected to have generic variants available in the next few years, which will likely cause a drop in prices, a scenario analysis was conducted in which the daily costs of ticagrelor and prasugrel were identical to clopidogrel costs (€0.05/day). Following this scenario analysis, the genotype-guided strategy remained cost-saving, with similar QALYs gained as compared to the base-case analysis. Therefore, it can be realistically assumed that extra costs associated with performing genetic testing will not change the results. The availability of generic ticagrelor and prasugrel will lower prices in the future, but it is uncertain to what extent. The scenario analysis performed is therefore on the conservative end of the spectrum. The second important factor driving the results of the current analysis is the distribution of the patients at the onset of the Markov model. Based on the results from the POPular Genetics trial, patients in the genotype-guided arm were in a more favourable health-state than patients in the standard treatment arm when they entered the Markov model, due to a lower incidence of stroke and MI in the genotype-guided cohort as compared to the standard treatment cohort. To account for the uncertainty that a genotype-guided strategy actually leads to numerically less stroke and less MI, as seen in the POPular Genetics trial, a scenario analysis was conducted in which there was no difference amongst the distribution of patients in the different health states when entering the Markov model between the two strategies. In this scenario analysis, the genotype-guided strategy remained cost saving while only a small difference in QALYs gained remained. This was expected, since the difference in QALYs is now only caused by a difference in minor bleeding events between both groups, which have a relatively low impact on long-term health utilities. These results demonstrate that the cost-savings and long-term benefits are based on the differences in the first year after genotype-guided treatment.

In the POPular Genetics trial, more than 95% of the included patients was Caucasian, while only a small proportion of patients was of Asian, Latin American or African descent [10]. The prevalence of *CYP2C19* loss-of-function alleles does not differ much between Africans, Americans and

Figure 2. Deterministic sensitivity analysis

Figure 2. One-way sensitivity analysis of a genotype-guided strategy versus standard treatment with ticagrelor or prasugrel. Horizontal bars indicate the range of cost-savings obtained when setting each individual variable at its maximum and minimum confidence interval. Since all results remain cost saving, the direction of the bar makes no difference. CV: cardiovascular, MI: myocardial infarction, NF: non-fatal

Figure 3. Probabilistic sensitivity analysis

Figure 3. Results of the probabilistic sensitivity analysis on the cost-effectiveness plane. The scatterplot depicts results of the Monte Carlo analysis (1000 iterations) when all model inputs are randomly varied between their confidence intervals. The black line depicts the willingness to pay €20,000/QALY. A genotype-guided strategy is cost-effective if it falls below the black line. QALY: quality-adjusted life year

Table 3. Lifetime cost-effectiveness results for base-case and scenario analyses

	Cost genotype-guided (€)	Cost standard treatment (€)	ΔCost (€)	QALYs genotype-guided	QALYs standard treatment	ΔQALY	ICER ^a (€/QALY)
Base-case							
Lifetime, discounted costs at 4%, QALYs at 1.5%	10,650,062	11,375,613	-725,551	11394.59	11385.60	8.98	NA
Scenario analyses							
1 year	1,080,346	1,442,899	-362,553	1702.92	1701.47	1.45	NA
5 years	2,874,516	3,370,275	-495,759	4709.45	4705.54	3.91	NA
10 years	5,577,006	6,187,410	-610,404	7671.43	7665.21	6.23	NA
25 years	10,471,360	11,194,994	-723,634	11314.19	11305.27	8.93	NA
Undiscounted	16,695,121	17,580,561	-885,440	12929.05	12918.72	10.33	NA
Costs & utilities discounted at 4%	10,650,062	11,375,613	-725,551	9443.80	9436.30	7.50	NA
Identical prices for P2Y ₁₂ inhibitors	10,347,722	10,658,977	-311,255	11394.59	11385.60	8.98	NA
No difference in health states at the start of the Markov model	11,098,092	11,375,613	-277,521	11385.61	11385.60	0.01	NA

Table 3. (see previous page) Cost-effectiveness analysis outcomes of the base-case and sensitivity analyses based on the POPular Genetics trial.
a: When a genotype-guided strategy resulted in cost-savings, the ICER could not be calculated.
ICER: incremental cost-effectiveness ratio, NA: not applicable, QALY: quality-adjusted life years

Europeans, but is a lot higher in Asian populations [4]. A higher prevalence of loss-of-function alleles means more people will remain on ticagrelor or prasugrel, which will negatively affect the results of the cost-effectiveness analysis in these countries. In addition, it is uncertain how this affects outcomes of the trial. On the other hand, in countries with a similar prevalence of loss-of-function alleles, one would expect similar clinical outcomes as in our trial and similar results of a cost-effectiveness analysis. However, we should be aware that healthcare costs can differ significantly between countries.

Comparison with other studies

Several health economic analyses concerning a genotype-guided P2Y₁₂ inhibitor strategy have been published in the past [14,26-29]. These analyses used extrapolated data from for instance the PLATO or Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON TIMI) 38 trial to build their decision tree, but they did not have data from a trial specifically investigating a genotype-guided strategy. A study by Lala et al., who based most of their model input parameters on the TRITON TIMI 38, found similar results as presented within the current study [14]. They found a genotype-guided strategy to be cost-saving, and an increase in QALYs compared to both a standard treatment with prasugrel and a standard treatment with clopidogrel. Our results are also in accordance with the cost-effectiveness analysis by Wang et al., which used data from the PLATO trial and found a genotype-guided strategy to be cost-saving and with improving QALYs as compared to standard treatment with ticagrelor [26]. In addition, they also concluded that compared to a standard treatment with clopidogrel, a genotype-guided strategy improved

QALYs at an ICER of \$2560. A cost-effectiveness analysis by Limdi et al. investigated whether universal ticagrelor treatment or genotype-guided de-escalation was cost-effective in acute coronary syndrome patients undergoing PCI as compared to universal clopidogrel treatment based on real-world data [27]. While both strategies increased QALYs compared to universal clopidogrel treatment, only genotype-guided de-escalation was cost-effective at an ICER of \$42,365. Similar as to our results, results from both Wang et al. and Limdi et al. are below the 'high value' limit of \$50,000/QALY as set by the ACC/AHA guideline [25].

In contrast to our results, two studies by Sorich et al. and Crespin et al., both based on results from the PLATO trial, found that ticagrelor treatment was cost-effective as compared to a genotype-guided strategy [28,29]. This means higher costs with additional QALYs gained. The fact that ticagrelor compared to the genotype-guided strategy resulted in QALYs gained, already explains the major difference between the results of Crespin et al. and Sorich et al. compared to the results of this study [28,29]. In addition, some important considerations with respect to the findings of Crespin et al. and Sorich et al. should be taken into account. Crespin et al. noted that ticagrelor would no longer be cost-effective if the hazard ratio for mortality between ticagrelor and clopidogrel is higher than 0.93 [29], which was the case in the POPular Genetics trial (HR 1.00) [10]. In the study by Sorich et al., the cost of ticagrelor was only 3 times the cost of clopidogrel while the Dutch tariffs indicate a 43.6 times higher cost of ticagrelor compared to clopidogrel [28]. Sorich et al. reported that this small price difference between clopidogrel and ticagrelor was one of the reasons as to why ticagrelor treatment was cost-effective compared to a genotype-guided strategy in their study (ICER of ticagrelor versus a genotype-guided strategy: AUS\$ 22,821). Furthermore, researchers indicated that when the hazard ratio between ticagrelor and clopidogrel would exceed 0.95, as it does in our study, the ICER would increase to over AUS\$ 50,000, making the cost-effectiveness of ticagrelor compared to a genotype-guided strategy highly questionable.

While the previously mentioned studies used data from the PLATO and TRITON TIMI 38 trial to build their study model, the respective study groups themselves also wrote health economic analyses. The health economic analysis of the PLATO trial, which compared ticagrelor to clopidogrel, noted, like Crespin et al., that most of the QALYs gained in the ticagrelor arm of their analysis were derived from the mortality benefit

from ticagrelor as compared to clopidogrel [12]. Most benefit in the TRITON TIMI 38 health economic analysis was derived from a reduction in MI in the prasugrel arm as compared to the clopidogrel arm [30]. In the POPular Genetics trial, no numerical benefit in both MI and mortality were seen in the standard treatment arm as compared to the genotype-guided arm [10]. Besides the much lower costs of clopidogrel, treatment compared to the other P2Y12 inhibitors and some savings on bleeding events, this is one of the most important reasons as to why there was no benefit for ticagrelor and prasugrel in our analysis.

Limitations

Our results should be interpreted in light of the following limitations. This cost-effectiveness analysis was based on data from STEMI patients undergoing primary PCI. Therefore, it is unknown if these results also apply to patients with another form of acute coronary syndrome and patients not undergoing PCI. In addition, a majority of the patients in the POPular Genetics trial were treated with ticagrelor. Since the recently published Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment (ISAR-REACT) 5 trial found that the use of prasugrel might be beneficial over the use of ticagrelor [31], both costs and event rates might be affected if more patients are treated with prasugrel instead of ticagrelor. This applies to both the standard treatment and genotype-guided groups, since 1/3 of patients in the genotyping group are still treated with ticagrelor or prasugrel. Concerning the health-economic analysis, the analysis is based on a healthcare perspective instead of a societal perspective, which is sometimes preferred. However, using a societal perspective would mean further assumptions regarding costs that had to be made. Besides, the only difference in our model was present in the first year during the decision tree, hence adding the same costs to both arms (treatment and control) was expected not to add additional value to the model nor affect the result of the analysis.

Conclusion

In STEMI patients undergoing primary PCI, a *CYP2C19* genotype-guided strategy compared to standard treatment with ticagrelor or prasugrel resulted in QALYs gained and cost-savings.

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Part III: Switching P2Y₁₂ inhibitors in the early phase after STEMI

Reloading when switching from ticagrelor or prasugrel to clopidogrel within 7 days after STEMI

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When switching from ticagrelor or prasugrel to clopidogrel early after myocardial infarction (MI), a loading dose is recommended [1, 2]. However, this recommendation is based solely on pharmacodynamics studies, whereas studies investigating the effects on clinical outcomes (i.e., stent thrombosis [ST] and bleeding) are lacking. In this pre-specified subanalysis of the POPular Genetics (Patient Outcome after Primary Percutaneous Coronary Intervention [PCI]) trial, we aimed to investigate the effect of loading with clopidogrel on clinical outcomes when switching from ticagrelor or prasugrel to clopidogrel early after MI.

Results of the POPular Genetics trial have been published recently [3]. Noncarriers of loss-of-function alleles switching to clopidogrel within seven days after primary PCI and with a known loading dose status were included for this analysis. The primary thrombotic outcome consisted of cardiovascular death, MI, definite ST, and stroke. The primary bleeding outcome consisted of Platelet Inhibition and Patient Outcomes (PLATO) major and minor bleeding. Both outcomes were assessed at 7 days after switching. T $\frac{1}{4}$ 0 was defined as the moment the first dose of clopidogrel was administered. Groups were compared using Student’s t-test, chi-square test, or 1-way analysis of variance. The log-rank test was used to calculate p values. p Values below 0.05 were considered statistically significant.

Six hundred eighty-eight patients, of whom 172 received a loading dose, were suitable for this analysis. Seventy-one received clopidogrel 300mg, and 101 received clopidogrel 600mg. Except for 7 patients who switched from prasugrel, the 681 other patients switched from ticagrelor to clopidogrel. Median time from primary PCI to switching was approximately 37 h [3]. Most patients receiving a loading dose when switching were included after publication of the European Society of Cardiology dual-antiplatelet therapy guideline in September 2017, whereas most patients that did not were included before that date. Probably because of this, there were some clear differences in procedural characteristics. Femoral access site (34.1% vs. 15.3%), periprocedural use of glycoprotein IIb/IIIa receptor blocker (46.6% vs. 29.8%), bivalirudin use (5.9% vs. 1.2%), bare-metal stent use (5.8% vs. 0%), and multivessel disease (52.1% vs. 40.6%) were more common in the patient group not receiving a loading dose. The thrombotic outcome did not occur within 7 days of switching in patients in the loading dose group, whereas it occurred in four patients in the no loading dose group (0% vs. 0.8%; p = 0.25) (Table 1). Thrombotic events occurred at the day of switching (stroke and recurrent MI), at day 3 (ST) and at day 4 (coronary artery bypass surgery-related stroke). The

bleeding outcome also did not occur in the loading dose group, whereas 3 patients in the no loading dose group experienced a bleeding event within 7 days after switching (0% vs. 0.6%; p $\frac{1}{4}$ 0.32), consisting of one coronary artery bypass grafting surgery-related PLATO major bleeding (0.2%; p $\frac{1}{4}$ 0.58) and two PLATO minor bleedings (0.4%; p $\frac{1}{4}$ 0.19). Bleeding events occurred 3 to 5 days after switching to clopidogrel.

In conclusion, we found that very low event rates irrespective of using a loading dose, while there were numerically less thrombotic and bleeding events in the loading dose group. These results give some support for using a clopidogrel loading dose. However, due to the low event rates and lack of randomization, this study should be considered as hypothesis generating, and definitive conclusions cannot be drawn. In addition, though clopidogrel may not provoke bleeding, these data cannot be extrapolated to prasugrel or ticagrelor loading doses. Furthermore, this study was performed in patients who were noncarriers of loss-of-function alleles, and results may therefore not be generalisable to carriers of loss-of-function alleles who are at increased thrombotic risk and might benefit more from a loading dose. Despite these limitations, this is the first study reporting clinical outcomes in patients using a loading dose or not. Furthermore, the decision to switch to clopidogrel was based on the trial protocol, whereas the decision to use a loading dose or not was homogenous among patients in each individual centre. These decisions were therefore less susceptible to bias.

Table 1. Outcomes after using a loading dose versus no loading dose when switching to clopidogrel.

	Loading dose (N=172)	No loading dose (N=516)	P value
Thrombotic outcome (cardiovascular death, MI, definite ST, stroke)	0 (0)	4 (0.8)	0.25
Combined bleeding outcome (PLATO major and minor bleeding)	0 (0)	3 (0.6)	0.32
PLATO major bleeding	0 (0)	1 (0.2)	0.58
PLATO minor bleeding	0 (0)	2 (0.4)	0.40

Table 1. Values are n (%) of patients. Outcomes are 7 days after switch. MI = myocardial infarction; PLATO = platelet inhibition and patient outcomes; ST = stent thrombosis.

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General discussion

The most important gene that influences platelet reactivity in patients treated with clopidogrel is the *CYP2C19* gene, which encodes the eponymous enzyme [1]. Since it was discovered that a standard dose of clopidogrel does not have the desired antiplatelet effect in all patients, alternative strategies for standard clopidogrel dosage have been investigated. However, the more potent P2Y₁₂ inhibitors prasugrel and ticagrelor proved beneficial in reducing atherothrombotic events, they also led to an increase in bleedings [2,3]. Furthermore, a reduction in atherothrombotic events in the past decade due to newer stents and improved secondary prevention [4] marked a turning point where focus has shifted from only preventing atherothrombotic events to preventing bleeding events as well. This thesis focusses on using *CYP2C19* genetic testing to de-escalate P2Y₁₂ inhibitor therapy. Most data is derived from the randomized POPular Genetics trial, which investigated whether a *CYP2C19* genotype-guided strategy is feasible in clinical practice and whether it can help to reduce bleeding complication rates without increasing atherothrombotic event rates as compared to treatment with prasugrel or ticagrelor.

De-escalation of P2Y₁₂ inhibitor therapy

The first two chapters of this thesis form the introduction. **Chapter 1** consists of a review regarding the rationale behind using *CYP2C19* genetic testing and how it can be used to either escalate or de-escalate antiplatelet therapy, depending on what standard treatment is used [5]. When escalating, patients are treated with clopidogrel and switch to ticagrelor or prasugrel if they carry a *CYP2C19* loss-of-function (LoF) allele. When de-escalating, patients are treated with ticagrelor or prasugrel and switch to clopidogrel if they do not carry a loss-of-function allele. This thesis focusses is on de-escalation of antiplatelet therapy, which is introduced by a review in **Chapter 2**. This review describes three different methods to de-escalate antiplatelet therapy; unguided, platelet function testing (PFT)-guided and genotype-guided. Results of the three corresponding randomized trials are presented [6]. All three methods share the same class IIb recommendation in the latest European Society of Cardiology (ESC) guideline for patients with an acute coronary syndrome without persistent ST-segment elevation to be used as an option to de-escalate treatment [7]. However, unguided de-escalation might not be the best option in all patients, as seen in observational data [8] and in patients carrying *CYP2C19* LoF alleles. This was seen in the recent TAILOR PCI trial and in earlier trials like the TRITON TIMI 38 as well [1,9]. The review also

provides pros and cons of using either genotyping or PFT and provides handles as to which patients could be selected for de-escalation and what strategy should be chosen when de-escalating to clopidogrel.

Feasibility of using genetic testing in clinical practice

In **Chapter 3**, the feasibility of using *CYP2C19* genetic testing is studied by presenting descriptive data from two different cohorts [10]. The first cohort consists of 2556 patients from the POPular Risk Score (PRiS) project and the second cohort consists of 1038 patients from the POPular Genetics trial. The PRiS was a single-centre prospective registry that included consecutive patients that underwent non-urgent percutaneous coronary intervention (PCI) [10]. Blood samples were obtained from all participants and tested for the *CYP2C19**2 and *3 loss-of-function (LoF) alleles three times daily by the on-site pharmacogenetic laboratory. The POPular Genetics trial was a multi-centre randomized controlled trial (RCT) that included ST-segment elevation myocardial infarction (STEMI) patients who underwent primary PCI [10]. Patients in the intervention arm were tested for the same LoF alleles as patients in the PRiS cohort. Testing was performed in a laboratory at the coordinating site using the same logistics as in the PRiS or by using a point-of-care (POC) system at each participating site. In the PRiS cohort, median time from blood collection to genotyping results was 4:04 hours. In the POPular Genetics, when on-site genotyping was used, median time from randomization to genotyping results was 2:04 hours when using the lab at the coordinating site or 2:24 hours when using a POC system. In both studies, results were available within 24 hours in more than 90% of the patients. Though the POC system produced an inconclusive result in 8% of the tests, meaning the test has to be done again, it has some distinct advantages over laboratory-based tests since these require more training, equipment and infrastructure. In clinical practice, POC systems can provide even greater speeds than in a trial since written informed consent is not necessary anymore and tests can be performed all day long. With the one-hour turnaround time of the test, results in STEMI patients who undergo primary PCI could be available almost as soon as they leave the Cath lab.

Clinical outcomes of *CYP2C19* genetic testing

The primary clinical outcomes of the POPular Genetics trial are reported in **Chapter 4** [11]. In the POPular Genetics trial 2488 patients were randomized between standard treatment with ticagrelor or prasugrel (n=1246) or genotype-guided treatment (n=1242). In the genotype-guided group, patients that carried a *CYP2C19**2 or *3 were treated with ticagrelor

or prasugrel, while noncarriers were de-escalated to clopidogrel within 48 hours after PCI. Follow-up duration of the trial was 12 months after primary PCI. The genotype-guided strategy was non-inferior compared to standard treatment regarding the first co-primary outcome defined as all-cause death, recurrent myocardial infarction, stent thrombosis, stroke and Platelet Inhibition and Patient Outcome (PLATO) major bleeding (HR = 0.87, $P_{\text{non-inferiority}} < 0.001$). The genotype-guided strategy was superior regarding the second co-primary outcome defined as PLATO major and minor bleeding (HR 0.78, $P = 0.04$). This was mainly driven by a reduction in PLATO minor bleedings. Based on these results, using a genotype-guided strategy for all STEMI patients that undergo PCI could be beneficial.

Critics of the strategy argue that in a post-hoc analysis of the PLATO trial, which included 7080 patients (recruited between 2006 and 2008), ticagrelor proved to be superior compared to clopidogrel irrespective of *CYP2C19* genotype [12]. However, regarding the thrombotic outcome, ticagrelor was not superior compared to clopidogrel in noncarriers of a LoF allele (HR = 0.86, $P = 0.06$). Since then, event rates have decreased considerably due to newer stents and improved secondary prevention. For instance, in the subgroup analysis of PLATO which included STEMI patients intended for reperfusion with primary PCI, a thrombotic event rate (consisting of cardiovascular death, MI and stroke) of 9.4% was found in the ticagrelor group [13]. That is almost three times the rate seen in POPular Genetics and other recently published trials like TAILOR PCI and TROPICAL ACS [9,14]. Therefore, a similar analysis is presented in **Chapter 5** [15]. It included 2429 patients, 821 noncarriers of LoF alleles treated with clopidogrel and 1608 patients treated with ticagrelor or prasugrel irrespective of their genotype. It found similar rates of atherothrombotic events (cardiovascular death, myocardial infarction, stent thrombosis, stroke) in both groups (HR = 1.14, $P = 0.62$) and a significant reduction in (mostly minor) bleedings in clopidogrel treated patients (HR = 0.74, $P = 0.03$). The on-treatment analysis found similar results with no differences in atherothrombotic events HR = 0.98, $P = 0.94$) and a similar reduction in bleeding events (HR = 0.69, $P = 0.008$) in clopidogrel treated patients. The results of these analyses in combination with the main trial results suggest the strategy of de-escalation is safe and could benefit patient outcomes.

Elderly patients

Higher age is an independent predictor of high on-treatment platelet reactivity when being treated with clopidogrel [16]. This is why **Chapter**

6 reports outcomes of *CYP2C19* genetic testing in patients aged 70 and older. In the POPular Age trial, which randomized non-STEMI and unstable angina patients aged 70 years and older to receive either ticagrelor/prasugrel or clopidogrel in combination with aspirin during one year [17], *CYP2C19* genetic testing was performed at the end of the trial. Data of these patients was combined with data from patients in the POPular Genetics trial aged 70 years and older to form the presented analysis. Furthermore, it contains subgroup analysis of the POPular Age and POPular Genetics separately. In the combined analysis, 1002 patients were included, 402 noncarriers of LoF alleles treated with clopidogrel and 600 patients treated with ticagrelor or prasugrel. In this small subgroup, results were comparable to the POPular Genetics with no difference in atherothrombotic event rates (HR = 1.00, $P = 0.98$) and less bleeding events in clopidogrel treated patients (HR = 0.83, $P = 0.23$), though this difference was not statistically significant. In addition, in the POPular Genetics subgroup analysis, age was not a significant interaction variable [11], suggesting results are generalizable across all ages. In the POPular Age trial, clopidogrel treated patients had lower bleeding event rates (HR = 0.71, 95% CI 0.54-0.94) and similar atherothrombotic event rates (HR = 0.92, 95% CI 0.65 – 1.34) as ticagrelor or prasugrel treated patients, while clopidogrel treated patients were not prospectively tested for the *CYP2C19* gene [17]. Therefore, one could question whether genetic testing provides any additional value in elderly patients. However, these results are in contradiction to results from the Bremen STEMI registry, TAILOR PCI trial and a genetic sub analysis of the TRITON TIMI 38 trial, which suggest clopidogrel treatment, is not the best option for all (elderly) patients and that genetic testing can help select patients that do benefit [1,9,18].

*CYP2C19**17 allele

While most of this thesis focusses on the *CYP2C19* LoF alleles, some argue that the *17 allele has a gain-of-function effect. Clopidogrel treated carriers of this allele would have less chance of an atherothrombotic event and a higher chance of a bleeding event than noncarriers of this allele. In addition to the previous sub analysis, **Chapter 5** also focusses on the existence of this effect. The subgroup analysis included clopidogrel treated noncarriers of LoF and compared 312 carriers of *17 alleles to 420 *CYP2C19**1/*1 patients. It found no evidence for a gain-of-function effect on clinical outcomes with similar rates of atherothrombotic events (HR = 0.95, $P = 0.90$) and a non-significant lower bleeding event rate (HR = 0.74, $P = 0.21$) in *17 carriers. These results are in accordance with a study by Lewis et al., which found that the *2 and *17 alleles were in complete

linkage disequilibrium [19]. This means the two alleles do not exist on the same chromosome. The gain-of-function effect of the *17 allele was mostly, if not entirely, derived from the non-existence of the *CYP2C19**2 variant.

Cost-effectiveness

With aging populations and advances in diagnostics and treatments of diseases, healthcare costs are rapidly increasing across the globe. We now live in an era where medical innovations and its associated costs have reached or even surpassed the economic capabilities of many modern countries. Therefore, the importance of health economic analyses has increased in the past decades. **Chapter 7** presents a cost-effectiveness analysis of implementing a *CYP2C19* genotype-guided strategy based on the POPular Genetics trial. While earlier studies have already shown this to be a cost-effective strategy [20-22], these studies based their assumptions on trials that did not specifically investigate a genotype-guided strategy. The current analysis compared two cohorts of 1000 patients with STEMI undergoing primary PCI. The first cohort was treated using a *CYP2C19* genotype-guided strategy, while the second was treated with ticagrelor or prasugrel. After a one-year decision tree based on the POPular Genetics trial, all patients entered a life-long Markov model to simulate costs and effects. In the base-case analysis, a genotype-guided strategy resulted in 8.98 quality-adjusted life-years (QALYs) gained and €725,550.69 in cost-savings (dominant) per 1000 patients. In scenario analyses simulating different time horizons, different discount rates, equal drug prices for all P2Y₁₂ inhibitors (simulating the availability of generic ticagrelor and prasugrel) and an equal distribution of atherothrombotic events at the beginning of the Markov model, the genotype-guided strategy remained dominant with cost-savings and increased QALYs. These results suggest a *CYP2C19* genotype-guided strategy does not only improve patient outcomes, but also reduces healthcare costs.

Switching P2Y₁₂ inhibitors in the early phase after STEMI

Clopidogrel, ticagrelor and prasugrel have different pharmacodynamics and kinetics. When switching between these drugs, especially in the early phase after myocardial infarction, it is important to have a sufficient reduction in platelet reactivity, while trying to keep the bleeding risk as low as possible. Therefore, the ESC has published a focused update on dual antiplatelet therapy, which provides recommendations on how to switch between the different drugs [23]. It recommends using a 600mg loading dose of clopidogrel 24 hours after last administration of ticagrelor or

prasugrel. However, this recommendation is based solely on pharmacodynamic studies that present only limited results on clinical outcomes. Therefore, **Chapter 8** reports on outcomes when switching from ticagrelor (and in a few cases prasugrel) to clopidogrel within one week after primary PCI [24]. This was done without a loading dose in 516 patients (mostly included earlier in the trial), while a loading dose of either 300mg or, in most cases 600mg, was used in 172 patients. In the group of patients that received a loading dose of clopidogrel, there were no atherothrombotic or bleeding events in the 7 days after switching, while in the patient group that received no loading dose, four patients experienced an atherothrombotic and three patients experienced a bleeding event. The non-randomized nature of this study and the small number of events restrict drawing any strong conclusions, but using a new loading dose does not seem to increase the risk of bleeding and might help to reduce atherothrombotic events as compared to not using a loading dose.

Future perspective

While in cancer treatment, genetic analysis of different types of cancer has already found widespread implementation to guide treatment, in the field of cardiovascular medicine, personalised therapies still have a limited role in clinical practice. For a widespread implementation of personalised antiplatelet strategies, several requirements have to be met; 1) it should improve clinical outcomes, 2) results have to be consistent and easy to interpret, 3) the test should be easy to perform, 4) results should be available within a reasonable amount of time and 5) costs have to be reimbursed.

The first requirement has partially been met. The first studies prospectively investigating a *CYP2C19* genotype-guided approach were published almost a decade ago and there has been an increasing interest amongst cardiologists in the strategy since then. While the POPular Genetics trial demonstrated a clear benefit in reducing bleeding event rates, it was not powered to detect differences in ischemic events. Probably, this is the reason that the latest NSTEMI guideline from the ESC only gives the strategy a class IIb recommendation (may be considered). For a higher recommendation in the guidelines, more data is needed. This could either be provided by additional, larger RCTs or by providing more registry data of the strategy in clinical practice. However, large RCTs are impossible to execute without financial support (mostly provided by the pharmaceutical industry) and currently no large trials are expected in the near future.

With a paucity of very large RCTs, meta-analysis could be considered the next best evidence. Recently, a meta-analysis by Pereira et al. was published. It included 15,949 patients (98% with acute coronary syndrome, 77% treated with PCI) and compared clopidogrel to ticagrelor or prasugrel separately in noncarriers of *CYP2C19* loss-of-function alleles and in carriers of loss-of-function alleles [25]. They found that ticagrelor and prasugrel compared with clopidogrel resulted in a significant reduction in atherothrombotic events in *CYP2C19* loss-of-function carriers (Relative risk 0.70, 95% CI, 0.59 – 0.83), but not in noncarriers (RR 1.0, 95% CI 0.80 – 1.25). The test for interaction was statistically significant ($p = 0.013$), suggesting this effect is caused by carrying *CYP2C19* loss-of-function alleles. Therefore, this meta-analysis, which compares the P2Y₁₂ inhibitors directly, demonstrated clopidogrel, in noncarriers of *CYP2C19* loss-of-function alleles, is as effective as ticagrelor and prasugrel. Thus providing more, though indirect, evidence that a genotype-guided strategy can be used. Another large meta-analysis, by Galli et al., was also published recently [26]. It compared guided therapy (both de-escalation and escalation strategies using genotyping or PFT) to standard treatment and found that guided therapy reduced major adverse cardiac events (RR 0.78, 95% CI, 0.63 – 0.95) and a trend towards a reduction in bleedings (RR 0.88, 95% CI 0.77 – 1.01). Comparing escalation and de-escalation strategies separately, on the one hand it found that a guided escalation strategy compared to standard clopidogrel treatment resulted in reductions in atherothrombotic outcomes without increasing bleeding event rates. On the other hand, it found that a guided de-escalation strategy compared to standard ticagrelor or prasugrel treatment resulted in a reduction in bleedings (driven by minor bleedings), without any differences in atherothrombotic outcomes. Findings of these meta-analyses might provide a reason for a higher recommendation in future guidelines.

The second requirement has been met by using genetic testing. A genotype-guided strategy has distinct advantages over, for instance, a PFT guided strategy. PFT requires a patient to already be on clopidogrel for a considerable amount of time before it can be tested. Additionally, many factors influence PFT and there is a large variability in results between different PFT tests. Genetic testing does not have these disadvantages as long as there is no contamination of the blood sample or buccal swab.

POC systems like the one from Spartan Bioscience are easy to use and require only limited training. Also, a buccal swab provides almost no burden for the patient. However, during the POPular Genetics trial, almost 10% of samples returned inconclusive and I noticed differences in error

rates from the tests between different centres. This could be due to incorrectly obtaining the buccal swab or incorrectly preparing the samples before testing. The inconclusive rate should be lower than 10% if it is to be widely used. This could be achieved by better training, or the inconclusive rate may improve simply due to more experience. Perhaps, newer techniques become available which reduce the chance of inconclusive results. Additionally, the buccal swabs cannot be stored in standard freezers, since they require lower temperatures (between -20° and -80° Celsius). For the test to be more accessible, this could also be improved upon. Therefore, despite a few shortcoming that have to be improved in the future, it is my opinion that the third requirement has also been met. Furthermore, laboratory based tests can also be used without additional burden for the patient if the blood sample is taken at the time of coronary angiography. However, the required expertise, and infrastructure is not available in all hospitals.

The fourth requirement has also been met. Both data from the POPular Genetics trial, PRiS and data from other groups investigating a genotype-guided strategy [27] have demonstrated that results can be available within hours. A POC system could be used 24 hours daily by personnel at the Cath lab or coronary care unit, while with a dedicated system, as seen in the St. Antonius hospital, even laboratory-based tests can be used with sufficient speed.

The fifth requirement, as of now, seems to be the largest obstacle for many hospitals. Implementing a genotype-guided strategy is associated with significant costs for hospitals. Cost-savings, however, do not end up in the hospital, but with the insurance companies or governments that pay for healthcare. In the United States, both Medicare and Medicaid already provide reimbursement, but this is not the case in the Netherlands and other European countries. The availability of cost-effectiveness analyses might help to convince policymakers in the government and insurance companies to change their strategy. While more data from meta-analyses and registries demonstrating a beneficial effect on hard clinical outcomes could also help tip the skill.

While this thesis focusses on using a genotype-guided strategy to personalize and de-escalate antiplatelet therapy after myocardial infarction, other methods of de-escalation are also investigated. Results from several RCTs investigating different options, like de-escalating from normal dose ticagrelor (90mg twice daily) or prasugrel (10mg once daily) to low dose ticagrelor (60mg twice daily) or prasugrel (5mg once daily) in combination with aspirin have been published in recent years.

Simultaneously, other trials investigated strategies where aspirin was discontinued after one to three months after PCI and thus patients were treated with ticagrelor or prasugrel monotherapy. Since none of these strategies have been compared head-to-head or to genotype or PFT-guided strategies, it is currently unknown, which provides the best outcomes for patients and what strategy is most practical. Only time will tell, but improvements in clinical outcomes are sure to continue.

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Nederlandse samenvatting

Plaatjesaggregatie remmers worden voorgeschreven aan patiënten met een myocardinfarct om de kans op een recidief te verkleinen. Patiënten worden behandeld met duale antiplaatjes therapie, bestaande uit aspirine en een P2Y₁₂ remmer. De Europese en Amerikaanse richtlijnen geven daarbij de voorkeur aan de sterkere P2Y₁₂ remmers ticagrelor of prasugrel in plaats van clopidogrel, omdat mensen die behandeld worden met deze sterkere middelen minder kans hebben op ischemische events zoals stent trombose, myocardinfarcten, herseninfarcten en overlijden. Behandeling met deze sterkere P2Y₁₂ remmers geeft echter ook een grotere kans op bloedingen. Ook bloedingen kunnen ernstige gevolgen hebben en zijn geassocieerd met slechtere uitkomsten, zoals bijvoorbeeld overlijden. Het is daarom belangrijk om deze te voorkomen.

Clopidogrel is een pro-drug welke gemetaboliseerd wordt door cytochroom P450 iso-enzymen om actief te worden. Het belangrijkste enzym in dit proces is het CYP2C19 enzym. Dit enzym wordt gecodeerd door het *CYP2C19* gen. Ongeveer 1/3 van de Kaukasische bevolking draagt allelen van het gen waardoor de metabolisering minder goed verloopt, zogenaamde verlies-van-functie allelen. Het *2 allel en in mindere mate het *3 allel zijn hiervan verreweg de meest voorkomende allelen. In dit proefschrift is onderzocht of een strategie waarbij gebruik wordt gemaakt van een genetische test voor het *CYP2C19* gen leidde tot minder bloedingen zonder dat er meer ischemische events waren. Dit werd vergeleken met de uitkomsten van een standaardbehandeling waarbij iedereen behandeld werd met ticagrelor of prasugrel. In de genotype-gestuurde strategie behandelden we patiënten die drager waren van verlies-van-functie allelen met ticagrelor of prasugrel, terwijl mensen die geen drager waren behandeld werden met clopidogrel. Daarnaast is gekeken of deze strategie praktisch haalbaar is in de praktijk en of deze strategie kosteneffectief is. Tenslotte is ook gekeken of er een nieuwe oplaaddosis van clopidogrel moet worden gegeven als er gede-escaleerd wordt van ticagrelor of prasugrel naar clopidogrel.

De inleiding van dit proefschrift bestaat uit **hoofdstuk 1** en **hoofdstuk 2**. Hoofdstuk 1 bevat een review waarin werd ingegaan op de grondgedachte achter het gebruik van *CYP2C19* genetische testen. Het beschrijft zowel onderzoek naar het escaleren van antiplaatjes therapie (switchen van clopidogrel naar ticagrelor of prasugrel wanneer iemand drager is van verlies-van-functie allelen) als naar het de-escaleren van antiplaatjes

therapie (switchen van ticagrelor of prasugrel naar clopidogrel als iemand geen drager is van verlies-van-functie allelen). In hoofdstuk 2 werd in een review specifiek ingegaan op het de-escaleren. Dit kan op drie verschillende manieren; 1) zonder te testen; 2) door gebruik te maken van plaatjes functie testen; en 3) door gebruik te maken van genetische testen. Op basis van drie verschillende gerandomiseerde onderzoeken hebben al deze methoden dezelfde klasse IIb indicatie gekregen (er kan overwogen worden deze strategie te gebruiken), in de laatste European Society of Cardiology (ESC) richtlijn voor patiënten met een acuut coronair syndroom zonder persisterende ST-segment elevaties. Echter, op basis van enkele andere onderzoeken, zoals de recent gepubliceerde TAILOR PCI en de oudere TRITON TIMI 38 studie, lijkt de-escaleren zonder gebruik te maken van een test niet de beste optie bij alle patiënten, met name bij dragers van *CYP2C19* verlies-van-functie allelen. Verder beschrijft de review de voor- en nadelen van een genetische test ten opzichte van een plaatjesfunctie test en op welke strategie gevolgd moet worden als er gede-escaleerd wordt.

Haalbaarheid van het implementeren van *CYP2C19* genetisch testen

In **hoofdstuk 3** werd de haalbaarheid van het implementeren van *CYP2C19* testen in de praktijk beschouwd, op basis van twee verschillende cohorten. Het ene cohort bestond uit 2556 patiënten uit de POPular Risk Score (PRiS) studie en het andere cohort bestond uit 1038 patiënten uit de POPular Genetics studie. De PRiS was een prospectieve registratie-studie uitgevoerd in één centrum en includeerde patiënten die een electieve percutane coronaire interventie (PCI) ondergingen. Bij alle patiënten werd bloed afgenomen en dit werd drie keer per dag in het farmacogenetisch lab getest op de aanwezigheid van de *CYP2C19**2 en *3 verlies-van-functie allelen. De POPular Genetics studie was een gerandomiseerde studie en includeerde patiënten met een ST-segment elevatie myocardinfarct die een primaire PCI ondergingen. In de interventiegroep werden patiënten op dezelfde allelen getest als in de PRiS studie. Testen werden in het coördinerende centrum volgens dezelfde methode uitgevoerd als in de PRiS studie of patiënten werden in het eigen ziekenhuis getest door gebruik te maken van een point-of-care (POC) systeem. In de PRiS was de mediane tijd van bloedafname tot de uitslag ruim 4 uur. In de POPular Genetics was de tijd van randomisatie tot het bekend worden van het *CYP2C19* genotype iets meer dan 2 uur wanneer patiënten in het eigen ziekenhuis getest werden, zowel bij tests via het lab als via het POC-systeem. In beide studies

was bij meer dan 90% van de patiënten de uitslag binnen 24 uur bekend. Bij het POC-systeem was ongeveer 8% van de uitslagen niet conclusief, waardoor de test opnieuw gedaan moest worden. Toch heeft het gebruik van POC-systemen voordelen doordat er minder training, apparatuur en infrastructuur vereist is. Daarnaast kunnen deze testen 24 uur per dag worden gebruikt, bijvoorbeeld door verpleegkundige of personaal op de hartkatheterisatie kamers. Als ze in de klinische praktijk worden gebruikt kan de uitslag al binnen 1 uur bekend zijn. Medicatie kan dan al volgens een *CYP2C19* genotype-gestuurde strategie worden aangepast zodra een patiënt de hartkatheterisatie kamer verlaat.

Klinische uitkomsten bij het gebruik van een *CYP2C19* genotype-gestuurde strategie

De primaire klinische uitkomsten van de POPular Genetics studie werden beschreven in **hoofdstuk 4**. In de POPular Genetics studie werden 2488 patiënten gerandomiseerd naar een standaardbehandeling met ticagrelor of prasugrel (n=1246) of naar een genotype-gestuurde strategie (n=1242). In de genotype-gestuurde strategie werden patiënten die dragers waren van een *CYP2C19**2 of *3 allel behandeld met ticagrelor of prasugrel en patiënten die geen dragers waren met clopidogrel. Deze verandering in medicatie gebeurde binnen 48 uur na de primaire PCI en follow-up van het onderzoek liep tot 12 maanden na de primaire PCI. De genotype-gestuurde groep was non-inferieur ten opzichte van de standaardbehandeling met betrekking tot de eerste co-primaire uitkomst bestaande uit overlijden, myocardinfarct, stent trombose, cerebrovasculair accident (CVA) en grote bloedingen gedefinieerd volgens Platelet Inhibition and Patient Outcome (PLATO) (HR 0.87, $P_{\text{non-inferieur}} < 0.001$). De genotype-gestuurde strategie was superieur met betrekking tot de tweede co-primaire uitkomst bestaande uit PLATO grote en kleine bloedingen (HR = 0.78, $P = 0.04$). Dit werd voornamelijk veroorzaakt door een vermindering van het aantal kleine bloedingen.

In **hoofdstuk 5** werden twee verschillende patiëntengroepen met elkaar vergeleken, namelijk patiënten die geen drager zijn van verlies-van-functie allelen behandeld met clopidogrel en patiënten die behandeld zijn met ticagrelor of prasugrel. Er werd gekeken naar dezelfde eindpunten als genoemd in de POPular Genetics studie. Er werden 2429 patiënten geïncludeerd, waarvan er 821 werden behandeld met clopidogrel en 1608 met ticagrelor of prasugrel. Er werd geen verschil gezien in het aantal

ischemische uitkomsten (cardiovasculair overlijden, myocardinfarct, stent trombose en CVA) tussen beide groepen (HR = 1.14, $P = 0.62$) en er was een significante vermindering van het aantal (meestal kleine) bloedingen (HR = 0.74, $P = 0.03$).

Omdat leeftijd een onafhankelijke voorspeller is voor onvoldoende plaatjesremming bij patiënten die behandeld worden met clopidogrel, is in **hoofdstuk 6** specifiek gekeken naar oudere patiënten. Een deel van de patiënten uit deze analyse komt uit de POPular Age studie, waarin patiënten van 70 jaar en ouder met een niet-ST-segment elevatie myocardinfarct of instabiele angina pectoris werden geïncludeerd. Zij werden gerandomiseerd tussen clopidogrel enerzijds of ticagrelor of prasugrel anderzijds. Vooraf werd geen genetische test verricht, maar bij de patiënten waar bloed van beschikbaar was werd aan het einde van het onderzoek getest op de *CYP2C19**2 en *3 verlies-van-functie allelen. Deze patiënten werden gecombineerd met de patiënten van 70 jaar en ouder uit de POPular Genetics studie en net als in hoofdstuk 5 werden patiënten zonder verlies-van-functie allelen behandeld met clopidogrel vergeleken met patiënten behandeld met ticagrelor. In totaal waren er 991 patiënten, 401 behandeld met clopidogrel en 590 met ticagrelor. In deze kleinere groep met ouderen waren er vergelijkbare resultaten als bij de gehele groep, echter was het verschil in bloedingen niet statistisch significant (ischemische uitkomsten HR = 1.00, $P = 0.98$, bloedingen HR = 0.83, $P = 0.23$). Tevens gaf leeftijd in de subgroep analyse van de POPular Genetics geen significante interactie. Daarom lijkt ook bij ouderen het gebruik van een *CYP2C19* genotype strategie voordelen te hebben.

Heeft het *CYP2C19**17 allel een versterkende werking

Hoewel het grootste deel van dit proefschrift zich focust op verlies-van-functie allelen, zijn er ook onderzoekers die beargumenteren dat het veel voorkomende *17 allel zorgt voor een sterkere werking van clopidogrel. In **hoofdstuk 5** werd daarom ook bekeken of dit effect zich zou uiten in klinische uitkomsten. In deze subgroep analyse zijn 732 patiënten geïncludeerd die behandeld waren met clopidogrel en die geen drager waren van een verlies-van-functie allel. Hiervan waren er 312 patiënten drager van één of twee *17 allelen en 420 patiënten hadden het *1/*1 genotype. Er werden geen aanwijzingen gevonden voor het bestaan van een versterkende werking van het *17 allel. Er werd een vergelijkbaar aantal ischemische uitkomsten gezien in beide groepen (HR = 0.95, $P =$

0.90) en een niet significant lager aantal bloedingen in *17 dragers (HR = 0.74, P = 0.21). Als er een versterkende werking zou zijn, zou juist verwacht worden dat er meer bloedingen zijn en minder ischemische uitkomsten.

Kosteneffectiviteit van het implementeren van een CYP2C19 genotype-gestuurde strategie

Hoofdstuk 7 behandelde de kosteneffectiviteit van het invoeren van een *CYP2C19* genotype-gestuurde strategie vergeleken met een standaardbehandeling met ticagrelor of prasugrel. In de analyse werden twee cohorten van elk 1000 patiënten, gebaseerd op de POPular Genetics studie, met elkaar vergeleken. Patiënten doorliepen eerst een 1-jaars beslisboom gebaseerd op de studie en kwamen daarna in een levenslang Markov model om kosten en effecten te simuleren. In de basisanalyse leidde de genotype-gestuurde strategie tot een toename in quality-adjusted life-years (QALYs) van 8.98 en een afname in de kosten van €725,550.69 euro per 1000 patiënten. Ook in verschillende scenario analyses, onder andere een waarin de medicijn kosten gelijk waren om de generieke beschikbaarheid van ticagrelor en prasugrel te simuleren, bleef de genotype-gestuurde strategie kosteneffectief.

Heropladen met clopidogrel na het switchen van P2Y12 remmers

In **hoofdstuk 8** werd gekeken of het gebruik van een nieuwe oplaaddosis van de clopidogrel, zoals wordt aangeraden in de huidige richtlijnen, effect heeft op de uitkomsten in de week na het myocardinfarct. Er waren 516 patiënten die geen oplaaddosis kregen en 172 patiënten die wel een oplaaddosis van 300mg of, in de meeste gevallen, 600mg kregen. In de groep die wel een oplaaddosis kreeg, ontwikkelde niemand een bloeding of een ischemische uitkomst, terwijl in de groep zonder oplaaddosis vier patiënten een ischemische uitkomst en drie patiënten een bloeding hadden. Doordat dit slechts kleine groepen zijn en de groepen niet gerandomiseerd waren kunnen er geen harde conclusies aan worden verbonden, maar het geven van een nieuwe oplaaddosis lijkt veilig.

Impact section

Relevance

Cardiovascular diseases remain the leading cause of death worldwide and can significantly reduce quality of life and economic output. In the Netherlands, more than 34 thousand people are diagnosed with myocardial infarction each year. It has been well established that in these patients, antiplatelet treatment with the P2Y₁₂ inhibitors ticagrelor, prasugrel or clopidogrel helps to prevent life threatening events like myocardial infarction, stent thrombosis and stroke. Unfortunately, this comes at the cost of increased bleeding complication rates. These occur in up to 30% (depending on the selected population) of the patients in the year after myocardial infarction and require medical attention. Therefore, it is important to reduce the amount of bleeding complications in order to improve quality of life, reduce healthcare costs and reduce overall mortality. Clopidogrel does not reach its optimal effect in approximately 30% of the population, which is mostly caused by mutations in the *CYP2C19* gene that encodes the eponymous enzyme. Patients treated with clopidogrel and carrying these loss-of-function mutations experience more atherothrombotic events, while the other P2Y₁₂ inhibitors lead to more bleeding complications. Therefore, this thesis aimed to investigate whether using *CYP2C19* genetic testing to guide antiplatelet therapy could improve patient outcomes by reducing bleeding complication rates without increasing atherothrombotic event rates like myocardial infarction, stroke and death.

Target groups

The results of this thesis are of interest to clinicians who are directly involved in the treatment of patients who have had an acute myocardial infarction. Primarily, this is the case with cardiologists who are responsible for implementing the strategy in clinical practice, but also for primary care physicians and specialists like surgeons who might have to treat bleeding complications that occur during treatment with P2Y₁₂ inhibitors. Furthermore, clopidogrel is used frequently by neurologists as well and *CYP2C19* genetic testing is of increasing interest in patients with stroke, especially in patients with recurrent strokes while being treated with clopidogrel. Results of this thesis could help to start new prospective trials to investigate whether *CYP2C19* genetic testing could improve outcomes in stroke patients as well. This thesis is also of interest to health insurance

companies and policy makers since a genotype-guided strategy could help to reduce healthcare costs while improving patient outcomes and quality of life.

Implication and implementation

A *CYP2C19* genotype-guided strategy resulted in fewer bleedings without an increase in events like myocardial infarction, stroke and death. These findings were published in the New England Journal of Medicine, which is one of the most impactful medical journals worldwide. It already has been cited in dozens of publications in the short period since its publication and has led to the genotype-guided strategy receiving a recommendation in the latest European Society of Cardiology guideline. This demonstrates the major impact of the trial and the interest for this topic of cardiologists and researchers worldwide. Many are interested in, or have already implemented the strategy in clinical practice. However, it is crucial that health insurance companies or governments reimburse the costs of the genetic test. The health economic analysis of the POPular Genetics demonstrated that a genotype-guided strategy results in improved quality of life, while reducing healthcare costs. Therefore, since in the Netherlands some hospitals have already implemented the strategy, insurance companies and governments are currently the ones saving costs, while hospitals face higher costs with buying the genetic tests. To ensure a broad implementation of the strategy in clinical practice, health insurance companies should cover these costs.

Curriculum vitae

Daniel Marcus Franciscus Claassens was born on March 14th 1993 in Rotterdam, the Netherlands. He attended the Grotius College in Delft between 2004 and 2010. In 2010 he obtained his Gymnasium diploma and in addition his International Baccalaureate A2 in English. After graduation, he studied medicine at Utrecht University. He obtained his bachelor degree in 2013 and his master degree in 2017. He quickly gained interest in the field of cardiology and started doing research at the St. Antonius Hospital in Nieuwegein while still in university under supervision of prof. dr. J.M. ten Berg. After graduation as a medical doctor, he became a fulltime PhD candidate at the department of cardiology in the St. Antonius Hospital. He was responsible for coordinating the international multicenter randomized POPular Genetics trial and worked under supervision of prof. dr. J.M. ten Berg, prof. dr. A.W.J. van 't Hof, dr. V.H.M. Deneer and dr. R.S. Hermanides. He held presentations at several national and international congresses, including a hotline session at the European Society of Cardiology congress in Paris in 2019. He won a price for best presentation at the St. Antonius wetenschapsavond in 2019 and he reached 2nd place for best research in the field of cardiovascular medicine in the Netherlands from the Werkgroep Cardiologische centra Nederland (WCN) 2019-2020.

In January 2020, he started working as a resident (ANIOS) at the department of cardiology in the St. Antonius Hospital and in December 2020, he started working as a resident (ANIOS) at the department of cardiology in the Isala. He will start his residency in cardiology (AIOS) at the department of internal medicine in the Isala in December 2021.

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