Draft Comparative Effectiveness Review

Number XX

Testing of CYP2C19 Variants and Platelet Reactivity for Guiding Antiplatelet Treatment

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Preface

The Agency for Healthcare Research and Quality (AHRQ) conducts the Effective Health Care Program as part of its mission to organize knowledge and make it available to inform decisions about health care. As part of the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Congress directed AHRQ to conduct and support research on the comparative outcomes, clinical effectiveness, and appropriateness of pharmaceuticals, devices, and health care services to meet the needs of Medicare, Medicaid, and the Children's Health Insurance Program (CHIP).

AHRQ has an established network of Evidence-based Practice Centers (EPCs) that produce Evidence Reports/Technology Assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care. The EPCs now lend their expertise to the Effective Health Care Program by conducting comparative effectiveness reviews (CERs) of medications, devices, and other relevant interventions, including strategies for how these items and services can best be organized, managed, and delivered.

Systematic reviews are the building blocks underlying evidence-based practice; they focus attention on the strength and limits of evidence from research studies about the effectiveness and safety of a clinical intervention. In the context of developing recommendations for practice, systematic reviews are useful because they define the strengths and limits of the evidence, clarifying whether assertions about the value of the intervention are based on strong evidence from clinical studies. For more information about systematic reviews, see http://www.effectivehealthcare.ahrq.gov/reference/purpose.cfm.

AHRQ expects that CERs will be helpful to health plans, providers, purchasers, government programs, and the health care system as a whole. In addition, AHRQ is committed to presenting information in different formats so that consumers who make decisions about their own and their family's health can benefit from the evidence.

Transparency and stakeholder input from are essential to the Effective Health Care Program. Please visit the Web site (http://www.effectivehealthcare.ahrq.gov) to see draft research questions and reports or to join an e-mail list to learn about new program products and opportunities for input. Comparative Effectiveness Reviews will be updated regularly.

We welcome comments on this CER. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to epc@ahrq.hhs.gov.

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Testing of CYP2C19 Variants and Platelet Reactivity for Guiding Antiplatelet Treatment

Structured Abstract

Objectives: This comparative effectiveness review evaluated the analytic validity, predictive value, and comparative effectiveness of two types of medical tests (genetic testing for CYP2C19 variants and phenotypic testing to measure platelet reactivity) to identify patients who are most likely to benefit from clopidogrel-based antiplatelet therapy and to guide antiplatelet therapy in patient populations who are eligible for clopidogrel treatment.

Data Sources: MEDLINE® (from inception to August 24, 2011), the Cochrane Central Trials Registry (through the 2nd quarter of 2011), and the Cochrane Database of Systematic Reviews (through the 2nd quarter of 2011) without any language restriction; the Human Genome Epidemiology Network (HuGENet) database and National Institutes of Health Genetic Association Database, using the same cutoff date (August 24, 2011); and the Food and Drug Administration Web site and ClinicalTrials.gov.

Review Methods: We used established systematic review methods to identify English-language articles describing studies performed in all relevant care settings, on the basis of predetermined eligibility criteria: adult patients with cardiovascular, cerebrovascular, or peripheral arterial disease who were candidates for or were receiving clopidogrel; use of genetic testing (for CYP2C19 variants) or phenotypic testing (for platelet reactivity). Studies had to report information on the analytic validity; predictive ability for intermediate (platelet reactivity) or clinical outcomes; use of tests to guide antiplatelet therapy; or adverse events from testing itself or from test-directed treatment.

Results: The literature search yielded 9441 unique citations, 842 of which were obtained in full text and reviewed. A total of 226 publications were judged to have met the inclusion criteria (some publications contributed data to multiple analyses).

Eighty-five studies provided information on the analytic validity of the tests of interest (5 for genetic testing; 80 for phenotypic testing). Test–retest reliability of genotyping assays appeared to be adequate; however, few studies were available. Agreement between assays for measuring platelet reactivity was poor to moderate; generally, agreement was higher between measurements obtained from the same assay using different agonist concentrations rather than between different assay types. Only 10 studies provided information on analytic test performance and reported variable results.

Fifty-six studies (in 51 publications) provided information on the ability of genetic testing for CYP2C19 variants to predict clinical outcomes or platelet reactivity during followup. The majority of studies were conducted in populations with ischemic heart disease. Loss-of-function alleles were associated with higher on-clopidogrel platelet reactivity and were statistically significantly associated with stent thrombosis (relative risk [RR]=1.56; 95 percent confidence interval [CI] 1.15, 2.11) and cardiovascular mortality (RR=2.40; 95 percent CI 1.05, 5.53). Gain-of-function alleles were significantly associated with reduced risk of major adverse cardiovascular events (MACE) (RR=0.80; 95 percent CI 0.70, 0.90). Studies on the predictive value of CYP2C19 variants were judged to have moderate risk of bias.

One hundred studies provided information on the ability of baseline on-clopidogrel platelet reactivity to predict clinical outcomes or platelet reactivity during followup. The majority of studies were conducted in populations with ischemic heart disease. Patients with high platelet reactivity at baseline were more likely to be clopidogrel nonresponders during followup. The ability to predict clinical outcomes was reported for various assays; the most commonly assessed were light-transmission aggregometry (46 studies); VerifyNow P2Y12 (28 studies); the vasodilator-stimulated phosphoprotein (VASP) assay (14 studies); Multiplate analyzer (12 studies); and Platelet Function Analyzer-100 (8 studies). Overall, studies suggested that increased on-clopidogrel reactivity was associated with increased rates of adverse cardiovascular outcomes. Results were sparse for most assay–outcome combinations. By meta-analysis, we found statistically significant associations between increased reactivity as measured by the VerifyNow assay and cardiovascular mortality (RR=2.50; 95 percent CI 1.28, 4.87) and MACE (RR=2.46; 95 percent CI 1.88, 3.22). We also found a significant association between platelet reactivity measured by the VASP assay and increased risk of stent thrombosis (RR=3.37; 95 percent CI 1.59, 7.11) and MACE (RR=2.57; 95 percent CI 1.21, 5.47). Studies reporting on the predictive effect of platelet reactivity assays were judged to have a moderate risk of bias.

Ten studies provided information on the use of genetic testing for CYP2C19 variants for guiding treatment choice (all were randomized treatment trials assessing effect modification by CYP2C19 status). Eleven studies provided information on the use of platelet reactivity measurements to guide antiplatelet therapy (5 were randomized trials of alternative testing strategies; 1 was a randomized treatment trial assessing effect modification by reactivity status; 5 were randomized treatment trials selecting patients on the basis of platelet reactivity levels). Studies had heterogeneous designs and compared different treatment strategies (8 compared clopidogrel dosing schemes; 2 compared clopidogrel to placebo; 6 assessed replacement agents in the place of clopidogrel; 5 evaluated the addition of a third agent to apirin plus clopidogrel treatment). Overall, there was insufficient evidence regarding the use of genotyping CYP2C19 variants or phenotypic testing for platelet reactivity to guide treatment selection.

Conclusions: We found evidence to support an association between loss-of-function CYP2C19 variants and increased risk of adverse cardiovascular outcomes. Similarly, we found evidence that high on-clopidogrel platelet reactivity is associated with an increased risk of adverse cardiovascular outcomes for at least some of the available assays. The strength of evidence regarding these prognostic effects is low because of concerns regarding selective outcome reporting and the relatively small number of studies reporting clinical outcomes. The strength of evidence regarding the use of genetic or platelet reactivity testing to guide antiplatelet treatment selection is insufficient, because studies reporting on clinical outcomes are few, have diverse designs, and included heterogeneous populations.

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Executive Summary

Background

Burden of Disease and Clinical Setting

Approximately 82 million Americans currently suffer from some form of cardiovascular disease.[1] Coronary heart disease alone is the cause of 1 of every 6 deaths in the United States; and stroke, 1 of every 18 deaths.[2] Every year more than a million Americans have a myocardial infarction and approximately 795,000 experience a first or recurrent stroke.[1] There were approximately seven million inpatient cardiovascular operations and procedures in the United States in 2007, of which one million were either percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) surgeries.[1]

Randomized controlled trials have established dual antiplatelet treatment (with clopidogrel and aspirin) as the current standard of care for medical and interventional management of acute coronary syndromes.[3] Dual antiplatelet treatment is also recommended for patients undergoing PCI[4] with placement of stents (either bare metal or drug eluting). Randomized controlled trials support the use of clopidogrel in patients who have experienced acute cardiovascular events (e.g., stroke) and those with peripheral arterial disease.[3,5-8] For patients with atrial fibrillation, vitamin K antagonists (e.g., warfarin) are the current treatment of choice; however, as many as 50 percent of patients eligible for vitamin K antagonist therapy do not receive it,[9-11] most often because of contraindications[12] (e.g., increased risk of bleeding). Evidence from trials suggests that for patients with such contradindications, the combination of clopidogrel and aspirin is more effective than aspirin alone for preventing thromboembolic disease.[13] Although clopidogrel-based antiplatelet therapy has been shown to improve patient outcomes in various settings, it appears to be associated with an increased bleeding risk compared with aspirin alone[3,14] or placebo.

Since the approval of clopidogrel by the Food and Drug Administration (FDA) for routine clinical use, the drug has become one of the most commonly prescribed agents in the United States. However, response to clopidogrel-based antiplatelet therapy is variable (both between patients and across multiple measurements within a patient), with some patients showing no platelet response to clopidogrel administration ("nonresponsiveness" or "resistance"). Alternatives to standard clopidogrel treatment include higher-dose clopidogrel regimens and the use of other antiplatelet agents, such as prasugrel or ticagrelor, which are not metabolized through the same pathways as clopidogrel. Prasugrel and ticagrelor have efficacy similar or superior to clopidogrel for preventing major adverse cardiovascular events (MACE). However, these drugs may increase the risk of bleeding complications.[15-18] Given the availability of alternative antiplatelet strategies and concern about adverse clinical outcomes in clopidogrel nonresponders, research has focused on methods to identify patients who are unlikely to benefit from clopidogrel-based treatment. The question of identifying the optimal antiplatelet therapy may also carry substantial cost implications because generic clopidogrel products will soon be available.

Clopidogrel Metabolism

To be biologically active, clopidogrel must be transformed to its active metabolite by CYP enzymes, primarily CYP2C19, which binds irreversibly to the $P2Y_{12}$ receptor (the adenosine diphosphate [ADP] receptor) on the surface of platelets and inhibits platelet aggregation for the life cycle of the platelet.[19,20] The CYP2C19 gene is highly polymorphic, with each variant designated by a number (e.g., "*1," "*2," and so on). CYP2C19*1 alleles lead to normal enzymatic activity (i.e., a normal-metabolizer phenotype). Other alleles, including the relatively common CYP2C19*2, *3, and *4 alleles, are all known to be "loss-of-function" alleles that lead to complete elimination of enzymatic activity (i.e., a poor-metabolizer phenotype). Finally, the relatively common CYP2C19*17 allele is known to be a "gain-of-function" allele that leads to increased enzymatic activity (i.e., an enhanced metabolizer phenotype). [21] The fact that each individual carries two CYP2C19 alleles results in additional variation in the observed metabolic phenotypes. For example, carriers of a *1/*2 genotype have an intermediate phenotype between that of *1/*1 and that of *2/*2. The functional status of some combinations of genotypes (usually combination of loss-of-function and gain-of-function alleles) is currently unknown; however, such genotypes are generally rare. A genomewide association study recently demonstrated that CYP2C19*2 is the main genetic determinant of variability in clopidogrel responsiveness.[22]

Predicting Response and Guiding Antiplatelet Treatment

There are currently two basic approaches to determine whether a patient will have a poor response to clopidogrel: (1) genetic testing to see whether the patient has a CYP2C19 genotype that is associated with reduced ability to metabolize clopidogrel (a poor-metabolizer phenotype), and (2) direct testing of the patient's blood while the patient is taking clopidogrel to see whether the platelets actually have become less prone to aggregate in response to specific agonists (phenotypic testing for platelet reactivity). Several recent studies have assessed the utility of these two approaches for predicting major clinical outcomes in patients receiving clopidogrel.[23-27]Although clinicians and patients may find it helpful to know the probability of future outcomes under clopidogrel therapy, medical tests are most valuable when they can be used to guide treatment decisions. Thus, beyond predictive ability, there is interest in evaluating whether genetic or phenotypic testing can improve patient outcomes by identifying patients who would benefit more (or experience less harm) by using treatment strategies other than standard clopidogrel-based treatment (e.g., using alternative clopidogrel dosing schemes or other antiplatelet agents). The observation that specific CYP2C19 variants or levels of on-treatment platelet reactivity above a threshold predict worse outcomes does not necessarily mean that changing treatment on the basis of these tests will improve outcomes. It is possible that the genotype or phenotype is simply a marker for poor outcome regardless of the treatment strategy used.

Genetic Tests for CYP2C19 Variants

Genetic testing for one or more genetic variants can be performed with various genotyping methods. Testing for CYP2C19 variants requires a sample of somatic genetic material, usually obtained from a blood sample or from buccal swabs. Because allelic variants at the CYP2C19 locus do not change over a person's lifetime, testing done at any time point is representative of the person's genotype.

Measurement of Platelet Reactivity

Phenotypic testing measures the reactivity of platelets while a patient is taking clopidogrel (on-clopidogrel platelet reactivity). Several assays for measuring platelet reactivity are available. These include rapid (point-of-care) platelet function assays (e.g., VerifyNow, Platelet Function Analyzer [PFA]-100, Plateletworks), measurements of mediators of reactivity (e.g., vasodilator-stimulated phosphoprotein phosphorylation [VASP] using flow cytometry), and functional assays (e.g., aggregometry using appropriate agonists). We refer to all these assays as "phenotypic tests," because they attempt to measure an intermediate clinical phenotype (platelet reactivity).[28]

Scope

We performed a comparative effectiveness review regarding the utility of testing for CYP2C19 variants and platelet reactivity for guiding antiplatelet treatment. This review evaluated the analytic validity, predictive utility, and comparative effectiveness of genetic and phenotypic tests as biomarker tests (and of relevant test-and-treat strategies) for guiding antiplatelet therapy in patient populations who are eligible for clopidogrel treatment.

Key Questions

On the basis of the original topic nomination and an extensive process of topic development and refinement, we formulated the following Key Questions to guide the review:

Key Question 1: In patient populations who are candidates for clopidogrel therapy, does genetic testing for CYP2C19 variants predict intermediate and clinical outcomes following treatment initiation?

a) What is the analytic validity (technical test performance) of the various assays used for CYP2C19 genetic testing?
b) What is the clinical validity (predictive accuracy) of genetic testing for predicting intermediate and clinical outcomes in patients who are receiving clopidogrel therapy?
c) Do the following factors modify the association between genetic test results and clinical outcomes?
    i. Comedications
    ii. Patient-level factors (e.g., race or ethnicity, age, sex, disease severity, or comorbidities)
    iii. Test-related factors (e.g., between-assay differences)
    iv. System-level factors (e.g., settings where testing is performed)

Key Question 2: In patient populations receiving clopidogrel therapy, does phenotypic testing of platelet reactivity predict intermediate and clinical outcomes?

a) What is the analytic validity (technical test performance) of the various assays used in phenotypic testing of platelet reactivity?
b) What is the clinical validity (predictive accuracy) of phenotypic testing for predicting intermediate and clinical outcomes in patients who are receiving clopidogrel therapy?
c) Do the following factors modify the association between phenotypic test results and clinical outcomes?
    i. Comedications

ii. Patient-level factors (e.g., race or ethnicity, age, sex, disease severity, or comorbidities)
iii. Test-related factors (e.g., between-assay differences)
iv. System-level factors (e.g., settings where testing is performed)

Key Question 3: What is the comparative effectiveness of alternative test-and-treat strategies (including a no-testing strategy) for therapeutic decisionmaking regarding antiplatelet therapy among patients who are candidates for clopidogrel-based treatment?

a) What is the comparative effectiveness of the following testing strategies on therapeutic decisionmaking, platelet reactivity during followup, and clinical outcomes in patients who are candidates for antiplatelet treatment?
    i. Genetic testing for CYP2C19
    ii. Genetic testing for CYP2C19 followed by phenotypic testing for platelet reactivity
    iii. Phenotypic testing for platelet reactivity
    iv. No testing
b) How do modifying factors (e.g., race or ethnicity, age, sex, comorbidities, diet, or the time between conducting the test and obtaining results) affect the association of alternative phenotypic or genetic test-and-treat strategies and patient outcomes? Alternative test-guided treatments can include nonclopidogrel antiplatelet agents or high-dose clopidogrel regimens.

Key Question 4: What are the potential adverse effects or harms from genetic or phenotypic testing *per se* or from test-directed treatments?

Analytic Framework

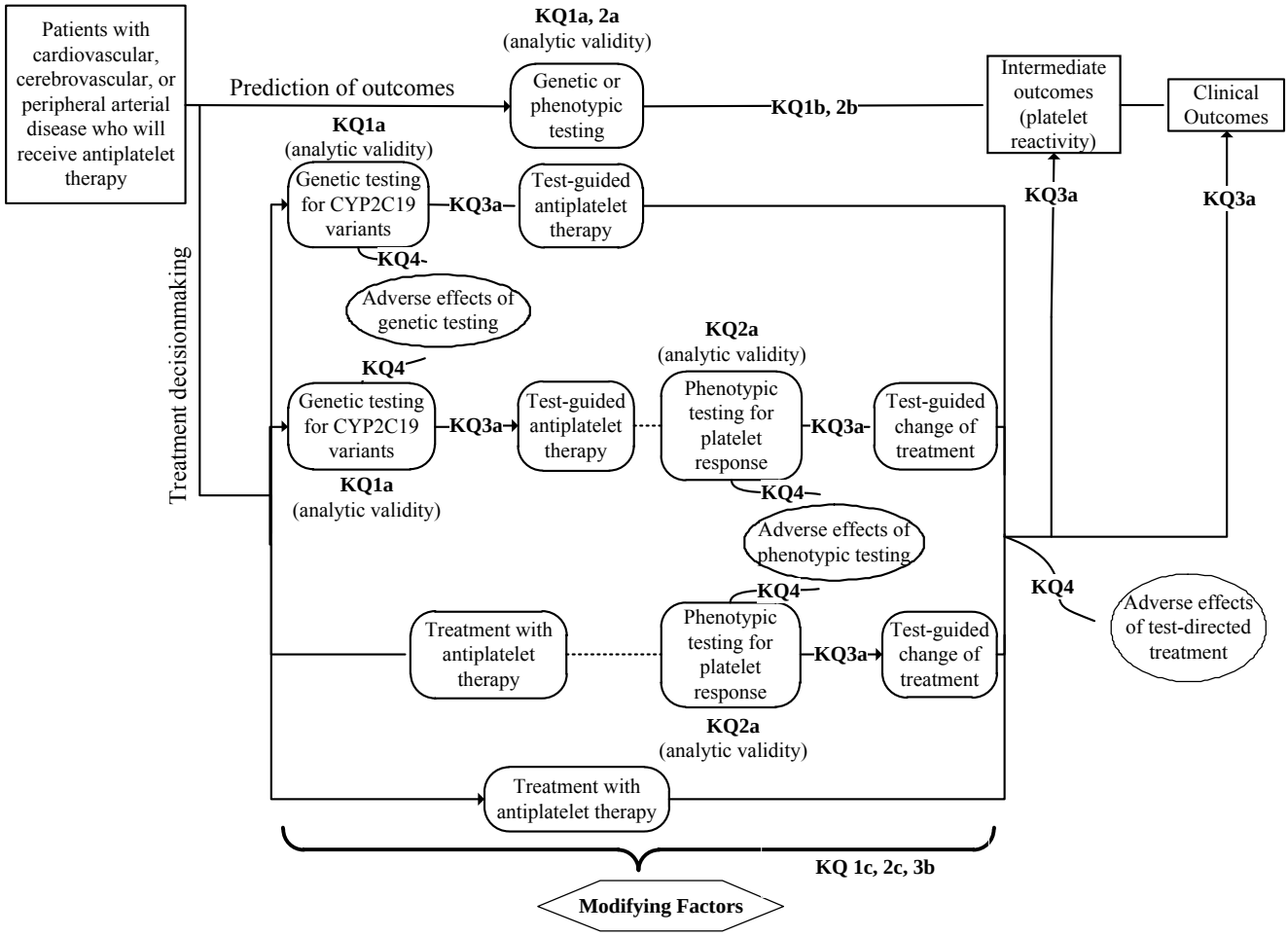
We developed an analytic framework (**Figure A**) that maps the Key Questions within the context of populations, interventions, comparators, and outcomes of interest, as well as the chain of logic that evidence must support to link the interventions to health outcomes. Briefly, the analytic validity (i.e., technical test performance) of the index tests of interest was reviewed in Key Questions 1a and 2a.The predictive ability of the tests was addressed in Key Questions 1b and 2b. We also considered the use of the tests to guide treatment decisionmaking (i.e., use of the tests as components of test-and-treat strategies), and the impact of this decisionmaking on clinical and intermediate outcomes, in Key Question 3a. Tests and test-directed treatments may be associated with harms; this was investigated in Key Question 4. Modifiers of the effects of testing on outcomes (both in terms of predictive ability and decisionmaking) were reviewed in Key Questions 1c, 2c, and 3b.

Regarding treatment decisionmaking, we conceptualized the analytic framework as a decision problem, wherein patients' disease can be managed with one of the following approaches (depicted from top to bottom in the flow diagram):

1. Undergo genetic testing and then base the treatment decision on the test results.
2. Undergo genetic testing and then base the treatment decision on the test results. After receiving therapy for an adequate period of time, undergo phenotypic testing for platelet reactivity and use the results to decide whether the treatment strategy should be modified.
3. Receive standard treatment directly and, after an appropriate amount of time, undergo phenotypic testing for platelet reactivity and use the test results to decide whether the treatment strategy should be modified. Use of phenotypic testing (but not genetic testing) as a monitoring test can be considered a variation of this strategy in which the test is repeatedly performed.

4. Receive antiplatelet therapy without undergoing any testing (the current standard of care).

Figure A: Analytic Framework.



KQ=Key Question.

Methods

Literature Search

We conducted literature searches for studies in MEDLINE® (from inception to August 24, 2011), the Cochrane Central Trials Registry (through the 2nd quarter of 2011), and the Cochrane Database of Systematic Reviews (through the 2nd quarter of 2011) without any language restriction. All searches will be updated (through the first quarter of 2012) while the draft report is under peer review and updated results will be presented in the final report. We also performed searches of the Human Genome Epidemiology Network (HuGENet) database and National Institutes of Health Genetic Association Database, using the same cutoff date (August 24, 2011). We did not contact authors and did not consider unpublished data other than that included in FDA documents or ClinicalTrials.gov.

Eligibility Criteria

We considered both comparative and noncomparative studies for Key Questions pertaining to predictive ability but focused on comparative studies of alternative test-and-treat strategies. We did not include non–English language studies but we recorded the number of studies that were excluded on the grounds of language for each Key Question. We excluded narrative reviews, editorials, letters to the editor, and other papers not presenting primary research data. We also excluded studies reporting exclusively on healthy individuals. Studies conducted in all relevant care settings (e.g., primary and secondary care or rural and urban clinics) were included. Study selection was not based on cointerventions.

Data Extraction

A single investigator extracted data from each study; quantitative results were verified by a second reviewer. Disagreements were resolved by consensus involving a third investigator. Data were extracted into standard forms; separate forms were generated for each Key Question. Extraction forms were piloted on three to five articles for each Key Question and revisions were made as needed. We extracted information on the following items: patient selection criteria, population characteristics, sample size, study design, analytic details, and outcomes.

Risk of Bias Assessment of Individual Studies

For assessing the risk of bias, we followed recently updated guidance from the Agency for Healthcare Research and Quality (AHRQ) *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* (hereafter referred to as the Methods Guide; available at www.effectivehealthcare.ahrq.gov/methodsguide.cfm).[29,30] We used different criteria for assessing the risk of bias (and when appropriate, the completeness of reporting) for each Key Question. Two independent reviewers evaluated the risk of bias for each study and disagreements were resolved by consensus including a third reviewer.

For studies of analytic validity, no established approach exists for assessing risk of bias or reporting quality. On the basis of a recent AHRQ Methods Report,[31] in which expert input and literature review methods were used to develop a preliminary list of quality and reporting criteria for studies of analytic validity,[a] we compiled a list of 11 items for assessing quality and reporting.

For studies of predictive ability (Key Questions 1b–c and 2b–c), we based our assessment on the recently proposed Quality Assessment of Diagnostic Accuracy Studies (QUADAS)–2 instrument,[32] a new version of the validated QUADAS list of quality items[33-35] for systematic reviews of medical tests. Briefly, the tool assesses four domains for risk of bias: patient selection, index test, reference standard test (outcome), and flow and timing. After scoring each item, a summary risk-of-bias assessment is performed for each of the four domains and then an overall determination (across the four domains) is made. We used arbitrary thresholds based on the number of items scored as having been adequately addressed (i.e., indicative of low risk of bias) to classify studies into three categories (A, B, or C) indicating low, moderate, and high risk of bias, respectively. This approach was used as a shorthand description of the available evidence; throughout the report we emphasize the component items that contributed to the summary rate.

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[a] The final proposed list of items incorporated assessment methods for analytic validity proposed by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group.

Finally, for studies providing information on test-and-treatment strategies (Key Questions 3 and 4) we used a combination of items from the QUADAS-2 tool and the Cochrane risk of bias tool.[36]

Data Synthesis

Qualitative Synthesis

We use tables and graphs to synthesize information across studies. Predefined subgroups of interest were those defined by race or ethnicity, sex, specific assay used, and clinical setting of test use (e.g., short-term administration of clopidogrel during treatment of acute cardiac events or PCI vs. chronic clopidogrel use).

Quantitative Synthesis

Meta-analysis

We performed random effects inverse-variance meta-analysis when at least three studies were available on sufficiently similar populations, using the same test, and assessed the same outcomes.[37] Between-study heterogeneity was assessed on the basis of the *Q* statistic[38] (considered statistically significant when its P value (P_Q) was less than 0.1). Between-study inconsistency was assessed using the I^2 index.[39] Before conducting any analyses, we decided not to combine studies of different phenotypic tests for platelet reactivity. This decision was based on the different principles of measurement implemented in different assays and our own findings on interassay agreement (presented under Key Question 2a), which indicated that agreement for identifying lack of response may be limited. Similarly, we decided not to combine randomized treatment trials providing information about effect modification because they had enrolled heterogeneous participant populations and compared different pairs of interventions (i.e., the magnitude and direction of effect modification by the tests of interest was likely to vary among different treatment comparisons).

For genetic variants, several genetic models of inheritance are possible. Following previous meta-analyses of CYP2C19 variants, we assumed a dominant model for all minor alleles (i.e., a model that assumes carriers of one and carriers of two minor alleles have the same phenotype).

Because the majority of studies reporting on clinical outcomes included in this report had longitudinal designs, the most appropriate statistical analyses would take into account the time to occurrence of events (e.g., by using survival analysis methods). For this reason, we used hazard or incidence rate ratios in our meta-analyses whenever available or extractable from the reviewed studies. When such statistics were not reported (and could not be calculated), we used risk (proportion) ratios because they approximate the relative incidence rate. For case–control and case-cohort studies, we used odds ratios because they are valid statistics for these designs and approximate the incidence rate ratio or risk ratio (depending on sampling methods).[40-42] For parsimony, we refer to all these statistics as relative risks (RRs).

Subgroup and Meta-regression Analysis and Small-Study Effects

To assess the impact of study-level characteristics on estimates of the effect size, we used univariable random effects meta-regression.[39] Subgroup and meta-regression analyses were performed for factors reported at the group level. We also explored temporal trends in the

reported effect sizes using meta-regression with year of publication as the covariate. We used Egger's regression-based test[43] to assess the presence of "small-study effects."[43,44]

Strength of the Body of Evidence

We graded the strength of the body of evidence for the Key Questions following the Methods Guide and recently updated recommendations for the Evidence-based Practice Center (EPC) program.[29,45] Briefly, the grading of the strength of evidence was based on four dimensions: risk of bias, consistency, directness, and precision. We assessed the consistency of the data as either "no inconsistency" or "inconsistency present" (or "not applicable" if only one study). The direction, magnitude, and statistical significance of all studies were evaluated in assessing consistency, and explanations were provided in the case of equivocal results.

We also assessed the precision and sparseness of the evidence. We considered evidence to be sparse if it was from only one study with a small sample size. Because this review assessed many outcomes, the evaluation of overall strength of evidence was based on patient-relevant clinical outcomes, which we broadly defined as any outcomes that affect the patient's well-being. We rated the strength of evidence as having one of the following four strengths (as per the AHRQ Methods Guide): high, moderate, low, or insufficient. Ratings were assigned on the basis of our level of confidence that the evidence reflected the true effect for the major comparisons of interest.

Assessing Applicability

We assessed applicability of the study findings on the basis of the individual study eligibility criteria and baseline characteristics of the included populations, following recommendations in the AHRQ Methods Guide and recently updated recommendations for the EPC program.[29,45] We did not assess the applicability of studies on the analytic validity of the tests of interest (Key Questions 1a and 2a) because technical test performance does not directly inform medical decisions (it is, however, a prerequisite for the clinical use of tests).[46]

For studies of predictive ability (Key Questions 1b–c and 2b–c), we judged applicability on the basis of study eligibility criteria (e.g., narrow vs. broad range of demographics [men only vs. men and women]), the use of tests that are not widely available (tests developed "in house" ["home-brew" assays]), and the assessment of outcomes that required laboratory measurement of platelet reactivity (rather than patient-relevant outcomes). We took a similar approach for studies considered relevant to test-and-treatment strategies (Key Questions 3 and 4).

Results

The literature search yielded 9441 citations (9351 from electronic databases, 77 from submission information packages, and 13 from hand searching). Of these, 842 articles were reviewed in full text. After full text review, 226 were judged to have met the inclusion criteria for one or more of the four Key Questions. We summarized the findings of the report according to the order of the Key Questions. Within each Key Question, results are organized for each appropriate subgroup on the basis of the populations assessed and clinical indications for clopidogrel use, index tests used, and outcomes assessed.

Key Question 1a: What Is the Analytic Validity (Technical Test Performance) of the Various Assays Used for CYP2C19 Genetic Testing?

Eligible Studies and Summary of Findings

We identified five studies reporting information on the analytic validity of genotyping methods for detecting CYP2C19 variants. Studies reported limited information regarding the methods used to assess technical test performance, possibly reflecting their focus on clinical (rather than analytic) validity. Three of the studies were conducted by the same team of investigators and reported laboratory and clinical findings on patients identified at a single center in Germany (these were participants in ongoing clinical trials); thus, at least partial overlap is likely. We also reviewed four FDA 510(k) summaries on genetic testing assays.

All five studies included patients undergoing PCI for coronary artery disease and all assessed test–retest reliability (repeat testing of samples for the detection of CYP2C19 polymorphisms). Four of the five reported that the concordance rate on repeat genotyping was 100 percent. The fifth study reported that the rate was "higher than 98%." Three of the five studies reported the proportion of retested samples (20 percent in all three cases), corresponding to 305 samples (in each of two studies) and 497 samples (in the third study). The remaining two studies did not report the proportion of retested samples (and the proportion could not be calculated).

FDA 510(k) summaries did not report analyses on samples from populations and gene of interest in our review. However, the documents provided evidence that genotyping methods have high test–retest reliability and indicated that rates of interassay agreement were high.

Risk of Bias and Completeness of Reporting

Studies reported limited information on the methods they used to assess analytic validity. This probably reflects the fact that the primary focus of all included publications was not the tests' analytic validity (but rather their clinical utility). Generally, studies provided adequate information on the genotyping methods used. However, they provided little information on the use of positive or negative control samples, the handling of uninterpretable results, and the test detection limits. No study reported information on the reproducibility of genotyping across operators or different instruments and none were conducted as part of interlaboratory standardization projects.

Key Question 1b: What is the Clinical Validity (Predictive Accuracy) of Genetic Testing for Predicting Intermediate and Clinical Outcomes in Patients Who Are Receiving Clopidogrel Therapy?

Eligible Studies

Of the 56 studies addressing Key Question 1b (in 51 publications; 5 publications described 2 studies each), the vast majority (54 [96 percent]) were of patients with ischemic heart disease; the majority of these were undergoing PCI and were receiving dual antiplatelet therapy with clopidogrel in combination with aspirin. The studies had intermediate to large sample sizes (median=281; 25th percentile=98; 75th percentile=1005; minimum=30; maximum=5148) and were conducted within the past decade (with 75 percent beginning enrollment after 2004), reflecting the relatively recent widespread availability of genetic testing for CYP2C19 variants. The majority of enrolled patients were men and the median age was 64.7 years. Across studies,

the median proportions of patients with dyslipidemia and hypertension were over 50 percent. The median proportion of patients with diabetes mellitus and smoking was approximately 25 percent. Overall, 95 percent of studies had a longitudinal (cohort) design; 14 of these were genetic substudies consisting of prospectively followed clopidogrel-treated groups from randomized trials.[b]

Overall, studies had moderate risk of bias (4 studies were rated as quality "A," 49 studies were rated as quality "B," and 3 were rated as quality "C"). We caution that this aggregate risk-of-bias rating can be misleading, especially in the presence of incomplete reporting.

The 56 studies used a variety of genotyping methods for identifying CYP2C19 variants. The most common were TaqMan genotyping (21 studies; 38 percent), polymerase chain reaction (PCR)–restriction fragment length polymorphism (7 studies; 13 percent), sequencing (4 studies; 7 percent), and real-time PCR (4 studies; 7 percent). In the majority of cases (49 studies; 88 percent), analyses were conducted on genetic material isolated from blood. Among the 36 studies that reported the genotyping success rate, the median was 100 percent (minimum=82 percent; maximum=100 percent). Violations of Hardy–Weinberg equilibrium (on the basis of an exact goodness-of-fit test) were not more common than would be expected by chance.

Summary of Findings

Findings are presented for studies providing information on the ability of genetic testing for CYP2C19 variants to predict clinical outcomes (27 studies) or platelet reactivity (40 studies) during followup.

Clinical Outcomes

Several clinical outcomes of interest were reported: all-cause mortality, cardiac mortality, acute coronary syndromes, stent thrombosis, stroke, MACE, bleeding events, and need for revascularization. Loss-of function alleles (CYP2C19*2 or *3) were statistically significantly associated with higher on-clopidogrel platelet reactivity and were statistically significantly associated with stent thrombosis (RR=1.56; 95 percent confidence interval [CI] 1.15, 2.11) and cardiovascular mortality (RR=2.40; 95 percent CI 1.05, 5.53). Gain-of-function alleles (CYP2C19*17) were statistically significantly associated with reduced risk of MACE (RR=0.80; 95 percent CI 0.70, 0.90). Studies on the predictive value of CYP2C19 variants were judged to have moderate risk of bias; there was some indication of systematic differences between larger and smaller studies (P=0.002, P=0.24, and P=0.007 for stent thrombosis, MACE, and bleeding, respectively). There was also substantial risk of selective outcome reporting.

One finding was of note from the two studies that enrolled patients other than those with ischemic heart disease. ACTIVE A (Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A), a study of patients with atrial fibrillation, reported a higher rate of major bleeding events among patients carrying no loss-of-function alleles than among those carrying such alleles (log-rank P=0.01); an independent analysis of the event rates yielded an RR of 2.36 (95 percent CI 1.21, 4.62).

[b] When appropriate, possible modification of the relative treatment effect by genotype status has been considered under Key Questions 3 and 4 of this report.

Intermediate Outcome: Platelet Reactivity

The intermediate outcome of platelet reactivity was reported either as a continuous variable (in 34 studies) or according to a threshold of reactivity (e.g., high vs. low; in 22 studies). The most common assays for assessing reactivity were light-transmission aggregometry (LTA), the VerifyNow P2Y12 assay, and the vasodilator-stimulated phosphoprotein (VASP) assay. For platelet reactivity as a continuous outcome, the mean or median reactivity was generally higher among clopidogrel-treated patients with one or two loss-of-function alleles compared to those with no loss-of-function alleles. For platelet reactivity as a categorical outcome, studies generally showed that platelet reactivity above the threshold used (or in higher quantiles compared to lower quantiles of reactivity) was more common in clopidogrel-treated patients with one or two loss-of-function alleles than those with no loss-of-function alleles. Because of the extensive differences among studies of either type of reactivity outcome, and the often incomplete reporting of numerical information, we did not perform meta-analyses for studies using reactivity as the outcome of interest.

Risk of Bias for Studies Reviewed for Key Question 1b

Overall individual studies had moderate risk of bias. The vast majority used longitudinal designs (not case–control) and few studies had substantial loss to followup. Inappropriate exclusions were uncommon; however, information on blinding was often not reported (particularly for the index test) or blinding was not used. Using the arbitrary cutoff values based on the number of adequately addressed risk-of-bias items, 4 studies were rated as quality “A”; 44 studies were rated as quality “B”; and 3 were rated as quality “C”.

For outcomes for which we performed meta-analysis, we assessed differences between more precise (larger) and less precise (smaller) studies using the Egger regression-based test for small-study effects. Overall, we found a statistically significant difference between smaller and larger studies on the effect size for stent thrombosis, MACE, and bleeding events across carriers of loss-of-function alleles. Although publication bias (i.e., selective publication of positive or extreme findings) is a potential explanation for these associations, other factors, such as “true” heterogeneity between smaller and larger studies, chance, or other sources of bias could also explain these results.

Key Question 1c: What Is the Impact of Modifying Factors on the Association Between Genetic Test Results and Clinical Outcomes?

We reviewed studies to identify any evidence that patient- or system-level factors or test characteristics could modify the predictive ability of genetic testing for CYP2C19 variants. We considered both within-study information (e.g., studies where the predictive effect of phenotypic testing was evaluated in two or more patient subgroups) and information across studies (through meta-regression analyses on study-level factors).

Effect Modification Within Studies

Ten studies reported information on modification of the predictive effect of the genetic test by various factors: proton-pump inhibitors, gene–gene interactions, indication for clopidogrel use (acute coronary syndrome vs. stable angina), whether patients required a loading dose or not (because they were on chronic clopidogrel therapy), duration of clopidogrel therapy, smoking status, body-mass index ($\geq$25 kg/m^2 vs. <25 kg/m^2), ancestry (European vs. Latin American), and

stent type (bare metal vs. drug eluting). Five of the studies used platelet reactivity as the outcome of interest and five reported information on clinical outcomes (MACE or bleeding events). Only one of the effects assessed was statistically significant (a multiplicative interaction between the *2 and *3 CYP2C19 alleles on on-clopidogrel platelet reactivity). Overall, the reported findings did not provide sufficient evidence to support or exclude a differential effect of CYP2C19 variants across any of the factors assessed in the studies we reviewed because few studies provided information on each modifier and the non–statistically significant results were generally too imprecise to rule out clinically meaningful effect modification.

Considering both within-study and across studies analyses, there is insufficient information to support or exclude the presence of substantial modification of the predictive effect of CYP2C19 variants by any of the investigated factors.

Effect Modification Across Studies

Potential modifiers of the predictive effect of genetic testing for CYP2C19 that were assessed across studies using subgroup analysis were disease subtype (acute coronary syndromes vs. mixed coronary artery disease populations), setting of care (PCI vs. other), race or ethnicity (white vs. East Asian), duration of followup (≤30 days vs. >30 days), and year when enrollment was started (continuous variable). Meta-regressions were performed for the two outcomes that were reported in 10 or more studies—MACE and stent thrombosis—and only for carriers versus noncarriers of CYP2C19 loss-of-function alleles. No statistically significant effects were found.

Key Question 2a: What is the Analytic Validity (Technical Test Performance) of the Various Assays Used in Phenotypic Testing of Platelet Reactivity?

Eligible Studies and Summary of Findings

We identified 80 studies reporting information on the analytic validity of assays for measuring platelet reactivity. We also reviewed 20 FDA 510(k) summaries on phenotypic testing assays. All published studies enrolled patients with ischemic cardiovascular disease. The five most commonly assessed assays were LTA, the VASP assay with flow cytometry, the VerifyNow P2Y12 assay, PFA-100, and thromboelastography. We summarized the reported information regarding analytic performance, interassay agreement, test reliability and assay variation, and correlations between assays applied to the same sample (by far the most common metric reported); no other aspect of analytic validity was evaluated in the studies.

Overall there appeared to be low to moderate agreement between assays. Agreement was generally greater between measurements obtained with the same assay using different agonist concentrations than between different assays.

Of the 10 studies providing information on analytic performance, reported analytic sensitivities ranged between 0.35 and 1.00; reported specificities ranged between 0.42 and 0.95. In studies reporting results across multiple cutoff values, a tradeoff between sensitivity and specificity was apparent (as expected). Overall, these results indicate poor agreement in sample classification (e.g., high vs. low reactivity) when one of the two tests compared was considered a gold standard.

Thirty-three studies provided information on interassay agreement. Overall, disagreements were relatively common between measurements obtained by different assays or by using different agonist concentrations within the same assays.

Thirty-four studies reported information on assay variability, although more than 90 percent did not describe the methods used. Variability or coefficient of variation results were less than 10 percent in all but two studies. These results need to be interpreted with caution, give the poor reporting of study methods and the fact that multiple studies were published by a limited number of investigative teams (we could not ascertain whether the studied populations are the same or similar).

Of the 56 studies reporting correlation values, only 1 used Lin's concordance correlation coefficient (an appropriate metric), reporting a high correlation (ρ=0.97) between observed and estimated platelet inhibition for the VerifyNow assay. The remaining studies used inappropriate metrics (e.g., Spearman or Pearson correlation coefficients or linear regression (which in the simple bivariate case of two measurements is equivalent to the Pearson correlation) or did not report the calculation method used.[47,48] The results indicated that the association between measurements obtained using different methods is relatively poor. However, given the inappropriateness of the methods used (for the purpose of assessing agreement), even high correlation values would not be considered indicative of good agreement.

None of the 20 FDA 510(k) summaries on phenotypic tests of platelet reactivity reported relevant analyses. Either no data were reported or the population or agonist used in test was not of interest, the analytic validity results were not reported for clopidogrel (rather, for a comedication), or the sample size was less than 10.

Risk of Bias and Completeness of Reporting

Studies reporting on assay variability were extremely poorly reported (in all but three studies, no information was provided on the study design, selection criteria, or measurement methods used; in more than 90 percent of the studies the number of replicate samples used for analyses was not reported). Thus it was impossible to assess the risk of bias of these studies. In addition, correlation estimates are practically uninformative for assessing agreement between assays. For these reasons, we limited the assessment of study quality to the 35 studies that reported information on analytic test performance or interassay agreement.

The 35 studies were generally of low methodological quality and poorly reported. All but one (a study published as a letter to the editor) provided adequate descriptions of the assays used. Information on the use of positive or negative controls was poorly reported. About half the studies did not report the criteria for positive, negative, or indeterminate results; no study reported the limits of detection or assay linearity range. Only a minority of studies assessed reproducibility of results on repeat testing, and no study assessed reproducibility across operators, instruments, or reagent lots or over time. Only one study was conducted as part of a multilaboratory collaboration; however, it did not provide details on the standardization procedures followed.

Key Question 2b: What Is the Clinical Validity (Predictive Accuracy) of Phenotypic Testing for Predicting Intermediate and Clinical Outcomes in Patients Who Are Receiving Clopidogrel Therapy?

Eligible Studies

Of the 100 studies addressing Key Question 2b, the vast majority (95 studies) were of patients with ischemic heart disease; 4 studies enrolled patients with cerebrovascular disease, and 1 study enrolled patients with peripheral artery disease. Among studies with ischemic heart

disease, 35 enrolled patients with acute coronary syndromes; the remaining either included patients with chronic stable coronary artery disease (58 studies) or mixed populations with chronic and acute presentations (7 studies). Of the 95 studies enrolling patients with ischemic heart disease, 89 (94 percent) included predominantly (>80 percent of the included population) patients undergoing PCI; 2 (2 percent), patients undergoing angiography; 2 (2 percent), patients undergoing CABG surgery; and 2 (2 percent), patients being treated with medical therapy without undergoing a procedure.

Studies reported information on a variety of assays for measuring platelet reactivity. The predictive ability of a single platelet function test was evaluated in 84 of the 100 studies, whereas 16 studies evaluated two or more tests. The tests that were used in the studies included LTA (46 studies; 46 percent), the VerifyNow assay (28 studies; 28 percent), the VASP assay (14 studies; 14 percent), the Multiplate analyzer (12 studies; 12 percent), the PFA-100 assay (8 studies; 8 percent), thromboelastography (5 studies; 5 percent), and other tests (7 studies; 7 percent). Detailed information on each test is presented separately under the discussion of individual assays. **Table A** summarizes information on the patient populations and outcomes assessed in the 100 studies.

Table A: Populations, Outcomes, and Strength of Evidence in Studies for Key Question 2b, According to Test Used**

Test Used Populations (no. of studies)	All-Cause Death	CV Death	ACS	ST	Stroke	Bleeding	MACE	Other Clinical Outcomes	Platelet Reactivity
LTA (n=46) IHD=45; PAD=1	IHD=10 [low]	IHD=8 [low]	IHD=17 [low]	IHD=18 [low]	IHD=11 [low]	IHD=6 [insufficient]	IHD=33 [low]	IHD=7 [insufficient]	IHD=9 [insufficient] PAD=1 [insufficient]
VerifyNow P2Y12 (n=28) IHD=25; CVD=3	IHD=5 [low]	IHD=6 [low]	IHD=14 [low]	IHD=9 [insufficient]	IHD=5 [insufficient]	IHD=8 [low]	IHD=17 [low]	IHD=3 [insufficient] CVD=3 [insufficient]	IHD=3 [insufficient]
VASP (n=14) IHD=14	IHD=3 [insufficient]	IHD=6 [insufficient]	IHD=6 [low]	IHD=7 [low]	IHD=1 [insufficient]	No studies	IHD=7 [low]	IHD=4 [insufficient]	IHD=6 [insufficient]
Multiplate analyzer (n=12) IHD=11; CVD=1	IHD=4 [insufficient]	IHD=4 [insufficient]	IHD=7 [insufficient]	IHD=7 [insufficient]	IHD=3 [insufficient]	IHD=5 [insufficient]	IHD=9 [insufficient] CVD=1 [insufficient]	IHD=4 [insufficient]	IHD=1 [insufficient]
TEG (n=5) IHD=5	IHD=1 [insufficient]	No studies	IHD=1 [insufficient]	No studies	No studies	IHD=2 [insufficient]	IHD=4 [insufficient]	No studies	No studies
PFA-100 (n=8) IHD=8	IHD=2 [insufficient]	IHD=2 [insufficient]	IHD=5 [insufficient]	IHD=2 [insufficient]	IHD=1 [insufficient]	IHD=1 [insufficient]	IHD=7 [low]	IHD=2 [insufficient]	IHD=1 [insufficient]
Other (n=7) IHD=7	IHD=2 [insufficient]	No studies	IHD=2 [insufficient]	IHD=1 [insufficient]	IHD=1 [insufficient]	IHD=2 [insufficient]	IHD=4 [insufficient]	IHD=2 [insufficient]	IHD=3 [insufficient]

ACS = acute coronary syndromes; CV = cardiovascular; CVD = cerebrovascular disease; IHD = ischemic heart disease; LTA = light-transmission aggregometry; MACE = major adverse cardiovascular events; PAD = peripheral arterial disease; PFA = Platelet Function Analyzer; ST=stent thrombosis; TEG = thromboelastography; VASP = vasodilator-stimulated phosphoprotein.
*Numbers indicate the number of available studies for each test–outcome combination in the population specified. (Studies could have involved more than one combination.) The ratings in brackets reflect our assessment of the strength of evidence for each test–outcome association.

For parsimony, we present here only results for the test–outcome combinations for which the strength of the overall body of evidence was judged to be at least "low."[c] (For test–outcome combinations for which strength of evidence was considered insufficient or was not assessed

[c] Please refer to the Methods section for details on our approach to rating the strength of evidence.

[i.e., when the outcome reported was the intermediate outcome of platelet reactivity during followup], see the full report.) The strength of evidence for all patient populations other than ischemic heart disease was judged to be insufficient (owing to the very few studies, all of which had small sample sizes for all assays); as such, findings summarized below pertain to patients with ischemic heart disease only.

LTA in Ischemic Heart Disease

Forty-five studies included patients with ischemic heart disease and reported information on the predictive value of LTA. Of these, 40 studies assessed the value of the test for predicting clinical outcomes and 9 studies the value for predicting platelet reactivity during followup (4 studies reported both clinical and intermediate outcomes). Thirty-two of the 45 studies enrolled patients with chronic stable coronary artery disease, 11 enrolled patients with acute coronary syndromes, and 2 enrolled mixed populations with chronic and acute presentations. Most studies used ADP as the agonist to assess reactivity but a few used ADP in combination with arachidonic acid (AA) to assess the response to both clopidogrel and aspirin.

All-Cause Mortality (10 Studies; 9 ADP, 1 ADP+AA)

Studies did not suggest an association between increased platelet reactivity by LTA and increased all-cause mortality in patients with ischemic heart disease; however, results were considered to have a low strength of evidence because of clinical heterogeneity that prevented quantitative synthesis.

Cardiovascular Mortality (8 Studies; 7 ADP, 1 ADP+AA)

Studies suggested an association between increased platelet reactivity by LTA and increased cardiovascular death in patients with ischemic heart disease; the strength of evidence for this association was considered low because of clinical heterogeneity that precluded quantitative synthesis.

Acute Coronary Syndromes (17 Studies; 17 ADP, 1 ADP+AA)

Overall, results provided some evidence of an association between increased platelet reactivity as measured by LTA and increased risk of acute coronary syndrome in patients with ischemic heart disease, but the strength of the evidence was limited by the heterogeneity of comparisons reported in the studies.

Stent Thrombosis (18 Studies; 16 ADP, 2 ADP+AA)

Eighteen studies reported information on the ability of platelet reactivity as measured by LTA to predict stent thrombosis. Three publications reported data from the same population. Taken together, the studies suggested an association between increased platelet reactivity and increased risk of stent thrombosis in patients with ischemic heart disease.

MACE (33 Studies; 31 ADP, 2 ADP+AA)

Three of the 33 studies reported data from the same population. Data for the longest followup time available were evaluated. All studies used ADP as the agonist to measure platelet reactivity; two studies used ADP in combination with AA to assess the response to both clopidogrel and aspirin. The majority of reviewed studies suggested an association between increased platelet reactivity by LTA and increased risk of MACE.

Stroke (11 Studies; 10 ADP, 1 ADP+AA)

The 11 reviewed studies did not suggest an association between increased platelet reactivity as measured by LTA and increased stroke in patients with ischemic heart disease; however, the strength of evidence was limited by between-study heterogeneity in reactivity measurements and uncertainty in study-specific estimates of effect.

VerifyNow P2Y12 in Ischemic Heart Disease

Twenty-five studies included patients with ischemic heart disease and reported information on the predictive value of the VerifyNow P2Y12 assay, which uses ADP as the agonist to assess platelet reactivity. Of these, 24 assessed the value of the test for predicting clinical outcomes and three for predicting platelet reactivity during followup (two studies reported both clinical and platelet reactivity outcomes). Of the 25 studies, 16 enrolled patients with chronic stable coronary artery disease, eight enrolled patients with acute coronary syndromes, and one enrolled a mixed population with chronic and acute presentations.

All-Cause Mortality (5 Studies; 4 ADP, 1 ADP+AA)

A meta-analysis of three studies that used ADP as the agonist and defined high platelet reactivity on the basis of platelet reactivity units found a summary RR of 1.39 (95 percent CI 0.73, 2.66); P=0.313, indicating a nonsignificant association between high platelet reactivity and all-cause mortality. There was little evidence of between study heterogeneity (P_Q=0.900; I^2=0 percent). Meta-analysis was not performed for the two other studies, which used percent platelet inhibition to define reactivity.)

Cardiovascular Mortality (6 Studies, All ADP)

A meta-analysis of the four studies that used cutoff values based on platelet reactivity units found a summary RR of 2.50 (95 percent CI 1.28, 4.87) (P=0.007), indicating a significant association between high platelet reactivity and cardiovascular mortality. There was little evidence of between study heterogeneity (P_Q=0.527; I^2=0 percent). The two studies not included in the meta-analysis did not report a significant association between higher platelet reactivity and increased cardiovascular mortality.

Acute Coronary Syndromes (14 Studies; 13 ADP, 1 ADP+AA)

Fourteen studies reported information on the ability of the VerifyNow P2Y12 assay to predict myocardial infarction in patients receiving clopidogrel-based treatment. Taken together, the studies suggested an association between increased platelet reactivity as measured by VerifyNow and increased rates of both periprocedural and nonperiprocedural acute coronary syndromes in patients with ischemic heart disease; however, the strength of evidence for this association was somewhat limited by variability in the metrics and thresholds used to define reactivity and heterogeneity of the included patient populations.

MACE (17 Studies; 16 ADP, 1 ADP+AA)

One study used both ADP and AA to identify a population of responders to both clopidogrel and aspirin. The study reported significantly higher odds of MACE in those who were clopidogrel nonresponders (irrespective of aspirin response status) as compared to those who were clopidogrel responders. A meta-analysis was done of 11 of the 16 remaining studies that enrolled nonoverlapping patient populations and used cutoff values for platelet reactivity based

on platelet reactivity units. The summary RR was 2.46 (95 percent CI 1.88, 3.22) (P<0.001) and there was little evidence of heterogeneity (P_Q=0.244; I^2=21 percent). Among the five studies not included in the meta-analysis, three used percentage of platelet inhibition to define platelet reactivity and two studies did not provide adequate data for inclusion. Among the three studies that used percentage of platelet inhibition to define platelet reactivity, two studies reported significantly higher rates of MACE at 6 months in those with a low response to clopidogrel; one study reported lower rates of MACE at 30 days in those with a low response to clopidogrel.

Bleeding Events (8 Studies; All ADP)

Three studies reporting results based on percent inhibition used different cutoff values for reactivity; thus they were not considered adequately similar for meta-analysis. Two of these studies reported lower rates of major and minor bleeding for patients with a low response to clopidogrel as compared to those with a normal response to clopidogrel; these differences were not statistically significant. The third study did not report any bleeding events. Of the remaining five studies reporting data on absolute on-clopidogrel reactivity levels, one reported that no events were observed (regardless of reactivity status), leaving four nonoverlapping studies for meta-analysis (three reporting information on bleeding events regardless of severity and all four reporting information on major bleeding events). The summary RR for all bleeding events was 1.02 (95 percent CI 0.79, 1.31) (P=0.881), with little evidence of heterogeneity (P_Q=0.891; I^2=0 percent). The summary RR for major bleeding events was 0.85 (95 percent CI 0.47, 1.52) (P=0.575), with substantial heterogeneity (P_Q=0.050; I^2=62 percent).

VASP Assay with Flow Cytometry in Ischemic Heart Disease

Fourteen studies included patients with ischemic heart disease and reported information on the predictive value of the VASP assay. Of these, 10 assessed the value of the test for predicting clinical outcomes, and 5 assessed the value for predicting platelet reactivity during followup (1 study reported both clinical and platelet reactivity outcomes). Of the 14 studies, 6 enrolled patients with acute coronary syndromes, 4 enrolled patients with chronic stable coronary artery disease, and 4 enrolled mixed populations with chronic and acute presentations.

Cardiovascular Mortality (6 Studies; All ADP)

Three of the six studies were nonoverlapping. The remaining three involved overlapping study populations and enrollment periods; in meta-analysis we used data from the publication reporting the largest number of events. A meta-analysis of the four studies, all of which used cutoff values based on the platelet reactivity index, found a non–statistically significant summary RR of 2.09 (95 percent CI 0.74, 5.88) (P=0.164). There was little evidence of between-study heterogeneity (P_Q=0.448; I^2=0 percent).

Acute Coronary Syndromes (6 Studies; All ADP)

One study reported that no events were observed (regardless of platelet reactivity status) and thus was not included in meta-analysis. Of the remaining five studies, two were nonoverlapping. The other three involved overlapping study populations and enrollment periods; in meta-analysis we used data from the publication reporting the largest number of events. A meta-analysis of the three studies, all of which used cutoff values based on the platelet reactivity index, found a summary RR of 1.47 (95 percent CI 0.77, 2.794) (P=0.246). There was little evidence of between-study heterogeneity (P_Q=0.372; I^2=0 percent).

Stent Thrombosis (7 Studies; All ADP)

One study reported that no events were observed (regardless of platelet reactivity status) and thus was not included in meta-analysis. Of the remaining six studies, three were nonoverlapping. The other three involved overlapping study populations and enrollment periods; in meta-analysis we used data from the publication reporting the largest number of events. A meta-analysis of the four studies found a summary RR of 3.37 (95 percent CI 1.59, 7.11) (P=0.015), indicating a statistically significant association between high platelet reactivity and stent thrombosis. There was evidence of moderate between-study heterogeneity (P_Q=0.044; I^2=56 percent).

MACE (7 Studies; All ADP)

Two publications involved overlapping study populations; in meta-analyses we included data from the publication reporting the largest total number of cardiovascular events. A meta-analysis of the six non-overlaping studies found a summary RR of 2.57 (95 percent CI 1.21, 5.47) (P=0.015), indicating a statistically significant association between high platelet reactivity measured by the VASP assay and MACE. There was evidence of moderate between-study heterogeneity (P_Q=0.044; I^2=56 percent).

Multiplate Analyzer in Ischemic Heart Disease

Eleven studies included patients with ischemic heart disease and reported information on the predictive value of the Multiplate analyzer assay. All 11 studies assessed the value of the test for predicting clinical outcomes; none assessed platelet reactivity during followup. Eight of the studies enrolled patients with chronic stable coronary artery disease; the remaining three included mixed populations with chronic coronary disease and acute coronary syndromes. The strength of evidence was judged to be insufficient for all clinical outcomes reported in studies of the Multiplate analyzer (all-cause mortality, cardiovascular death, acute coronary syndromes, stent thrombosis, stroke, bleeding events, and MACE).

Thromboelastography in Ischemic Heart Disease

Five studies included patients with ischemic heart disease and reported information on the predictive value of thromboelastography. All five assessed the value of the test for predicting four clinical outcomes (all-cause mortality, acute coronary syndromes, MACE, and bleeding events); none assessed platelet reactivity during followup. Of the five studies, four enrolled patients with chronic stable coronary artery disease; the remaining study included patients with acute coronary syndromes. The strength of evidence was judged to be insufficient for all four clinical outcomes reported.

PFA-100 in Ischemic Heart Disease

Eight studies included patients with ischemic heart disease and reported information on the predictive value of the PFA-100 assay. One of the studies also assessed (separately) a variation of the PFA-100 assay called the INNOVANCE PFA P2Y assay. Seven studies (including the one using both PFA-100 and INNOVANCE PFA) assessed the value of the assays predicting clinical outcomes (usually MACE) and the remaining study assessed the value for predicting platelet reactivity during followup. Four studies enrolled patients with acute coronary syndromes; the remaining four enrolled patients with chronic stable coronary artery disease.

Only one study used measurements of platelet reactivity from both PFA-100 and INNOVANCE PFA P2Y assays (used separately); the other studies used PFA-100 with a combination of collagen and epinephrine or collagen and ADP to assess platelet reactivity. Because of the limited number of available studies and heterogeneity in the methods used to define high platelet reactivity, we did not perform meta-analyses of predictive ability. In general, the reviewed studies supported an association between increased platelet reactivity as measured by the PFA-100 assay and increased risk of MACE among patients with ischemic heart disease. The strength of evidence for other outcomes reported in studies of the PFA-100 assay was considered insufficient (all-cause mortality, cardiovascular death, acute coronary syndromes, stent thrombosis, stroke, and bleeding events).

"Comparative Studies" of Test Performance

Sixteen studies reported information on the predictive value of two or more tests applied to the same population (we refer to these studies as "comparative studies"). Although some of these studies performed informal comparisons between the predictive value of tests (e.g., by comparing effect sizes of metrics for predicting clinical outcomes), no study was specifically designed and analyzed in a way that permitted formal comparisons between tests. Eleven of the comparative studies (all in populations with ischemic heart disease) reported extractable information on clinical outcomes for at least one of the assessed tests; sometimes information was reported for one test but not for others, leaving a total of nine comparative studies with adequate information.

The data could not be quantitatively synthesized, however, because the studies involved several assays being applied to the same patient population, in which case results are likely to be correlated (since the population is shared and since assays done on samples of the same blood will yield correlated—if not identical—results). This within-study correlation of results cannot be accounted for by using aggregate data extracted from the available published studies.

MACE (Comparative Studies)

Seven studies reported comparative information regarding the ability of assays measuring platelet reactivity to predict MACE. The most commonly used test was LTA, which was compared to various other assays (thromboelastography and VerifyNow P2Y12 were the most common comparators, used in three and two studies, respectively). Overall, point estimates were similar between alternative test methods (within each study) and CIs were overlapping, suggesting that the predictive ability of the compared tests is fairly similar.

Acute Coronary Syndromes (Comparative Studies)

Two studies reported comparative information regarding the ability of assays measuring platelet reactivity to predict acute coronary syndromes. Both studies compared LTA and VerifyNow P2Y12; one study also assessed four other assays. Overall, point estimates were similar between alternative test methods (within each study) and CIs were fairly wide and overlapping. These findings suggest that the predictive ability of the compared assays may be similar but that there is substantial uncertainty regarding relative test performance.

Stent Thrombosis (Comparative Studies)

Three studies reported comparative information regarding the ability of assays measuring platelet reactivity to predict stent thrombosis for patients undergoing PCI with stent implantation.

All studies compared LTA against other reactivity assays (VerifyNow P2Y12 was the comparator in two studies; all other comparisons were unique). Overall, point estimates for the predictive ability of alternative test methods were variable (within each study); however, CIs were extremely wide (and overlapping), suggesting that there is substantial uncertainty regarding the relative predictive ability of the compared tests for stent thrombosis and that there is insufficient evidence on comparative test performance for this outcome.

Risk of Bias for Studies Reviewed for Key Question 2b

Overall, studies were considered to have a moderate risk of bias. All studies used a longitudinal design (not case–control, per our inclusion criteria) and no studies had substantial loss-to-followup. Inappropriate exclusions were uncommon, but information on blinding was often not reported (particularly for the index test) or not used. Using the arbitrary cutoff values based on the number of adequately addressed risk-of-bias items, 21 studies were rated as quality "A"; 67 studies were rated as quality "B"; and 12 were rated as quality "C." A more detailed discussion of risk of bias, focusing on the individual items assessed, is presented in the full text of the report.

Assessing Small-Study Effects

For the two assays for which we performed meta-analyses—VerifyNow P2Y12 and VASP assay—we assessed differences between more precise (larger) and less precise (smaller) studies using the Egger regression-based test for small study effects. Outcomes assessed in meta-analyses were all-cause mortality, cardiovascular mortality, MACE, and bleeding events for VerifyNow P2Y12 and cardiovascular mortality, acute coronary syndromes, stent thrombosis, and MACE for the VASP assay. Small-study effects were statistically significant for the VerifyNow P2Y12 studies included in meta-analyses of all-cause mortality and MACE, as well as VASP studies included in the meta-analysis of cardiovascular mortality. We caution that these significant effects should not be overinterpreted as proof for the presence of publication bias, given both the statistical properties of the Egger test and the fact that alternative causes (true heterogeneity of smaller and smaller studies, chance, or other biases) can also produce statistically significant results.

Key Question 2c: What Is the Impact of Modifying Factors on the Association Between Phenotypic Test Results and Clinical Outcomes?

We reviewed studies to identify any evidence that patient- or system-level factors or test characteristics could modify the predictive ability of phenotypic testing for platelet reactivity. As for Key Question 1c, we considered both within-study information (e.g., studies where the predictive effect of phenotypic testing was evaluated in two or more patient subgroups) and information across studies (through meta-regression analyses on study-level factors).

Effect Modification Within Studies

In total, five studies reported information on effect modification of the predictive effect of platelet reactivity. All studies reported information on clinical outcomes. Only a small subset of the eligible studies provided information adequate to statistically assess effect modification, and selective reporting is highly likely.

Studies assessed the following factors as potential modifiers: the use of glycoprotein IIb/IIIa inhibitors as an adjunct treatment for PCI (in two studies), diabetes mellitus (in two studies), and chronic kidney disease (in one study). Two studies used the VASP assay to assess platelet reactivity, two used the VerifyNow P2Y12 assay (one of which also used VerifyNow ASA[d]), and one used LTA (with ADP as the agonist). Statistically significant interaction effects were reported only in a single study; this study assessed whether the predictive value of the VASP assay differed depending on whether patients with coronary artery disease had or did not have coexisting chronic kidney disease. The study found that high on-clopidogrel platelet reactivity had statistically significantly greater effects on several clinical outcomes (all-cause mortality, cardiac death, and a composite outcome of all-cause mortality, myocardial infarction, or target-lesion revascularization) in patients with chronic kidney disease than in those without chronic kidney disease.

Effect Modification Across Studies

We evaluated the following study-level modifiers of the predictive effect of platelet reactivity: disease subtype (acute coronary syndromes vs. coronary artery disease), duration of followup (≤30 days vs. >30 days), and year when enrollment was started (continuous variable). There was no variability across studies with respect to setting of care (all involved interventional procedures). Meta-regressions were performed to ascertain the degree of effect modification by these potential modifiers for outcomes reported in at least 10 studies considered to be adequately similar and involving the same phenotypic test. The only outcome and test combination with sufficient data was MACE (a composite) in studies of the VerifyNow P2Y12 assay. None of the potential modifiers evaluated were found to significantly affect the assay's predictive ability with respect to the composite outcome.

In general, information on effect modification was limited, both within and across studies. Few studies reported information on the same potential effect modifiers, results were imprecise, and selective reporting was highly likely. Information across studies was also limited by the number of available studies on each test and outcome of interest. It is unclear whether the predictive effect of phenotypic testing differs across patient subgroups.

Key Question 3a: What Is the Comparative Effectiveness of Alternative Testing Strategies for Therapeutic Decisionmaking?

We grouped the studies we identified for this Key Question into three categories:

1. *Randomized trials of test-and-treat strategies*: These studies randomize patients to alternative management strategies, at least one of which is based on a test of interest. Patients are then followed up for intermediate or clinical outcomes.
2. *Randomized treatment trials that evaluate treatment-effect modification:* These are randomized studies in which patients in all groups undergo the test of interest at baseline. Treatment assignment is based on randomization and thus is independent of test results. Because these studies include both test-positive and test-negative patients in each treatment arm, they can be used to assess test result × treatment interactions.

[d] This assay uses arachidonic acid as the agonist, to measure "aspirin resistance."

3. *Randomized trials with test-based selection:* These studies select patients on the basis of baseline test results and then randomize them into non–test-based treatment groups. When properly randomized and conducted, these studies can provide unconfounded estimates of the treatment effect conditional on a particular test result.

Genetic Testing for CYP2C19 Variants

No studies compared testing for CYP2C19 variants with alternative testing strategies to guide treatment decisionmaking. No studies were randomized trials of alternative treatment strategies selecting patients for inclusion on the basis of CYP2C19 genotype.

Randomized Trials Reporting Information on Treatment-Effect Modification by CYP2C19 Genotype Status

We identified 11 publications (reporting on 10 study populations) describing randomized controlled trials that provide information on effect modification by CYP2C19 variants. Five studies (reported in six publications)[e] provided information on clinical outcomes, five on intermediate outcomes (platelet reactivity during followup), and one on both types of outcome.

Clinical Outcomes

Of the five studies providing clinical outcome information, four were large (>1000 participants), multicenter, randomized trials of clopidogrel-based treatment versus alternative treatments (two compared aspirin plus clopidogrel vs. aspirin monotherapy, one compared aspirin plus clopidogrel vs. aspirin plus prasugrel, and one compared aspirin plus clopidogrel vs. aspirin plus ticagrelor); each of these had at least one outcome event. A smaller, single-center fifth study of patients followed for 30 days reported that no clinical outcomes of interest were observed.

Of the four larger studies, one included patients with NSTE acute coronary syndromes (the CURE [Clopidogrel in Unstable Angina to Prevent Recurrent Events] trial), one involved patients with ST-elevation or non–ST-elevation acute coronary syndromes (the PLATO [Platelet inhibition and patient Outcomes] trial), one included those with moderate-to-high-risk acute coronary syndromes who were undergoing PCI (TRITON-TIMI 38 [Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38]), and one enrolled patients with atrial fibrillation who were not candidates for vitamin K antagonist therapy (ACTIVE A). CURE and ACTIVE A compared aspirin plus clopidogrel (at standard doses) with aspirin monotherapy, TRITON-TIMI 38 compared aspirin plus clopidogrel versus aspirin plus prasugrel, and the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. All trials were designed and powered to detect the main effect of antiplatelet therapy but were not specifically powered to detect heterogeneity of treatment effects and typically included only a subsample of the overall trial population.

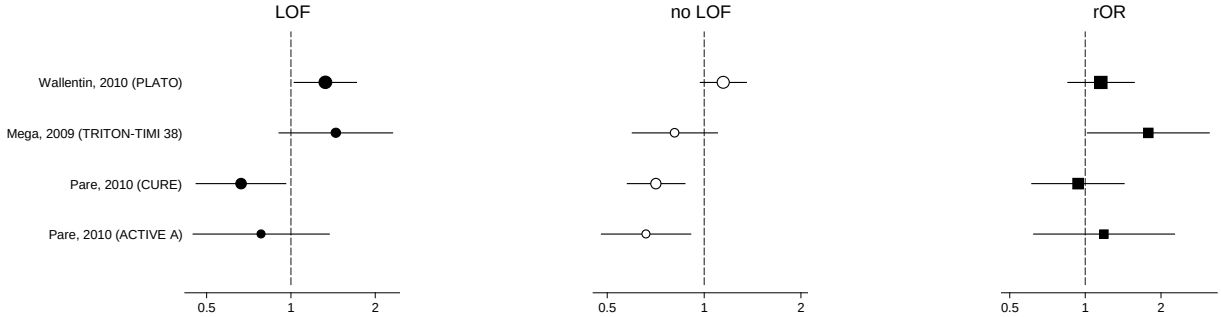
The CURE, PLATO, and ACTIVE A trials did not find statistically significant effect modification by CYP2C19 genotype for any of their efficacy outcomes. The genetic substudy of TRITON-TIMI 38 reported statistically significant treatment-effect heterogeneity among

[e] Four publications reported information on a single population each and one publication reported information on two independent populations.

genotype groups (at least one loss-of-function allele vs. none; P=0.046), with prasugrel being superior to clopidogrel among carriers of loss-of-function CYP2C19 alleles.

Because of the large differences in included populations, treatments compared, and exposure and outcome definitions among studies reporting on treatment-effect modification by CYP2C19 variants on clinical outcomes, we did not perform a meta-analysis. Given that the drugs compared (clopidogrel, prasugrel, and ticagrelor) have different mechanisms of action, it is plausible that interaction effects could have different magnitudes or directions across studies. For purposes of illustration, we used the counts reported in the studies to compare the treatment effect among carriers of CYP2C19 loss-of-function alleles versus noncarriers (i.e., those with normal or gain-of-function alleles). The relative treatment effect (i.e., the relative odds ratio) across the genotype groups—the equivalent of the genotype × treatment interaction—was statistically significant in only one study (**Figure B**).

Figure B: Results from Large Randomized Trials Assessing Effect Modification by CYP2C19 Variants on MACE



Forest plots of treatment effects (odds ratios) on major adverse cardiovascular events (MACE) among carriers of at least one loss-of-function (LOF) allele (left panel); treatment effects (odds ratios) among noncarriers of LOF alleles (middle panel); and relative effects (relative odds ratios [rOR]) comparing the treatment effect among LOF carriers and LOF noncarriers (right panel). The CURE and ACTIVE A trials compared aspirin plus clopidogrel versus aspirin monotherapy; the TRITON-TIMI 38 trial compared aspirin plus clopidogrel versus aspirin plus prasugrel; the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. Point estimates for treatment effects are shown as black circles (carriers) or white circles (noncarriers); point estimates for relative treatment effects are shown as black squares. Horizontal lines denote 95 percent confidence intervals for all estimates. Vertical dashed lines denote no effect.
ACTIVE A = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; PLATO = Platelet inhibition and patient Outcomes trial; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction.

Intermediate Outcome: Platelet Reactivity During Followup

Six studies assessing treatment-effect modification by CYP2C19 variants provided information on platelet reactivity during followup as an intermediate outcome. All six were based on randomized trials comparing clopidogrel-based treatment to alternative therapies, had small to moderate sample sizes (range, 60 to 474 participants), and enrolled heterogeneous populations: two included patients with stable coronary artery disease, one included patients with myocardial infarction undergoing PCI, one included patients undergoing elective PCI, one enrolled patients with coronary artery disease receiving drug-eluting stents, and one included "high-risk" patients undergoing PCI. All had short followup periods (<7 days to 6 weeks).

Four of the six studies did not provide a rationale for the genotype grouping. The studies differed in the alleles genotyped and the genotype groupings used, leading to heterogeneity in the

exposure definition. Platelet reactivity during followup was assessed by the VerifyNow P2Y12 assay in all six studies, as well as by LTA in three studies and the VASP assay (based on flow cytometry) in two studies. The overall results were variable and incomplete reporting often precluded the quantitative assessment of test × treatment interactions. Because of the differences in designs, populations, treatments compared, and followup durations among the included studies, we did not perform a meta-analysis.

Risk of Bias for Studies of Treatment-Effect Modification

The risk of bias of individual studies was variable. Generally, studies reporting on clinical outcomes (which were generally larger and had longer followup) rather than platelet reactivity as an outcome used robust methods for randomization and allocation concealment. However, these studies included only a small proportion of the patients included in the corresponding parent trials (15 to 40 percent in trials reporting clinical followup of more than a month). In contrast, in studies reporting information on laboratory outcomes (which tended to be smaller with short followup periods), 79 to 100 percent of the patients enrolled in the parent randomized trials were also included in the genetic substudies. These smaller studies provided adequate descriptions of the methods used for generating the randomization sequence but did not provide sufficient information to assess methods of allocation concealment.

Phenotypic Testing for Platelet Reactivity

Studies of Test-and-Treat Strategies

We identified five randomized studies directly comparing alternative test-and-treat strategies. Four compared VASP assay–guided therapy versus non–test-guided therapy; the fifth compared VerifyNow P2Y12–guided therapy versus non–test-guided therapy. The four evaluating the use of the VASP assay were of moderate size (the smallest enrolled 153 patients; the largest, 429 patients); three were multicenter studies and 1 was a single-center investigation. The single study assessing VerifyNow was smaller (had 60 patients) and had been conducted in a single research center.

All five studies directly comparing alternative test-and-treat strategies assessed patients undergoing PCI; four enrolled patients with stable coronary artery disease or acute coronary syndromes and one enrolled exclusively patients undergoing elective stenting. Four studies selected patients on the basis of a baseline assessment of platelet reactivity (enrolling only those with reactivity above a predetermined threshold); the fifth study included all patients, regardless of baseline reactivity. The experimental groups in four studies (three using the VASP assay and one using the VerifyNow assay) employed repeat reactivity monitoring at multiple time points with modification of the administered clopidogrel dose on the basis of test results. The fifth study performed only a single assessment of platelet reactivity, after 15 days of treatment, with subsequent treatment modification in patients found to have reactivity values above a predefined threshold. Control groups were given clopidogrel-based therapy at standard doses. Studies had relatively short followup durations (three had maximum followup of 30 days; one, 6 months; and one, a year). Only two studies, conducted by the same investigator group but with nonoverlapping enrollment periods, reported a prospective power calculation (and both had attained the enrollment goal).

Clinical Outcomes

All five studies comparing alternative test-and-treat strategies reported information on cardiovascular mortality and MACE; four also reported on recurrent acute coronary syndromes, two on all-cause mortality, three on stent thrombosis, and two on repeat revascularization or myocardial infarction. Overall, the studies had short followup durations and included moderate numbers of participants; thus, the outcome rates were low and relative effect estimates (when possible to calculate) were often extreme (e.g., odds ratios <0.5) and had substantial uncertainty (wide CIs). Studies generally indicated that the groups with test-based monitoring had better outcomes (lower event rates) than the groups without test-based monitoring; however, the differences were often not statistically significant. Meta-analyses were not performed, owing to the differences in the populations included, interventions compared, and durations of followup used.

Intermediate Outcome: Platelet Reactivity During Followup

Four of the five studies directly comparing alternative test-and-treat strategies reported information on platelet reactivity as an intermediate outcome. Although results generally indicated that platelet reactivity at the last followup assessment was lower in the groups that received test-based treatment than in those that received standard treatment, reporting was often incomplete and precluded statistical comparisons between groups. Furthermore, studies had short followup periods and it was unclear whether the observed differences in reactivity affected clinical outcomes.

Assessment of Risk of Bias of Individual Studies

Studies comparing alternative test-and-treat strategies based on phenotypic testing for platelet reactivity had moderate risk of bias. Because studies were prospectively conducted and because phenotypic testing is performed immediately after sample collection, the index test was assessed without knowledge of clinical or intermediate outcomes in all cases. However, information to judge whether outcomes were assessed without knowledge of the index-test result was often not reported. Subjects and personnel were not blinded and reporting was incomplete regarding the methods of generating the randomization sequence and concealing allocation. In one study we observed substantial discrepancies between the statistical results provided by the authors and results from analyses that we performed using the reported event counts (despite using the same statistical procedures described in the paper's methods section).

Studies of Treatment-Effect Modification by Baseline Platelet Reactivity

We identified a single study reporting information on effect modification by baseline platelet reactivity in patients randomized to alternative antiplatelet therapies. Four academic centers in France enrolled a total of 56 patients with unstable angina or non–ST-elevation myocardial infarction who received a loading dose of 900 mg of clopidogrel. Platelet reactivity was then assessed with four assays (LTA with 5 μM ADP, LTA with 20 μM ADP, VerifyNow P2Y12, and the VASP assay using flow cytometry). The study used a randomized cross-over design comparing aspirin plus prasugrel and aspirin plus clopidogrel (14 days of exposure to each treatment).

Clinical Outcomes

The study did not report patient-relevant clinical outcomes stratified by platelet reactivity status at baseline.

Intermediate Outcome: Platelet Reactivity During Followup

The study reported the response rate among "poor responders" to the clopidogrel loading dose during prasugrel-based therapy and during clopidogrel-based therapy. Generally the response rates were higher during prasugrel therapy, regardless of the assay used to assess platelet reactivity. However, the study did not report the response status during followup for patients who were "responders" to the clopidogrel loading dose. Thus, the interaction between post–loading dose response to clopidogrel and treatment assignment could not be assessed.

Assessment of Risk of Bias

The study was considered to have a high risk of bias because incomplete outcome information was reported and information on the generation of the randomized sequence and allocation concealment was unclear.

Studies with Phenotypic Test–Based Selection of Patients

Five studies met our inclusion criteria and reported information on the comparative effectiveness of treatments administered to patients selected on the basis of baseline platelet reactivity. The sample sizes ranged from 52 to more than 2000 participants and all five studies were relatively recent (published in 2008–2011). Four studies were performed mainly or exclusively in the PCI setting; the fifth study included patients with stable coronary artery disease. On-clopidogrel platelet reactivity was used as a selection criterion in all studies; it was assessed using the VerifyNow P2Y12 assay in two studies (in one of them, it was used together with VerifyNow AA, an assay of the platelet response to aspirin), LTA in two studies, and the VASP assay with flow cytometry in one study. The treatment comparisons were between standard-dose clopidogrel-based therapy and high-dose clopidogrel in two studies, addition of a glycoprotein IIb/IIIa inhibitor in two studies, and ticagrelor the remaining study.

Clinical Outcomes

Clinical-outcome comparisons between the randomized treatment groups were reported in three of the five studies.

One study (the GRAVITAS [Gauging Responsiveness with a VerifyNow Assay—Impact on Thrombosis and Safety] trial, enrolling 2214 randomized patients) included patients who had undergone PCI for stable coronary artery disease or NSTE acute coronary syndrome and showed increased on-clopidogrel reactivity on the VerifyNow P2Y12 assay. The patients were randomized to high-dose clopidogrel or standard-dose clopidogrel, both in combination with aspirin. After 6 months of followup, there was no statistically significant difference between the randomized groups in the rate of cardiovascular death, nonfatal myocardial infarction, stent thrombosis, all-cause mortality, or composite cardiovascular outcomes (either cardiovascular death or nonfatal myocardial infarction, or cardiovascular death, nonfatal myocardial infarction, or stent thrombosis). The study also included followup information for a randomly selected group of patients with low platelet reactivity at baseline who were treated with standard-dose clopidogrel (see the Results section for Key Question 1b for details).

Two smaller studies (of <150 patients each) with 1-month followup assessed the impact of treatment with a glycoprotein IIb/IIIa inhibitor in addition to standard treatment. Both studies used the VerifyNow P2Y12 assay to assess platelet reactivity. One demonstrated a statistically significant improvement in survival (freedom from cardiovascular death) in a time-to-event analysis and a statistically significant reduction in the risk of the composite outcome of death, periprocedural myonecrosis, stent thrombosis, or recurrent acute coronary syndrome. Other comparisons were either not statistically significant or the data reported were inadequate for estimating an effect size (and the associated P value).

Because the patient populations were heterogeneous, selected on the basis of different inclusion criteria, and assessed using different therapeutic regimens, we did not perform meta-analyses for any of the outcomes reported.

Intermediate Outcome: Platelet Reactivity During Followup

Two small studies, each including fewer than 100 patients, reported information on intermediate outcomes during 1 month of followup. The outcomes were assessed using different assays and were heterogeneously reported.

Assessment of Risk of Bias of Individual Studies

We performed a detailed assessment of 17 risk-of-bias items for studies comparing treatment effects in patients selected on the basis of phenotypic testing for platelet reactivity. Overall, the risk of bias varied across the randomized studies. The large, multicenter GRAVITAS trial had low risk of bias, both regarding aspects related to the index test of interest as well as general aspects of randomized trial design (e.g., generation of the randomization sequence and allocation concealment). In contrast, smaller studies with short-term followup were generally considered to have a higher risk of bias, owing to problems in the application of the tests of interest (e.g., an unclear rationale for the thresholds used) or incomplete reporting of outcomes. Furthermore, these studies often did not provide information sufficient to judge their risk of bias regarding general aspects of randomized trial design.

Key Question 3b: How Do Modifying Factors Affect the Association of Alternative Test-and-Treat Strategies and Patient Outcomes?

Only two of the studies relevant to Key Question 3a provided information about the use of testing for clinical decisionmaking with data stratified by patient characteristics—ancestry in one and baseline percent inhibition of on-clopidogrel reactivity in the other. Neither factor appeared to statistically significantly affect study results relevant to the use of testing to guide antiplatelet therapy.

Key Question 4: What Are the Potential Adverse Effects or Harms from Genetic or Phenotypic Testing *per se* or from Test-Directed Treatments?

Harms of Test-Directed Treatment

All studies addressing Key Question 4 were also included in Key Question 3a; assessment of the risk of bias of individual studies is addressed in that section.

Genetic Testing for CYP2C19 Variants

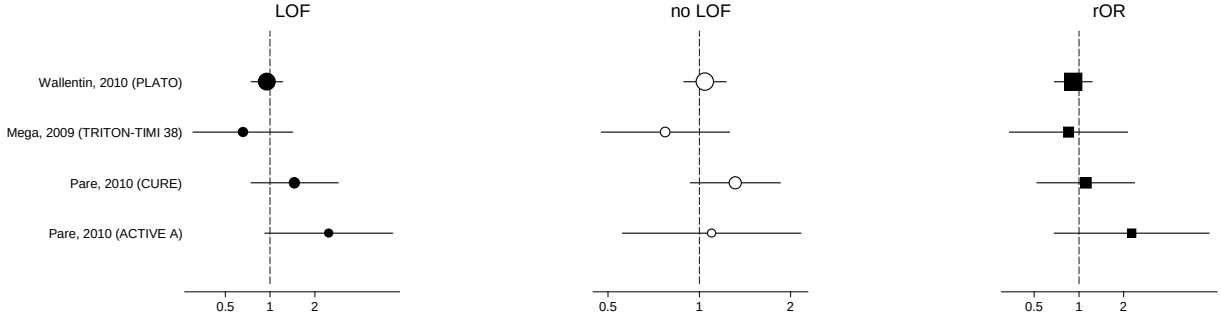
No studies compared testing for CYP2C19 variants with alternative testing strategies (including a no-testing strategy) to guide treatment decisionmaking. No studies were randomized trials of alternative treatment strategies selecting patients for inclusion on the basis of CYP2C19 genotype.

Studies of Treatment-Effect Modification by CYP2C19 Genotype Status

Five studies (reported in four publications) provided information on treatment-effect modification of bleeding outcomes by CYP2C19 status. Four were based on large randomized trials of clopidogrel-based therapy that included more than 1000 patients in their genetic substudies. Of these, one study enrolled patients with NSTE acute coronary syndromes, one enrolled those with acute coronary syndromes undergoing PCI, one involved patients with ST-elevation or non–ST-elevation acute coronary syndromes, and one enrolled persons with atrial fibrillation who were not appropriate candidates for vitamin K antagonist treatment. The fifth study was a small genetic substudy of 126 patients with myocardial infarction who were randomized to either aspirin plus high-dose maintenance clopidogrel or to triple-antiplatelet therapy (aspirin, clopidogrel, and cilostazol). With a followup of only 30 days, the study reported that no major bleeding events by Thrombolysis in Myocardial Infarction (TIMI) criteria were observed in either group. The four larger studies compared the effect of alternative treatment strategies (stratified by CYP2C19 genotype) on safety outcomes (in all four studies, bleeding events). Treatment effects were not statistically significant within genotype groups, with a single exception (for major hemorrhagic events among patients classified as "intermediate metabolizers" in the study of patients with atrial fibrillation). The test for interaction (i.e., a test for heterogeneity of treatment effects across genotype groups) was not statistically significant for any of the reported comparisons. This indicates that the impact of the compared treatments on bleeding events was not significantly different across patient groups defined by CYP2C19 genotype.

Because of the large differences in populations included, treatments compared, and exposure and outcome definitions among studies reporting on treatment-effect modification by CYP2C19 variants, we did not perform a meta-analysis. However, we used the counts reported in the studies to compare the treatment effect among carriers of CYP2C19*2 or *3 (loss-of-function alleles) versus noncarriers (i.e., carriers of CYP2C19*1 or *17 [normal and gain-of-function alleles, respectively]); the odds ratios for the treatment effect within each genotype subgroup and relative odds ratios comparing the treatment effect across genotype groups showed that treatment effect modification was non–statistically significant in all four studies (**Figure C**).

Figure C: Bleeding Events in Large Randomized Trials Reporting Information on Effect Modification by CYP2C19 Variants



Forest plots for treatment effects (odds ratios) on bleeding outcomes among carriers of at least one loss-of-function (LOF) allele (left panel); treatment effects (odds ratios) among noncarriers of LOF alleles (middle panel); and for relative effects (relative odds ratios [rOR]) comparing the treatment effect among LOF carriers and LOF noncarriers (right panel). The CURE and ACTIVE A trials compared aspirin plus clopidogrel versus aspirin monotherapy; the TRITON-TIMI 38 trial compared aspirin plus clopidogrel versus aspirin plus prasugrel; the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. Point estimates for treatment effects are shown as black circles (carriers) or white circles (noncarriers); point estimates for relative treatment effects are shown as black squares. Horizontal extending lines denote 95 percent confidence intervals for all estimates. Vertical dashed lines denote no effect.
ACTIVE A = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; PLATO = PLATelet inhibition and patient Outcomes trial; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction.

Phenotypic Testing for Platelet Reactivity

Studies of Test-and-Treat Strategies

Five studies comparing alternative test-and-treat strategies provided information on harms of test-directed treatment. Briefly, the studies randomized patients into groups receiving clopidogrel at a dose guided by phenotypic testing or into groups receiving standard dose clopidogrel. Four of the studies used the VASP assay to monitor response and modify treatment accordingly; the fifth study used the VerifyNow P2Y12 assay. No study identified a statistically significant difference between any study arms in the risk of bleeding events (the only treatment-related harms reported in the included studies). The studies had short followup durations (1 year in one study; 6 months in another; and 30 days in the remaining three) and few events were observed, particularly severe or major bleeding outcomes. Consequently, data were sparse and CIs around effect estimates were wide, indicating substantial uncertainty.

Studies of Treatment-Effect Modification by Baseline Platelet Reactivity

The single study reporting on treatment-effect modification by baseline on-clopidogrel platelet reactivity reported that no treatment discontinuations, non–CABG-related major bleeding events (by Thrombolysis in Myocardial Infarction [TIMI] criteria), or severe or life-threatening bleeding events (by GUSTO [Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries] criteria) were observed during the trial. Additional information on minor bleeding events or deaths (one death, due to cancer) was not stratified by baseline reactivity status.

Studies with Phenotypic-Test–Based Selection of Patients

Of the five studies that reported results from randomized trials with phenotypic testing–based patient selection, three reported information on treatment-related harms. The large randomized GRAVITAS trial compared high-dose clopidogrel (in 1109 patients) versus standard-dose clopidogrel (in 1105 patients); all the patients had high on-clopidogrel platelet reactivity as measured by the VerifyNow P2Y12 assay. The study found no statistically significant difference between the two treatment groups for any bleeding event (hazard ratio for high-dose vs. standard-dose therapy, 1.19; 95 percent CI 0.93, 1.53) (P=0.18) after 6 months of followup. Two other smaller studies with short followup durations (1 month in both cases) reported that no major bleeding events were observed in the whole study population (regardless of baseline platelet reactivity status).

Harms of Testing *per se*

We found no studies reporting on the harms of the testing process for CYP2C19 genotyping or measuring platelet reactivity in the populations of interest. However, one study comparing VASP-guided therapy with standard clopidogrel dosing in the PCI setting noted that patients in the test-guided arm had a longer time from clopidogrel loading to PCI than patients in the non–test-guided treatment arm (P<0.001). The delay was due to the need for repeat testing and treatment modification until a predefined reactivity threshold was reached in the test-guided group. It is unclear whether this delay resulted in harm to patients.

Discussion

Clopidogrel is used extensively in the interventional management of coronary artery disease and the treatment and secondary prevention of acute coronary syndromes.[49] Furthermore, it is used for the management of patients undergoing neurointervention (with stent placement), for the prevention of stroke in patients with atrial fibrillation who are not candidates for vitamin K antagonist therapy, and for the management of selected patients with peripheral arterial disease. However, response to clopidogrel therapy—as assessed by *ex vivo* studies of platelet function—is variable among patients and over time (within a patient). Some patients experience little suppression of platelet reactivity (despite being compliant to treatment) while others experience more profound suppression that may increase their risk of bleeding. Given the availability of several therapeutic options for antiplatelet treatment (e.g., increasing the loading or daily maintenance dose of clopidogrel or using adjunctive or replacement therapies such as prasugrel, ticagrelor, or cilostazol), there is interest in reliably identifying patients who are less likely to respond to standard clopidogrel treatment, as well as those who are most likely to respond to alternative treatments. This report reviewed the evidence of the effectiveness and comparative effectiveness of two types of tests that have been extensively evaluated as biomarkers for outcome prognosis for patients receiving clopidogrel therapy and as biomarkers of treatment response: genetic testing for CYP2C19 variants and phenotypic testing for on-clopidogrel platelet reactivity.

Key Findings and Assessment of the Strength of Evidence

Table B presents a summary of the report's key findings. When appropriate, results are presented separately for each of the populations and outcomes of interest. We did not assess the strength of evidence for studies of analytic validity (because analytic validity is a prerequisite for

the clinical use of the tests and because no framework exists for assessing the strength of evidence for analytic validity studies) or for studies exclusively assessing platelet reactivity as an outcome (because platelet reactivity measurements during followup are not usually performed as part of clinical care and because platelet reactivity is not a patient-relevant outcome). Instead, we focus here on the body of evidence pertaining to predictive effects, treatment decisionmaking, and harms as related to patient-relevant clinical outcomes. Please see the Methods section for a detailed discussion of our approach to rating the strength of evidence.

Table B: Key Findings from This Review and Assessment of Strength of Evidence

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
1a: What it the analytic validity of tests for genotyping CYP2C19 variants?	NA	Genotyping for any CYP2C19 variant	NA	SOE = NA • Few studies provided information on analytic validity specifically using samples obtained from patient populations relevant to this review. • When available, data were limited to test–retest reliability. • There was limited information comparing the validity of different genetic testing assays. • However, based on data on healthy volunteers (not reviewed in this report), the analytic validity of genotyping assays can be considered robust.
1b: What is the predictive value of genetic testing for CYP2C19 variants?	Ischemic heart disease	Genotyping for LOF CYP2C19 variants	Stent thrombosis	SOE = *Low* • Meta-analysis of 13 studies found a statistically significant association. • RR=1.56 (95 percent CI 1.15, 2.11) • There was little evidence of heterogeneity (I^2=0%); but the test for small-study effects was statistically significant. • There was some concern about selective outcome reporting. • Studies reported few outcome events and the summary estimate was imprecise.
			MACE	SOE = *Low (for lack of association)* • Meta-analysis of 15 studies did not identify a statistically significant association. • RR=1.09 (95 percent CI 0.93, 1.29) • There was some evidence of heterogeneity (I^2=33%) and small-study effects.
			All other clinical outcomes	SOE = *Insufficient* • Few studies reported information for noncomposite clinical outcomes other than stent thrombosis. • There was substantial concern about selective outcome reporting. • Study-specific and meta-analysis estimates (when performed) indicated substantial uncertainty.
		Genotyping for GOF CYP2C19 variants	MACE	SOE = *Low* • Meta-analysis of 4 studies found a statistically significant protective effect (for carriers vs. noncarriers). • RR=0.80 (95 percent CI 0.70, 0.90) • There was substantial concern about selective outcome reporting.
			All other clinical outcomes	SOE = *Insufficient* • Few studies provided relevant information. • There was substantial concern about selective outcome reporting. • Study-specific and meta-analysis estimates (when performed) indicated substantial uncertainty.
	Patients undergoing neurointervention	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • Only a single small study was available; it did not report information on clinical outcomes.
	Patients with atrial fibrillation	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • Only a single study was available. • Estimates were imprecise owing to the small number of outcome events observed.
1c: What factors affect the predictive value of genetic testing for CYP2C19 variants?	All patient populations	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • 10 studies provided information on effect modification; no factor affected predictive performance statistically significantly regarding clinical outcomes; no factor was assessed by more than 3 studies, giving rise to concerns about selective outcome reporting.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• Effect modification by study-level factors was not statistically significant and CIs were wide for all genotype–outcome pairs assessed.
2a: What is the analytic validity of tests for on-clopidogrel platelet reactivity?	NA	All assays used to measure on-clopidogrel platelet reactivity	NA	SOE = NA • Few studies reported information on analytic sensitivity and specificity, possibly reflecting the research community's belief that there is no good reference standard assay for platelet reactivity. • Agreement ranged from poor to moderate and was variable between tests. The highest agreement was observed between applications of the same assay with different concentrations of agonists, rather than between different assays.
2b: What is the predictive ability of phenotypic testing for platelet reactivity?	Ischemic heart disease	LTA	All-cause mortality	SOE = *Low* • 10 studies using heterogeneous methods to define increased reactivity were available. • These studies support an association between increased platelet reactivity measured by LTA and mortality.
			Cardiovascular mortality	SOE = *Low* • 8 studies using heterogeneous methods to define increased reactivity were available. • Studies provided evidence of an association between increased reactivity and cardiovascular mortality; however, clinical heterogeneity precluded firm conclusions. • There was some concern about selective outcome reporting.
			Acute coronary syndromes	SOE = *Low* • 17 studies using heterogeneous methods to define increased reactivity were available. • Studies often found statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions. • There was some concern about selective outcome reporting.
			Stent thrombosis	• 18 studies using heterogeneous methods to define increased reactivity were available. • Studies often found statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions. • There was some concern about selective outcome reporting.
			Stroke	SOE = *Low (for lack of association)* • 11 studies using heterogeneous methods to define increased reactivity were available. • Studies generally did not report statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions or quantitative synthesis to increase precision.
			MACE	SOE = *Low* • 33 studies using heterogeneous methods to define increased reactivity were available. • The majority of reviewed studies suggested a statistically significant association between increased platelet reactivity measured by LTA and composite cardiovascular events. • Definitions of composite outcomes where often heterogeneous.
			All other clinical outcomes**	SOE = *Insufficient* • Clinical and population heterogeneity or small number of studies limited our ability to draw conclusions
		VerifyNow	All cause mortality	SOE = *Low (for lack of association)* • 5 studies were available. Meta-analysis of 3 studies did not find an association between increased reactivity measured by VerifyNow and all-cause mortality.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• RR=1.39 (95 percent CI 0.73, 2.66) • The summary estimate was imprecise and 95% CI did not rule out clinically meaningful effects.
			Cardiovascular mortality	SOE = *Low* • 6 studies were available. Meta-analysis of 4 studies found a statistically significant association with little evidence of heterogeneity. • RR=2.50 (95 percent CI 1.28, 4.87) • The CI of the summary estimate indicated substantial uncertainty. • There was some concern about selective outcome reporting.
			Acute coronary syndromes	SOE = *Low* • 14 studies using heterogeneous methods to define increased reactivity were available. • Studies generally suggested an association between increased reactivity as measured by VerifyNow and acute coronary syndromes, both periprocedurally and during longer followup.
			Bleeding events (major and all levels of severity combined)	SOE = *Low (for lack of association)* • 8 studies were available. • Meta-analysis of 3 studies with data on any bleeding event did not find an association between increased reactivity measured by VerifyNow. • RR=1.02 (95 percent CI 0.79, 1.31). There was little evidence of heterogeneity (I^2=0 percent). • Meta-analysis of 4 studies with data on major bleeding events did not find an association between increased reactivity measured by VerifyNow. • RR=0.85 (95 percent CI 0.47, 1.52). There was substantial evidence of heterogeneity (I^2=62 percent). • For major bleeding events the summary estimate was imprecise and the 95% CI did not rule out clinically meaningful effects.
			MACE	SOE = *Low* • 17 studies were available. Meta-analysis of 11 studies identified a statistically significant association. • RR=2.46 (95 percent CI 1.88, 3.22) • There was little evidence of statistical heterogeneity (I^2=21 percent) and studies used fairly similar definitions of increased reactivity. • The test for small-study effects was statistically significant.
			All other clinical outcomes**	SOE = *Insufficient* • Clinical heterogeneity or small number of studies limited our ability to draw conclusions
		VASP assay	Cardiovascular mortality	SOE = *Low (for lack of association)* • 6 studies were available. Meta-analysis of 4 studies did not identify a statistically significant association. • RR=2.09 (95 percent CI 0.74, 5.88) • Although the test for heterogeneity was nonsignificant, point estimates from individual studies ranged from strong protective effects to strong harmful effects. • The meta-analytic summary point estimate was far from the null and its CI was wide (imprecise). • Clinically significant effects could not be ruled out.
			Acute coronary syndromes	SOE = *Low (for lack of association)* • 6 studies were available. Meta-analysis of 3 studies did not identify a statistically significant association. • RR=1.47 (95 percent CI 0.77, 2.79)

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• The test for heterogeneity was non-significant but point estimates from individual studies were highly variable. • The meta-analytic summary point estimate was far from the null and its CI was wide (imprecise). • Clinically significant effects could not be ruled out.
			Stent thrombosis	SOE = *Low* • 7 studies were available. Meta-analysis of 4 studies identified a statistically significant association. • RR=3.37 (95 percent CI 1.59, 7.11) • There was little evidence of statistical heterogeneity and studies used fairly similar definitions of increased reactivity. • The summary estimate was imprecise but the lower bound was consistent with a 59% increase in risk in the high-reactivity group. • There was some concern about selective outcome reporting.
			MACE	SOE = *Low* • 7 studies were available. Meta-analysis of 6 studies identified a statistically significant association. • RR=2.57 (95 percent CI 1.21, 5.47) • There was evidence of statistical heterogeneity. • The summary estimate was imprecise but the lower bound was consistent with a 21% increase in risk in the high-reactivity group. • There was some concern about selective outcome reporting.
			All other clinical outcomes**	SOE = *Insufficient* • Few studies reported information. • Clinical heterogeneity or small number of studies limited our ability to draw conclusions .
		PFA-100	MACE	SOE = *Low* • 7 of the 8 studies on this assay reported information on composite clinical outcomes. • Heterogeneity in the methods used to define increased reactivity precluded definitive conclusions; however, studies generally indicated an association between increase platelet reactivity as measured by the PFA-100 assay and composite clinical outcomes.
			All other clinical outcomes	SOE = *Insufficient* • Few of the available studies reported information on other outcomes. • There was concern about selective outcome reporting.
		All other assays	All clinical outcomes	SOE = *Insufficient* • Few studies were available. • When ≥2 studies were available for the same outcome they used heterogeneous metrics or thresholds to define increased reactivity or used different agonists for *ex vivo* stimulation of platelets.
	Patients with cerebrovascular disease	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • Only 4 studies, using diverse assays to measure reactivity, were available. • Studies were fairly small.
	Patients with peripheral arterial disease	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • Only a single small study was available.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
2c: What factors affect the predictive value of phenotypic testing for platelet reactivity?	All patient populations	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • 5 studies provided information on effect modification; no factor was assessed by more than 2 studies. • Effect modification by study-level factors could not be assessed for most assay–outcome pairs; when such analysis was possible (for VerifyNow MACE), results indicated substantial uncertainty.
3a: What is the comparative effectiveness of alternative test-and-treat strategies	Ischemic heart disease	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • 3 studies provided information on treatment effect modification; no studies directly compared testing strategies; no studies randomized patients selected on the basis of genotype status. • Studies compared different antiplatelet treatments and produced heterogeneous results. • Study-specific estimates were imprecise.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • The 5 randomized studies of testing strategies were small, had different designs, and produced extreme results with considerable statistical uncertainty. • Only a single study of effect modification was identified; the study had been terminated early and did report information on clinical events. • Studies of test-based patient selection assessed different treatments.
	Atrial fibrillation	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • Only one study providing information on effect modification by CYP2C19 status was identified. • The study did not find evidence of effect modification by genotype status but there was considerable statistical uncertainty in the study estimates.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
	Other patient populations	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
3b: What factors modify the comparative effectiveness of alternative test-and-treat strategies?	All patient populations	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • 2 studies provided information on effect modification; each assessed different effect modifiers; no statistically significant interactions were reported.
4: What are the harms of testing? What are the harms of test-directed treatment?	All patient populations	Genetic testing for CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • 4 studies assessed treatment effect modification by genotype status; no studies directly compared testing strategies; no studies randomized patients selected on the basis of genotype status. • Studies compared different antiplatelet treatments and had heterogeneous results. • No studies provided direct information on the harms of testing *per se*.
		Phenotypic testing for	All clinical	SOE = *Insufficient*

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
		platelet reactivity (all assays assessed)	outcomes	• The 5 randomized studies of testing strategies were small, had different designs, and produced extreme results with considerable statistical uncertainty (and in several cases simply did not report any outcome events). • Only a single study of effect modification was identified; safety outcomes either did not occur (regardless of reactivity status) or results were not stratified by reactivity group. • Studies of test-based patient selection assessed different treatments.

CI = confidence interval; GOF = gain-of-function; LOF = loss-of-function; MACE = major adverse cardiovascular events; NA = not applicable; RR = relative risk; SOE = strength of evidence.

In our comparative effectiveness review, we have synthesized more publications than previous reviews,[a] with generally similar findings. Regarding the predictive effects of CYP2C19 genotype status, existing systematic reviews have reached similar conclusions to ours, both in magnitude and direction. Also consistent with our findings, previous analyses have suggested that selective outcome reporting and publication bias may have affected meta-analytic estimates.[23,50]

Compared to previous systematic reviews regarding platelet reactivity assays, our review includes a much larger number of studies and considers multiple assays assessing on-clopidogrel platelet reactivity using agonists to stimulate platelets *ex vivo*. In contrast to previous meta-analyses, we did not combine results across different assays (i.e., across tests using different measurement principles), different agonist concentrations, or different calculation methods or cutoff values for defining high reactivity. We believe that this choice is supported by our review of analytic validity that found low to moderate agreement between different assays. Of note, our analyses relevant to the VerifyNow P2Y12 assay include almost double the number of studies included in a recently published meta-analysis of individual data on the same assay.[51] Despite differences in selection criteria and analysis methods, our findings were similar, identifying a fairly strong association between platelet reactivity as measured by the VerifyNow P2Y12 assay and adverse cardiovascular outcomes.

To our knowledge, this is the first review to have comprehensively evaluated the use of genetic and phenotypic testing to guide clinical decisionmaking. We developed a structured approach that considered different experimental designs (randomized trials of alternative test-and-treatment strategies; randomized treatment trials assessing effect modification by biomarkers; and randomized treatment trials using the biomarkers to select patients for inclusion). Although the studies we identified were too diverse to support firm conclusions on the value of the tests of interest, we believe that our methodologic approach will be helpful as the evidence base continues to grow (e.g., it will be applied in our updated literature review).

Applicability

The majority of included studies (almost all available studies, for all Key Questions) enrolled patients with ischemic heart disease. Other populations who are potential candidates for antiplatelet therapy (e.g., patients with cerebrovascular disease, peripheral arterial disease, or atrial fibrillation) were included in a minority of studies only. This imbalance is not unexpected, given that clopidogrel's primary indications pertain to ischemic heart disease populations. However, it is probably not prudent to extrapolate findings from studies of ischemic heart disease to other patient populations. Given that a large number of studies included patients undergoing PCI, applicability may also be limited to noninterventional settings.

Particularly for genetic testing for CYP2C19 variants, patient race or ethnicity may be an important effect modifier, because the prevalence of variant alleles is substantially different

[a] The EPC recognizes that several studies relevant to the topic have been published since the conduct of the literature search conducted as the basis for this report. Per our protocol, we plan to update our literature searches (through the first quarter of 2012) and include any additional relevant studies in our analyses. For example, we anticipate that the updated review will include the results from the recently published genetic substudy of the CHARISMA trial (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance, reporting on effect modification by CYP2C19 variants) and the RAPID GENE randomized trial (Reassessment of Anti-Platelet Therapy Using an Individualized Strategy Based on Genetic Evaluation, comparing genetic testing for CYP2C19 variants with standard care for patients undergoing PCI).

among racial and ethnic groups (e.g., *2 variants are much more common in East Asian populations than others). We found little evidence that prognostic effects were different across ethnic or racial groups, but data were limited. Although consistency of genetic effects across racial groups has been demonstrated for several gene-disease associations[52], more evidence is needed for patient populations underrepresented in this review (e.g., blacks and East Asians).

The majority of studies were conducted in tertiary (usually academic) medical centers. Studies of treatment-effect modification by CYP2C19 genotype were based on large randomized trials, and findings may not be generalizable to everyday care settings. Because patient information on preexisting vascular disease in studies of predictive effects was generally incompletely reported, it is unclear whether patients in the included studies are representative of those seen in clinical practice. Nonetheless, the distribution of risk factors for ischemic vascular disease (male sex, hyperlipidemia, diabetes, hypertension, smoking, etc.) appeared to be representative of contemporary patient populations, and the majority of studies were conducted in recent years.

Implications for Clinical and Policy Decisionmaking

Despite the availability of a large literature on the use of genetic testing of CYP2C19 variants and phenotypic testing of platelet reactivity for predicting outcomes in patients receiving clopidogrel-based therapy, studies provided limited information on the value added by these tests over ascertainment of conventional risk factors in the populations of interest (e.g., clinical or laboratory information or disease-specific predictive scores). Furthermore, there was little comparative evidence that could be used to identify the most informative test or combination of tests for predicting clinical outcomes. This and other limitations of the existing literature may reduce the potential for clinical application of the tests reviewed herein as predictive markers for patients on clopidogrel-based antiplatelet therapy. The available evidence was even more limited regarding the use of either type of testing to guide the choice of antiplatelet therapy.

Limitations of the Evidence

On the basis of the large number of reviewed studies, we believe that the evidence regarding genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity (for guiding antiplatelet treatment and predicting outcomes in patients who receive it) is limited in the following ways:

- Lack of comparative studies evaluating the relative predictive ability of alternative assays for measuring platelet reactivity, genetic testing of CYP2C19 variants, or combinations of these tests.
- Lack of separation of development (“training”) and assessment (“test”) samples when developing predictive markers.
- Paucity of studies evaluating the impact of test-guided treatment selection on the basis of CYP2C19 genotyping or reactivity measurements.
- Limited number of studies providing information on treatment effect modification by CYP2C19 genotype status or baseline on-clopidogrel platelet reactivity.
- Heterogeneity in exposure definitions, because not all studies genotype the same CYP2C19 variants and because studies use different assays, metrics, and cutoff values to define increased platelet reactivity.

- Selective outcome reporting is a concern for the association between test results and clinical outcomes; most studies report information on composite clinical outcomes but rarely provide results for the component clinical events.

Ongoing Research

A search on May 3, 2012, in the ClinicalTrials.gov registry identified 173 potentially relevant records. After full text review, 28 records of studies that can be expected to provide information relevant to Key Questions 3 and 4 of this report were identified. None of the studies had posted their results in the database. Two of the studies have been published in peer reviewed journals (after our last search) and we anticipate including them in the update of the present report.

Future Research

This review has identified substantial gaps in the literature on genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity, both as biomarkers of future outcomes among patients who are receiving clopidogrel therapy and—more importantly—as tests for guiding treatment selection for patients who are candidates for antiplatelet treatment. We believe that the following evidence gaps may represent fruitful areas for future research:

- *Analytic validity of phenotypic testing:* Future studies using rigorous methods to inform the analytic validity of tests for measuring platelet reactivity are needed, particularly with regard to test–retest reliability, interassay agreement, and analytic performance.
- *Predictive accuracy, with a focus on comparative predictive performance:* Although we identified several studies reporting on the predictive value of the tests of interest, studies had several limitations in their design and analysis methods (see the Limitations of the Evidence Base section above). Thus, large-scale prospective studies of the tests of interest are needed to derive reliable estimates of predictive performance.
- *Direct comparisons of methods for test-guided treatment selection:* Even if the predictive value of tests were established, this information is often inadequate as a basis for treatment decisionmaking. The most promising tests could be prioritized for assessment in directly comparative studies of testing versus no testing for guiding treatment choice. Although costly and time-consuming, such studies can provide unconfounded estimates of the relative benefits and harms of the compared strategies.[53]
- *"Repurposing" completed randomized trials to assess effect modification:* An approach to assessing effect modification by genotype status is to repurpose already completed randomized trials, in which the drugs of interest were tested against a suitable comparator, by genotyping samples from enrollees. Results of genetic analyses can be associated with the prospectively recorded clinical outcomes.[54,55] When samples are available from all participants in a randomized controlled trial (or a random sample thereof), and appropriate methods to control for multiple testing are implemented, such studies can support valid inference on treatment effect modification.
- *Monitoring of platelet reactivity to guide treatment:* Strategies of monitoring platelet reactivity can be conceptualized as "dynamic treatment regimes"[56-58] (i.e., rules for sequential decisionmaking based on the evolution of reactivity measurements over time; obviously such methods are not applicable to CYP2C19 testing).

Conclusions

In summary, we found limited evidence on the analytic validity of genetic testing for platelet reactivity. However, using evidence from other populations and genetic variants, we believe that the available assays for CYP2C19 genotyping have adequate technical test performance. In contrast, we found a large body of evidence on the analytic validity of assays for measuring platelet reactivity, suggesting that interassay agreement is poor to moderate. No phenotypic assay can be considered a "gold standard" test.

We found some evidence supporting a significant association between loss-of-function CYP2C19 alleles and increased risk of stent thrombosis and cardiovascular mortality, as well as a significant association between gain-of-function alleles and reduced risk of MACE. The interpretation of these associations should be cautious, given the potential for selective reporting and small-study effects to have affected study results. Furthermore, the applicability of findings to patient populations other than those with ischemic coronary artery disease (particularly those undergoing revascularization procedures) was limited. We also found evidence supporting an association between high on-clopidogrel platelet reactivity measured by LTA, VerifyNow P2Y12, or the VASP assay and various adverse clinical outcomes. Our confidence in these findings is limited by the relatively small number of studies available for each test–outcome combination, the potential for selective outcome reporting, and the common lack of separation between the populations used to derive test thresholds of optimal predictive value and those used to assess predictive value at these thresholds.

Regarding the use of genetic testing for CYP2C19 variants to guide treatment choice, we reviewed four large randomized trials reporting information on effect modification on clinical outcomes by CYP2C19 alleles and several smaller trials mostly reporting on platelet reactivity as the outcome of interest. All but one study did not find evidence of statistically significant effect modification by genotype status. Given the heterogeneity of patient populations, treatments, and outcomes assessed, as well as the lack of studies directly comparing alternative testing strategies, we believe there is insufficient evidence regarding the use of CYP2C19 genotyping for guiding treatment choice.

Finally, regarding the use of platelet reactivity measurement for guiding antiplatelet treatment choice, we found five randomized studies directly comparing alternative test and treat strategies, one study assessing effect modification by baseline on-clopidogrel reactivity, and three randomized trials of antiplatelet therapy using platelet reactivity to select patients with high reactivity. Taken together, these studies provided insufficient evidence for the utility of platelet reactivity testing to guide antiplatelet therapy because short follow-up periods and low numbers of outcome events resulted in imprecise and often implausibly extreme estimates of benefit.

Additional research is needed to better establish the predictive value and clinical utility for treatment decisionmaking, both for genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity, focusing on standardizing testing methods and assessing the relative impact of testing strategies on patient-relevant clinical outcomes in large, well-conducted clinical trials.

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Acronyms

ACTIVE A	Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A trial
ADP	Adenosine diphosphate
AHRQ	Agency for Healthcare Research and Quality
CABG	Coronary artery bypass graft
CI	Confidence interval
CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Events trial
EPC	Evidence-based Practice Center
FDA	Food and Drug Administration
GRAVITAS trial	Gauging Responsiveness with a VerifyNow Assay—Impact on Thrombosis and Safety trial
GUSTO criteria	Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries criteria
LTA	Light-transmission aggregometry
MACE	Major adverse cardiovascular events
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
PFA	Platelet Function Analyzer
PLATO trial	PLATelet inhibition and patient Outcomes trial
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies 2
RR	Relative risk
TRITON-TIMI 38 trial	Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38
VASP	Vasodilator-stimulated phosphoprotein

Introduction

Platelets play a role in the development of atherosclerotic vascular diseases such as acute and chronic coronary artery disease, ischemic cerebrovascular disease (i.e., ischemic stroke or transient ischemic attack), and peripheral arterial disease. Specifically, platelet activation and aggregation, and the interaction of platelets with blood cells and the endothelium, contribute to the pathophysiology of these diseases. Furthermore, platelets participate in thrombus formation in the setting of atrial fibrillation.[1] Because of the importance of platelets in disease processes that often culminate in major adverse clinical events (e.g., myocardial infarction, ischemic stroke, or cardiovascular death), there is a strong rationale for the development of therapies specifically targeting platelet function for the primary and secondary prevention of cardiovascular disease. Because patient response to antiplatelet treatments is variable, there is also great interest in developing biomarkers to predict treatment response and guide treatment selection.

Burden of Disease and Clinical Setting

Approximately 82 million Americans currently suffer from some form of cardiovascular disease.[2] Coronary heart disease alone is the cause of 1 of every 6 deaths in the United States; and stroke, 1 of every 18 deaths.[3] In spite of widespread prevention efforts, it is estimated that every year more than a million Americans have a myocardial infarction and approximately 795,000 Americans experience a first or recurrent stroke.[2] There were approximately seven million inpatient cardiovascular operations and procedures in the United States in 2007, of which one million were either percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG) surgeries.[2]

Randomized controlled trials have established dual antiplatelet treatment with clopidogrel and aspirin as the current standard of care for medical and interventional management of acute coronary syndromes.[4] Dual antiplatelet treatment is also recommended for patients undergoing PCI[5] with placement of stents (either bare metal or drug eluting). Randomized controlled trials support the use of clopidogrel in patients who have experienced acute cardiovascular events (e.g., stroke) and those with peripheral arterial disease.[4,6-9]For patients with atrial fibrillation, vitamin K antagonists (e.g., warfarin) are the current treatment of choice; however, as many as 50 percent of patients eligible for vitamin K antagonist therapy do not receive it,[10-12] most often because of contraindications[13] (e.g., increased risk of bleeding). Evidence from trials suggests that for patients with such contradindications, the combination of clopidogrel and aspirin is more effective than aspirin alone for preventing thromboembolic disease.[14] Although clopidogrel-based antiplatelet therapy has been shown to improve patient outcomes in various settings, it appears to be associated with an increased bleeding risk compared with aspirin alone[4,15] or placebo.

Since the approval of clopidogrel by the Food and Drug Administration (FDA) for routine clinical use, the drug has become one of the most commonly prescribed agents in the United States. However, patient response to clopidogrel-based antiplatelet therapy is variable (both between patients and across multiple measurements within a patient), with some patients showing no platelet response to clopidogrel administration (often termed clopidogrel "nonresponsiveness" or "resistance"). Alternatives to standard clopidogrel treatment include higher-dose clopidogrel regimens and the use of other antiplatelet agents, such as prasugrel or

ticagrelor, which are not metabolized through the same pathways as clopidogrel. Prasugrel and ticagrelor have efficacy similar or superior to clopidogrel for preventing major adverse cardiovascular events (MACE). However, these drugs may increase the risk of bleeding complications.[16-19] Given the availability of alternative antiplatelet strategies and concern about adverse clinical outcomes in clopidogrel nonresponders, research has focused on methods to identify patients who are unlikely to benefit from clopidogrel-based treatment.

Clopidogrel Metabolism

To be biologically active, clopidogrel must be transformed to the active metabolite R-130964 by members of CYP enzyme system, primarily the enzyme CYP2C19. R-130964 acts by binding irreversibly to the $P2Y_{12}$ receptor (the adenosine diphosphate [ADP] receptor) on the surface of platelets and inhibits platelet aggregation for the life cycle of the platelet.[20,21] Platelet aggregation in a patient returns to pretreatment levels approximately 5 days after clopidogrel is stopped, owing to the production of new (noninhibited) platelets by the hematopoietic system.[22,23]

The CYP2C19 gene is highly polymorphic, with more than 35 identified variants. Following the recommendations of the Human Cytochrome P450 Allele Nomenclature Database,[a] each of these variants is designated by a number (e.g., "*1," "*2," and so on). CYP2C19*1 alleles lead to normal enzymatic activity (i.e., a normal metabolizer phenotype). Some alleles, including the relatively common CYP2C19*2, *3, and *4 alleles, are all known to be "loss-of-function" alleles that lead to complete elimination of enzymatic activity (i.e., a poor metabolizer phenotype). The relatively common CYP2C19*17 alleles are known to be "gain-of-function" alleles that lead to increased enzymatic activity (i.e., an enhanced metabolizer phenotype).[24] The fact that each individual carries two CYP2C19 alleles results in combinations of alleles of varying enzymatic activity and leads to additional variation in the observed metabolic phenotypes. For example, carriers of a *1/*2 genotype have an intermediate metabolizer phenotype between that of *1/*1 (normal metabolizer) and that of *2/*2 (nonmetabolizer). The functional status of some combinations of genotypes (usually combination of loss-of-function and gain-of-function alleles) is currently unknown; however, such genotypes are generally rare. A genomewide association study recently demonstrated that CYP2C19*2 is the main genetic determinant of variability in clopidogrel responsiveness, accounting for 12 percent of the total observed variation in this trait in a selected white population.[25]

Predicting Response and Guiding Antiplatelet Treatment

There are currently two basic approaches to determine whether a patient will have a poor response to clopidogrel: (1) genetic testing to see whether the patient has a genotype that is associated with reduced ability to metabolize clopidogrel (a poor-metabolizer phenotype), and (2) direct testing of the patient's blood while the patient is taking clopidogrel to see whether the platelets actually have become less prone to aggregate in response to specific agonists (phenotypic testing for platelet reactivity).

Several recent studies have assessed the utility of these two approaches for predicting major clinical outcomes in patients receiving clopidogrel.[26-30] Although clinicians and patients may find it helpful to know the probability of future outcomes under clopidogrel therapy, medical tests are most valuable when they can be used to guide treatment decisions. Thus, beyond predictive

[a] Available at: http://www.cypalleles.ki.se/; last accessed April 18, 2012.

ability, there is interest in evaluating whether genetic or phenotypic testing can improve patient outcomes by identifying patients who would benefit more (or experience less harm) by using treatment strategies other than standard clopidogrel-based treatment (e.g., using alternative clopidogrel dosing schemes or other antiplatelet agents). The observation that specific CYP2C19 variants or levels of on-treatment platelet reactivity above a threshold predict worse outcomes does not necessarily mean that changing treatment on the basis of these tests will improve outcomes. It is possible that the genotype or phenotype is simply a marker for poor outcome regardless of the treatment strategy used. Therefore the evidence of the test's impact on treatment decisions and subsequent patient outcomes must be considered separately from outcome prediction.

Randomized controlled trials of testing strategies versus no-testing strategies can address the question of whether the use of testing affects clinical outcomes.[31] When such comparative studies are not available, evidence can be obtained by repurposing completed randomized controlled trials for which baseline samples are available to perform genetic analyses.[32,33]

Genetic Tests for CYP2C19 Variants

Genetic testing for one or more genetic variants can be performed with various genotyping methods, such as restriction-fragment-length polymorphism analysis, other polymerase chain reaction (PCR)–based methods, or single nucleotide polymorphism (SNP) microarray methods. Testing for CYP2C19 variants requires a sample of somatic genetic material, usually obtained from a blood sample or from buccal swabs. Because allelic variants at the CYP2C19 locus do not change over a person's lifetime, testing done at any time point is representative of the person's genotype. Several studies have evaluated associations between loss-of-function CYP2C19 alleles and major adverse cardiovascular events as well as intermediate endpoints (e.g., platelet reactivity). Experimental studies of healthy volunteers, as well as studies of patients with cardiovascular disease, have also assessed whether CYP2C19 genotypes can be used to predict the phenotype of on-clopidogrel platelet reactivity and whether the association of genotype and phenotype can be modified by alternative treatment strategies (e.g., higher clopidogrel dosing or use of prasugrel or ticagrelor).[34-36]

Measurement of Platelet Reactivity

Phenotypic testing measures the reactivity of platelets while a patient is taking clopidogrel (on-clopidogrel platelet reactivity). Several assays for measuring platelet reactivity are available. These include rapid (point-of-care) platelet function assays (e.g., VerifyNow, Platelet Function Analyzer [PFA]-100, Plateletworks), measurements of mediators of reactivity (e.g., vasodilator-stimulated phosphoprotein [VASP] phosphorylation using flow cytometry), and functional assays (e.g., turbidimetric, impedance, and conductance aggregometry using appropriate agonists). We refer to all these assays as "phenotypic tests," because they attempt to measure an intermediate clinical phenotype (platelet reactivity).[37] Studies using such assays have demonstrated that platelet response to clopidogrel is variable. These phenotypic tests may be highly useful for investigating the potential influence of genetic variants on platelet reactivity but cannot replace clinical outcomes as measures of the clinical utility of testing. In addition, studies have assessed different assays for measuring platelet reactivity as predictive tests for clinical outcomes among patients treated with clopidogrel and for guiding antiplatelet treatment selection. Reliable phenotypic tests that are easily performed during routine care could potentially replace genotypic testing because platelet reactivity reflects the combined impact of

genotype (not limited to CYP2C19 testing) and environmental exposures on clopidogrel pharmacodynamics.

Modifiers of the Predictive Ability and Clinical Utility of Tests

Proton-pump inhibitors (PPIs) are often prescribed along with antiplatelet therapy to limit the potential for gastrointestinal bleeding complications. Because CYP2C19 is the key enzyme in the metabolism of several PPIs, it has been hypothesized that coadministration of these drugs could inhibit the activation of clopidogrel.[38] A recent systematic review that examined studies investigating the association between PPI use and adverse cardiovascular events among patients receiving clopidogrel concluded that PPI use was associated with an approximately 40 percent increase in the risk of major adverse cardiovascular outcomes and an 18 percent increase in mortality.[27] However, no systematic review has assessed the interaction of PPIs with the clopidogrel treatment effect within categories defined by CYP2C19 status or platelet reactivity.

Other potential modifiers of the utility of genetic and phenotypic test results include the specific indication for clopidogrel use (because the predictive ability of testing may vary among patient populations), race or ethnicity (because of the varying prevalence of CYP2C19 alleles among different groups), comorbid conditions (that may affect the baseline event rate or serve as markers for the coadministration of drugs metabolized by CYP2C19), baseline disease severity, sex, and age.

Current Uncertainties Regarding Genetic and Phenotypic Testing

There are several areas of uncertainty regarding the use of both genetic tests for CYP2C19 variants and phenotypic tests to assess on-clopidogrel platelet reactivity. First, there is some controversy regarding the ability of these tests to predict clinical outcomes in patients who are receiving clopidogrel: several studies have reported significant associations between CYP2C19 status or platelet reactivity and clinical outcomes, however concerns have been raised regarding the potential for selective outcome reporting and publication bias to have affected study results.[26,39] Second, there are conflicting views on whether the results of these tests can be used to guide therapeutic decisionmaking for antiplatelet therapy with some investigators advocating clinical use,[40,41] while others suggesting that the tests are not ready for clinical application.[42] Third, the modifiers of these tests' effects, both in terms of predictive ability and therapeutic decisionmaking, also have not been fully evaluated.

Scope of the Review

The purpose of this review was to systematically evaluate the analytic validity, predictive utility, and comparative effectiveness of two types of biomarker tests (and relevant test-and-treat strategies) for guiding antiplatelet therapy in patient populations who are eligible for clopidogrel treatment. The impact of biomarkers such as genotype or phenotype on patient-relevant outcomes is indirect; any effects of testing are mediated through the impact of test results on clinical thinking and therapeutic decisionmaking. In the case of antiplatelet therapy, the effects of treatment on clinical outcomes are believed to be mediated through platelet reactivity (a surrogate outcome). We aimed to assess the impact of testing on both intermediate and patient-relevant clinical outcomes.

Key Questions

On the basis of the original topic nomination and an extensive process of topic development and refinement, we formulated the following Key Questions to guide the review. These questions broadly follow the "ACCE framework," covering Analytic validity, Clinical validity, Clinical utility, and test-related harms, as proposed by the Evaluation of Genomic Applications in Practice and Prevention Working Group of the Centers for Disease Control and Prevention.

Key Question 1: In patient populations who are candidates for clopidogrel therapy, does genetic testing for CYP2C19 variants predict intermediate and clinical outcomes following treatment initiation?

d) What is the analytic validity (technical test performance) of the various assays used for CYP2C19 genetic testing?
e) What is the clinical validity (predictive accuracy) of genetic testing for predicting intermediate and clinical outcomes in patients who are receiving clopidogrel therapy?
f) Do the following factors modify the association between genetic test results and clinical outcomes?
    v. Comedications
    vi. Patient-level factors (e.g., race or ethnicity, age, sex, disease severity, or comorbidities)
    vii. Test-related factors (e.g., between-assay differences)
    viii. System-level factors (e.g., settings where testing is performed)

Key Question 2: In patient populations receiving clopidogrel therapy, does phenotypic testing of platelet reactivity predict intermediate and clinical outcomes?

d) What is the analytic validity (technical test performance) of the various assays used in phenotypic testing of platelet reactivity?
e) What is the clinical validity (predictive accuracy) of phenotypic testing for predicting intermediate and clinical outcomes in patients who are receiving clopidogrel therapy?
f) Do the following factors modify the association between phenotypic test results and clinical outcomes?
    v. Comedications
    vi. Patient-level factors (e.g., race or ethnicity, age, sex, disease severity, or comorbidities)
    vii. Test-related factors (e.g., between-assay differences)
    viii. System-level factors (e.g., settings where testing is performed)

Key Question 3: What is the comparative effectiveness of alternative test-and-treat strategies (including a no-testing strategy) for therapeutic decisionmaking regarding antiplatelet therapy among patients who are candidates for clopidogrel-based treatment?

c) What is the comparative effectiveness of the following testing strategies on therapeutic decisionmaking, platelet reactivity during followup, and clinical outcomes in patients who are candidates for antiplatelet treatment?
    v. Genetic testing for CYP2C19
    vi. Genetic testing for CYP2C19 followed by phenotypic testing for platelet reactivity
    vii. Phenotypic testing for platelet reactivity

viii. No testing

d) How do modifying factors (e.g., race or ethnicity, age, sex, comorbidities, diet, or the time between conducting the test and obtaining results) affect the association of alternative phenotypic or genetic test-and-treat strategies and patient outcomes? Alternative test-guided treatments can include nonclopidogrel antiplatelet agents or high-dose clopidogrel regimens.

Key Question 4: What are the potential adverse effects or harms from genetic or phenotypic testing *per se* or from test-directed treatments?

Methods

This comparative effectiveness review evaluated the analytic validity, predictive value, and comparative effectiveness of two types of medical tests (genetic testing for CYP2C19 variants and phenotypic testing to measure platelet reactivity) for patients who are candidates for or are already receiving antiplatelet therapy. A primary focus was the evaluation of test-guided therapeutic decisionmaking on patient-relevant clinical outcomes.

We performed a systematic review of the published literature using established methodologies as outlined in the Agency for Healthcare Research and Quality (AHRQ) *Methods Guide for Effectiveness and Comparative Effectiveness Reviews* (hereafter referred to as the Methods Guide; available at http://www.effectivehealthcare.ahrq.gov/methodsguide.cfm). The main sections in this chapter reflect the elements of the protocol established for the comparative effectiveness review; certain methods map to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.[43] All methods and analyses were determined a priori. The protocol was developed with input from external clinical and methodological experts and in consultation with officers from AHRQ; it was posted online to solicit additional public comments.

AHRQ Task Order Officer

The AHRQ Task Order Officer (TOO) assigned to this project was responsible for overseeing all aspects of this report. The TOO facilitated a common understanding among all parties involved in the project, resolved ambiguities, and fielded all queries from the Evidence-based Practice Center (EPC) regarding the scope and processes of the project. The TOO and other staff at AHRQ reviewed the report for consistency, clarity, and to ensure that it conforms to AHRQ standards.

External Stakeholder Input

An initial set of questions for evidence review were nominated to the Effective Healthcare Program by a Federal agency. During a topic refinement phase, the initial questions that had previously been nominated for this report were refined with input from a panel of Key Informants. The Key Informants included a general internist, a cardiologist, a representative from the FDA, a representative from the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group, health care payers (one public and one private), and a patient representative. After a public review of the proposed Key Questions, a group of experts was convened to form the Technical Expert Panel (TEP), which served in an advisory capacity to help refine the Key Questions, identify important issues, and define parameters for the review of evidence. TEP members included a representative of the EGAPP Working Group (who also nominated the topic and participated in Topic Refinement), experts in cardiovascular disease (including an interventional cardiologist), experts on the tests of interest, and a methodologist with expertise in health technology assessment. Discussions among the EPC, TOO, Key Informants, and, subsequently, the TEP occurred during a series of teleconferences and via email. In addition, input from the TEP was sought during compilation of the report when questions arose about the scope of the review.

Key Questions

Four Key Questions were posed. Key Questions 1 and 2 pertains to the analytic validity and predictive validity of the index tests of interest. Key Question 3 pertains to the comparative effectiveness of alternative test-and-treatment strategies (including a no-testing strategy). Finally, Key Question 4 pertains to the harms of test-directed treatment and testing *per se*. The complete Key Questions are presented at the end of the Introduction section.

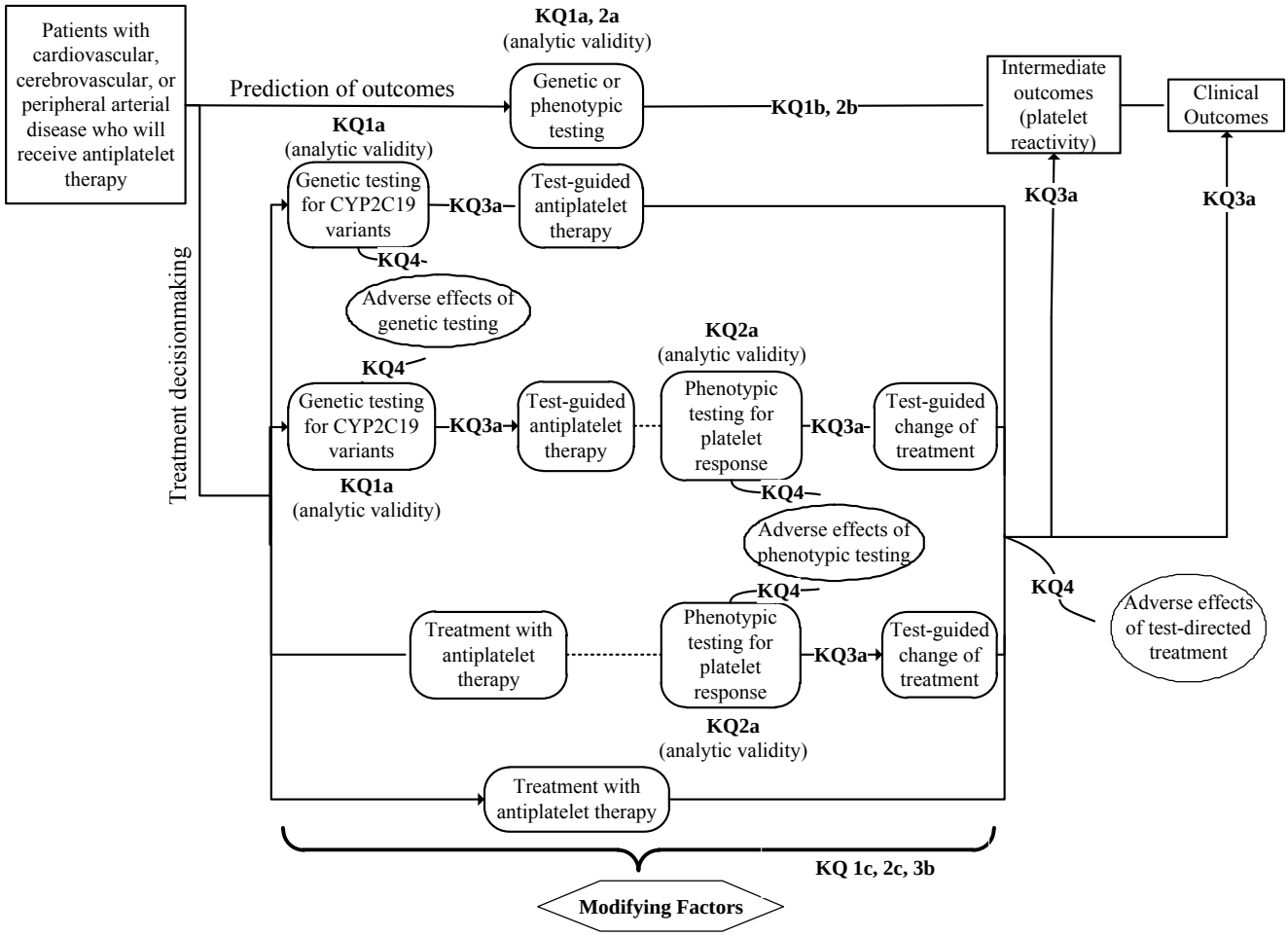
Analytic Framework

We developed an analytic framework (**Figure 1**) that maps the Key Questions within the context of populations, interventions, comparators, and outcomes of interest, as well as the chain of logic that evidence must support to link the interventions to health outcomes. Briefly, the analytic validity (i.e., technical test performance) of the index tests of interest was reviewed in Key Questions 1a and 2a.The predictive ability of the tests was addressed in Key Questions 1b and 2b. We also considered the use of the tests to guide treatment decisionmaking (i.e., use of the tests as components of test-and-treat strategies), and the impact of this decisionmaking on clinical and intermediate outcomes in Key Question 3a. Tests and test-directed treatments may be associated with harms; this was investigated in Key Question 4. Modifiers of the effects of testing on outcomes (both in terms of predictive ability and decisionmaking) were reviewed in Key Questions 1c, 2c, and 3b.

Regarding treatment decisionmaking, we conceptualized the analytic framework as a decision problem, wherein patients' disease can be managed with one of the following approaches (depicted from top to bottom in the flow diagram):

1. Undergo genetic testing and then base the treatment decision on the test results.
2. Undergo genetic testing and then base the treatment decision on the test results. After receiving therapy for an adequate period of time, undergo phenotypic testing for platelet reactivity and use the results to decide whether the treatment strategy should be modified.
3. Receive standard treatment directly and, after an appropriate amount of time, undergo phenotypic testing for platelet reactivity and use the test results to decide whether the treatment strategy should be modified. Use of phenotypic testing (but not genetic testing) as a monitoring test can be considered a variation of this strategy in which the test is repeatedly performed.
4. Receive antiplatelet therapy without undergoing any testing (the current standard of care).

Figure 1: Analytic Framework.



KQ = Key Question.

Literature Search and Abstract Screening

We conducted literature searches for studies in MEDLINE® (from inception to August 24, 2011), the Cochrane Central Trials Registry® (through the 2nd quarter of 2011), and the Cochrane Database of Systematic Reviews (through the 2nd quarter of 2011) without any language restriction. All searches will be updated (through the first quarter of 2012) while the draft report is under peer review and updated results will be presented in the final report. Our search included terms for the populations, tests, and drugs of interest (see **Appendix A** for complete search strings, which were extensively validated against previous reviews on the tests of interest). We also performed searches of the Human Genome Epidemiology Network (HuGENet) database and National Institutes of Health Genetic Association Database, using the same cutoff date (August 24, 2011).

A common set of 200 abstracts was first screened by four investigators and discrepancies were discussed in order to standardize screening practices and ensure understanding of the criteria by all team members. Two hundred additional abstracts were screened by all investigators to ensure that selection criteria were standardized. The remaining citations were

split into nonoverlapping sets, each screened by a single reviewer. Abstracts were manually screened, using *Abstrackr*.[44] Reviewers were specifically instructed to be inclusive in order to increase the sensitivity of abstract screening. Abstracts considered not relevant by a reviewer were rereviewed by a second team member. We retrieved the full text of articles for citations considered potentially relevant by at least one investigator.

We asked technical experts to provide additional citations of potentially relevant articles. Additional studies were identified through the perusal of reference lists of eligible studies, published clinical practice guidelines, and relevant narrative and systematic reviews. We also performed a targeted search of the FDA Web site (with the last search performed on April 25th, 2012). On the basis of preliminary searches conducted during topic refinement, we provided the Scientific Resource Center (SRC, an entity within the Effective Health Care Program independent of the EPC) with a list of relevant technologies and manufacturers. The SRC solicited information from the manufacturers and organized all obtained material into submission information packages (SIPs), which were then forwarded to the EPC for review. All articles identified through sources other than electronic database searches were reviewed for eligibility in full text, using the same criteria as for articles identified through our database searches.

Finally, we searched the ClinicalTrials.gov Web site (with the last search performed on May 3rd, 2012) to identify ongoing comparative trials of test-and-treat strategies for guiding antiplatelet treatment. We did not contact authors and did not consider unpublished data other than that included in FDA documents or ClinicalTrials.gov.

Study Selection and Eligibility Criteria

Full-text articles were reviewed independently by two investigators to determine eligibility. Disagreements regarding inclusion or relevance to a specific question were resolved by consensus including at least one additional senior investigator.

We considered both comparative and noncomparative studies for Key Questions pertaining to predictive ability but focused on comparative studies of alternative test-and-treat strategies. Below we detail the study selection criteria for each Key Question. The criteria are also summarized in **Table 1**.

We did not include non–English language studies but we recorded the number of studies that were excluded on the grounds of language for each Key Question. We excluded narrative reviews, editorials, letters to the editor, and other papers not presenting primary research data. We also excluded studies reporting exclusively on healthy individuals. **Appendix B** lists all the studies excluded after full-text screening and the reason for exclusion.

Populations and Conditions of Interest

For all Key Questions, the populations of interest consisted of adult patients with cardiovascular, cerebrovascular, or peripheral arterial disease who were candidates for or were receiving clopidogrel. Specifically, we included studies enrolling: (1) patients with acute coronary syndromes, including those who had experienced a myocardial infarction (ST-elevation or non–ST-elevation), or patients who had unstable angina; (2) patients who were undergoing PCI for acute coronary syndromes, those who had undergone PCI with stent implantation (of either bare-metal or drug-eluting stents), or those who had undergone CABG (and had a contraindication to acetylsalicylic acid); (3) patients with a previous ischemic stroke or transient ischemic attack; (4) patients with established peripheral arterial disease; and (5) patients with atrial fibrillation for whom vitamin K antagonist therapy was not suitable.

Interventions

For Key Questions 1 and 2 we reviewed studies of genetic testing (for CYP2C19 variants) or phenotypic testing. Genetic variants of interest were all variants of the CYP2C19 locus, including loss- and gain-of-function alleles. Phenotypic tests of interest were those assessing on-clopidogrel platelet reactivity (the degree to which platelets are able to be activated by an agonist). After consultation with the TEP and the TOO, it was decided that tests of platelet activation (where an agonist is not used) would not be reviewed, because they are not in wide clinical use and are less standardized than tests of reactivity.

For Key Questions 3 (comparative effectiveness of test-and-treat strategies) and 4 (harms) we reviewed studies of management strategies involving genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity, followed by therapeutic management decisions based on test results. Potential test-and-treat strategies included testing for CYP2C19 genetic variants, testing for platelet reactivity, or both, to guide the choice among alternative antiplatelet treatment strategies (including standard clopidogrel dosing, increased clopidogrel dosing, and use of non–clopidogrel-based antiplatelet therapies such as ticagrelor or prasugrel).

Comparators

For Key Questions 1 and 2 (predictive effects), a common implicit comparator is not performing any testing; in studies where information was reported on the predictive effect of testing without comparisons against alternative tests, we considered the comparison to be testing versus no testing (implicit). However, we included studies comparing the predictive accuracy of more than one genetic or phenotypic test applied to the same patient population.

For Key Questions 3 (comparative effectiveness of test-and-treat strategies) and 4 (harms), we considered as comparators either a no-testing strategy or alternative test-and-treat strategies.

Outcomes

For Key Questions 1a and 2a (analytic validity) we considered the following outcomes: analytic accuracy (e.g., analytic sensitivity and specificity), analytic precision, test detection limits, dilution linearity, test–retest reliability (e.g., intra-assay agreement, measurement reproducibility), interassay agreement, interlaboratory comparisons (e.g., interlaboratory agreement, measurement reproducibility), and the proportion of nonevaluable samples.

For Key Questions 1b–c and 2b–c we considered both intermediate outcomes (platelet reactivity, when used as a biomarker of outcome status) and clinical outcomes: overall mortality, myocardial infarction (fatal or nonfatal), ischemic stroke (fatal or nonfatal), cardiovascular mortality, stent thrombosis (for patients with implanted stents), combinations of the above (composite clinical outcomes), bleeding events (categorized by severity and by the organ system affected), and health-related and overall quality of life.

For Key Question 3 we considered intermediate outcomes (platelet reactivity, when used as a biomarker of outcome status, and impact on therapeutic decisionmaking [i.e., change in clinical decisions on the basis of test results) and clinical outcomes (see previous paragraph).

Finally, for Key Question 4, we considered adverse effects of test-directed treatment (including bleeding events and others such as gastrointestinal events and liver toxic effects) and adverse effects of testing *per se* (such as test-related anxiety and adverse events secondary to venipuncture).

Study Designs

Key Questions 1 and 2

For Key Questions 1a and 2a, we reviewed studies of analytic validity (single laboratory studies or interlaboratory comparisons) reporting on metrics of intra-assay variability, reliability, analytic sensitivity against a reference standard, or agreement between methods.

For Key Questions 1b–c and 2b–c, we included prospective or retrospective cohort studies using the index tests of interest to predict outcomes. For Key Question 1b we also considered case–control studies. We excluded case–control studies from Key Question 2b because platelet reactivity is modified by coronary events, is variable over time, and is affected by treatments that are bound to be systematically different between cases and controls [i.e., platelet reactivity in the cases is modified by post-event exposures (e.g., treatment instituted after an ischemic event) and may be nonrepresentative of reactivity levels preceding the event of interest]. For analyses of predictive ability, we also included clopidogrel-treated arms of comparative studies (of clopidogrel versus alternative strategies). We included studies assessing a single index test as well as those assessing more than one test (directly comparative studies). For Key Questions pertaining to effect modification (1c, 2c, and 3c) we required that studies reported formal interaction tests or allowed for the calculation of statistics that compare the test effect among strata of the modifier of interest.

Key Questions 3 and 4

For Key Questions 3 and 4 we included randomized and nonrandomized comparative studies of test-and-treat strategies in unselected populations. Because such studies are rare we also considered "repurposed" randomized controlled trials.[b32,33,45] These studies are randomized investigations comparing alternative therapeutic interventions (e.g., clopidogrel-based vs. non–clopidogrel-based management). Biological samples are collected from all patients (all comparison groups) and are analyzed for one or more biomarker of interest. Because for each patient there is information on biomarker status, treatment assignment, and outcomes, the studies can provide information on effect modification (i.e., the presence of a differential treatment effect in biomarker-"positive" vs. biomarker-"negative" patients). This effect modification provides information about whether the biomarker can be used to guide therapeutic decisionmaking. In addition, we included studies where patients were selected on the basis of tests of interest (e.g., platelet reactivity above some threshold) and then randomly assigned to one of at least two alternative treatment strategies. For this study design we only considered randomized comparative studies (parallel arm or cross-over) because they can provide information on the noncompounded effect of treatment conditional on the test result. For Key Question 4 we planned to include noncomparative studies reporting on the harms of testing *per se*, but none of these studies were identified.

Sample Size and Timing

For Key Questions relevant to analytic validity, we used a minimum sample size of 50 patients (or data points, depending on the design and analysis of the primary studies). This

[b] These studies are also referred to as "prospective–retrospective" studies in the oncology literature, which is where the design has been applied mostly (presumably because of the availability of archived tissue samples in randomized trials of cancer therapeutics).[33]

minimum sample size is often recommended for statistical comparisons of analytic validity (e.g., test–retest reliability or Bland–Altman test for agreement between continuous measurements). The precision of estimates of analytic validity depends on the specific statistic used; generally, a sample size of 50 excludes studies that are too small to be informative without being otherwise restrictive. For all other Key Questions (1b–c, 2b–c, 3, and 4), we included studies reporting on at least 10 patients (in total or, when there were ≥2 study groups, per group), because smaller studies produce very imprecise estimates.

We included studies of any duration of followup, including those that did not report this information. We considered short-term and long-term outcomes separately (using a cutoff time of 30 days, wherever appropriate). For patients undergoing invasive or interventional procedures (e.g., PCI or CABG), we also considered periprocedural events separately.

Settings

Studies conducted in all relevant care settings (e.g., primary and secondary care or rural and urban clinics) were included. Study selection was not based on cointerventions.

Data Extraction

A single investigator extracted data from each study; quantitative results were verified by a second reviewer. Disagreements were resolved by consensus involving a third investigator. Data were extracted into standard forms; separate forms were generated for each Key Question. Extraction forms were piloted on three to five articles for each Key Question and revisions were made as needed. We extracted information on the following items: patient selection criteria, population characteristics, sample size, study design, analytic details, and outcomes.

Summary Tables

We created separate summary tables for each index test of interest and each outcome assessed; these are in the Results sections below. Summary tables succinctly report measures of the main comparisons evaluated. We included bibliometric information (first author, year, and MEDLINE unique identification number) and data on study design, location, and sample size; the index tests used; patient characteristics; duration of followup; and items related to risk of bias (see below). In addition, for each outcome of interest, we recorded appropriate estimates of the effect size (e.g., odds ratios for binary outcomes, hazard ratios for time-to-event analyses, mean differences for continuous outcomes) used to compare groups of interest. We also recorded appropriate measures of statistical uncertainty around these estimates (e.g., 95% CIs, standard deviations, or standard errors).

Table 1: Summary of the Selection Criteria Used

Key Question	Population*	Intervention	Comparator	Outcomes	Study Design	Sample Size
KQ1a	Adult patients with vascular disease who were candidates for or were receiving clopidogrel	Genetic testing of CYP2C19 variants	No testing or other predictive tests	Analytic accuracy, analytic precision, test detection limits, dilution linearity, test–retest reliability, interassay agreement; interlaboratory comparison, and proportion of nonevaluable samples for genetic test	Laboratory studies reporting on assay variability, reliability, accuracy, or agreement	≥50 data points
KQ1b	Same as above	Phenotypic testing of on-clopidogrel platelet reactivity	No testing or other predictive tests	Intermediate outcomes (e.g., platelet reactivity as a biomarker of outcome status) Clinical outcomes: overall or cardiovascular mortality, fatal or nonfatal MI or ischemic stroke, stent thrombosis, bleeding, and health-related and overall quality of life	Prospective or retrospective cohort or case–control studies using genetic test to predict outcomes, comparative studies (of clopidogrel vs. alternative treatment), or clopidogrel-based treatment groups from RCTs	≥10 patients
KQ1c	Same as above	Genetic testing of CYP2C19 variants	No testing or other predictive tests	Same as for KQ1b	Same as for KQ1b but in addition, formal interaction tests or statistics comparing the test effect among strata of the modifier of interest must have been reported or calculation permitted	≥10 patients
KQ2a	Same as above	Phenotypic testing of on-clopidogrel platelet reactivity	No testing or other predictive tests	Analytic accuracy, analytic precision, test detection limits, dilution linearity, test–retest reliability, interassay agreement; interlaboratory comparison, and proportion of nonevaluable samples for phenotypic test	Laboratory studies reporting on assay variability, reliability, accuracy, or agreement	≥50 data points
KQ2b	Same as above	Genetic testing of CYP2C19 variants	No testing or other predictive tests	Intermediate outcomes (e.g., platelet reactivity as a biomarker of outcome status) Clinical outcomes: overall or cardiovascular mortality, fatal or nonfatal MI or ischemic stroke, stent thrombosis, bleeding, and health-related and overall quality of life	Prospective or retrospective cohort studies using phenotypic test to predict outcomes, comparative studies (of clopidogrel vs. alternative treatment), orclopidogrel-based treatment groups from RCTs	≥10 patients
KQ2c	Same as above	Phenotypic testing of on-clopidogrel platelet reactivity	No testing or other predictive tests	Same as for KQ2b	Same as for KQ2b but fin addition, normal interaction tests or statistics comparing the test effect among strata of the modifier of interest must have been reported or calculation permitted	≥10 patients
KQ3a	Same as above	Management strategies involving	No-testing strategy or	Intermediate outcomes (e.g., platelet reactivity as a biomarker of outcome status)	Randomized and nonrandomized comparative studies of test-and-	≥10 patients per group

		CYP2C19 testing, followed by treatment decision on basis of test result**	alternative test-and-treat strategy	Impact on therapeutic decisionmaking Clinical outcomes: overall or cardiovascular mortality, fatal or nonfatal MI or ischemic stroke, stent thrombosis, bleeding, and health-related and overall quality of life	treat strategies in unselected populations or "repurposed" RCTs (e.g., clopidogrel-based vs. non–clopidogrel-based management; see text for more information)	
KQ3b	Same as above	Management strategies involving phenotypic testing of on-clopidogrel platelet reactivity, followed by treatment decision on basis of test result**	No-testing strategy or alternative test-and-treat strategy	Same as for KQ 3a	Same as for KQ3a	≥10 patients per group
KQ4	Same as above	Same as for KQ3a and 3b	Same as for KQ3a and 3b	Adverse effects of test-directed treatment (e.g., bleeding, gastrointestinal event, liver toxic effect) and of testing (e.g., test-related anxiety, events secondary to venipuncture)	Same as for KQ3a; also noncomparative studies reporting harms of testing	≥10 patients (per group, for comparative studies)

KQ = Key Question; MI = myocardial infarction; RCT = randomized controlled trial.
*The population of interest was the same for all the Key Questions and included cardiovascular, cerebrovascular, or peripheral arterial disease (acute coronary syndromes; percutaneous coronary intervention or coronary artery bypass grafting with a contraindication to acetylsalicylic acid; previous ischemic stroke or transient ischemic attack; established peripheral arterial disease; and atrial fibrillation with contraindications to vitamin K antagonist treatment).
**Potential test-and-treat strategies included testing for CYP2C19 genetic variants, testing for platelet reactivity, or both, to guide selection among alternative antiplatelet treatment strategies (including standard clopidogrel dosing, increased clopidogrel dosing, and non–clopidogrel-based antiplatelet therapies such as ticagrelor or prasugrel).

Data Synthesis

Qualitative Synthesis

We summarized the findings of the report according to the order of the Key Questions. Within each Key Question, results were organized for each appropriate subgroup on the basis of the populations assessed and clinical indications for clopidogrel use (e.g., acute coronary syndromes, stroke, atrial fibrillation not suitable for vitamin K antagonists, etc.), index tests used, and outcomes assessed. Data are presented in evidence tables and are summarized narratively in the full text. We use tables and graphs (histograms, weighted scatterplots, and others) to synthesize information across studies.

Predefined subgroups of interest were those defined by race or ethnicity, sex, specific assay used, and clinical setting of test use (e.g., short-term administration of clopidogrel during treatment of acute cardiac events or PCI vs. chronic clopidogrel use).

Quantitative Synthesis

Meta-analysis

We performed meta-analysis when at least three studies were available on sufficiently similar populations, using the same test, and assessed the same outcomes. Sufficient similarity was judged on the basis of the clinical heterogeneity of patient populations, interventions, and testing strategies, as well as the methodological heterogeneity of study designs and outcomes reported. The determination on the appropriateness of meta-analysis was made before any statistical analysis was conducted; we did not base the decision to perform meta-analysis on statistical criteria for heterogeneity. Such criteria are often inadequate (e.g., low power when the number of studies is small) and do not account for the ability to explore and explain heterogeneity by examining study-level characteristics. We provide our rationale for performing (or not performing) meta-analysis on a case-by-case basis in the Results section. Of particular note was our decision not to combine studies of different phenotypic tests for platelet reactivity. This decision was based on the different principles of measurement implemented in different assays and our own findings on interassay agreement (presented under Key Question 2a), which indicated that agreement for identifying lack of response may be limited. Similarly, we decided not to combine randomized treatment trials providing information about effect modification because they had enrolled heterogeneous patient populations and compared different pairs of interventions (i.e., the magnitude and direction of effect modification by the tests of interest was likely to vary among different treatment comparisons).

All meta-analyses were performed using random effects inverse-variance models that account for unexplained heterogeneity between studies. Fixed effects models were evaluated in sensitivity analyses. We estimated between-study heterogeneity using the DerSimonian–Laird method.[46] Between-study heterogeneity was assessed on the basis of Cochran's chi-square–based Q statistic.[47] Heterogeneity was considered statistically significant when the P value of the Q statistic (P_Q) was less than 0.1. Between-study inconsistency was assessed using the I^2 index.[48] I^2 values higher than 50% were considered to indicate moderate inconsistency; values above 75%, severe inconsistency. These cutoff values are arbitrary and I^2 estimates are typically associated with substantial uncertainty.[49]

Genetic Model and Measures of Association

In the case of biallelic loci dominant, recessive, additive, or co-dominant genetic models of inheritance are possible. In most cases (including all CYP2C19 variants) the "true" underlying genetic model is not known with certainty. Some experimental evidence indicates that the loss-of-function trait for the CYP2C19 gene has a dominant or additive mode of inheritance. Similar data exist for the gain-of-function (*17) allele. Following previous meta-analyses on the same variants, we used a dominant model for all minor alleles (i.e., a model that assumes carriers of one and carriers of two minor alleles have the same phenotype). In addition to having been used by other investigators, the dominant genetic model allows the inclusion of data from the maximum possible number of studies (because data in relevant publications are often inadequate to perform analyses under an additive model).

Because the majority of studies reporting on clinical outcomes included in this report had longitudinal designs, the most appropriate statistical analyses take into account the time to occurrence of events (e.g., by using survival analysis methods). For this reason, we used hazard or incidence rate ratios in our meta-analyses whenever available or extractable from the reviewed studies. When such statistics were not reported (and could not be calculated), we used risk (proportion) ratios because they approximate the relative incidence rate. For case–control and case-cohort studies we used odds ratios (reported or calculated) because they are valid statistics for these designs and approximate the risk ratio or incidence rate ratio (depending on sampling methods).[50-52] For parsimony, we refer to all these statistics as relative risks (RRs).

Subgroup and Meta-regression Analysis

To assess the impact of study-level characteristics on estimates of the effect size, we performed univariable (one study-level characteristic used as a predictor at a time) random effects meta-regression.[48] We did not perform analyses using averages of patient-level measurements as predictors (e.g., mean age or percent smokers) because these are prone to ecological bias.[53] Subgroup and meta-regression analyses were performed for prespecified factors if reported at the group level (i.e., race or ethnicity, sex, specific assay used, and clinical setting of test use) and for quality items included in our quality assessment. We also explored temporal trends in the reported effect sizes using meta-regression with year of publication as the covariate.

Small-Study Effects

We used Egger's regression-based test[54] to assess the presence of "small-study effects"[55]—that is, differences between larger (more precise) and smaller (less precise) studies. Although this test is often referred to as a test for "publication bias,"[54] theoretical and empirical studies show that the test cannot differentiate publication bias from "true" heterogeneity between smaller and larger studies.[56,57] Furthermore, selective outcome reporting, other biases, or chance can also lead to significant results. Because of these reasons, we interpreted the results of tests for small-study effects with caution.[58]

Sensitivity Analyses

When meta-analyses were conducted, we performed the following sensitivity analyses, as appropriate: leave-one-out meta-analysis, analysis under a fixed effects model, and analysis after excluding the first study of a specific association (to assess "first-study effects"[59,60]).

Population Overlap Across Publications

We took particular care to avoid double counting (both in qualitative and quantitative analyses) when published papers reported on potentially (fully or partially) overlapping patient populations. Potential overlap was assessed on the basis of the sampling population of each study, the enrollment period for each publication, the patient selection criteria, and information on overlap provided by the authors in the published papers. We used a conservative approach of considering as potentially overlapping the populations of studies conducted by the same investigators when overlap could not be ruled out on the basis of the above criteria. In the presence of suspected overlap we based our analysis on the study reporting the largest number of outcome events (typically, the study reporting on the longest followup for longitudinal studies).

Software

All analyses were performed using Stata IC, version 12.1 (Stata Corp., College Station, TX). All tests were two-sided (except those for heterogeneity) and statistical significance was defined as a P value of less than 0.05. We did not perform any adjustments for multiple comparisons.

Risk of Bias and Completeness of Reporting of Individual Studies

For assessing the risk of bias, we followed recently updated guidance from the Methods Guide.[61,62] We used different criteria for assessing the risk of bias (and when appropriate, the completeness of reporting) for each Key Question.

For studies of analytic validity, no established approach exists for assessing risk of bias or reporting quality. On the basis of a recent AHRQ Methods Report,[63] in which expert input and literature review methods were used to develop a preliminary list of quality and reporting criteria for studies of analytic validity,[a] we compiled a list of 11 items for assessing quality and reporting. Because not all criteria were relevant to the index tests of interest to this report, we constructed a modified list of 11 criteria, which are provided in **Appendix Table D2** (for Key Question 1a) and **Appendix Table E4** (for Key Question 2a). Each item was rated as "Yes," "No," "Unclear/Not reported," or "Not applicable".

For studies of predictive ability (Key Questions 1b–c and 2b–c), we based our assessment on the recently proposed Quality Assessment of Diagnostic Accuracy Studies (QUADAS)–2 instrument,[64] a new version of the validated QUADAS list of quality items[65-67] for systematic reviews of medical tests. Briefly, the tool assesses four domains for risk of bias: patient selection, index test, reference standard test (outcome), and flow and timing. Each domain is evaluated according to a set of relevant "signaling" questions answered as "Yes," "No," or "Unclear/Not reported. The complete tool and the operational definitions we used for each item are presented in **Appendix Table D16** (for Key Question 1b) and **Appendix E** (for Key Question 2b). After scoring each item, a summary risk-of-bias assessment is performed for each of the four domains and then an overall determination (across the four domains) is made. We used arbitrary thresholds based on the number of items scored as having been adequately addressed (i.e., indicative of low risk of bias) to classify studies into three categories (A, B, or C) indicating low, moderate, and high risk of bias, respectively. This approach was used as a shorthand description of the available evidence; throughout the report we emphasize the component items that contributed to the summary rate.

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[a] The final proposed list of items incorporated assessment methods for analytic validity proposed by the EGAPP Working Group.

Finally, for studies providing information on test-and-treatment strategies (Key Questions 3 and 4) we used a combination of items from the QUADAS-2 tool and the Cochrane risk of bias tool.[68] The complete list of items assessed is provided in **Appendix Tables F1-4**. Each item was labeled as "Yes," "No," or "Unclear/Not reported." Two independent reviewers evaluated the risk of bias for each study and disagreements were resolved by consensus including a third reviewer.

Grading the Body of Evidence

We graded the strength of the body of evidence for the Key Questions following the Methods Guide and recently updated recommendations for the EPC program.[69,70] Briefly, the grading of the strength of evidence was based on four dimensions: risk of bias, consistency, directness, and precision. The risk of bias assessment of individual studies was performed as described in the preceding section.

We assessed consistency of the data as either "no inconsistency" or "inconsistency present" (or "not applicable" if only one study). We did not use rigid counts of studies as standards of evaluation (e.g., four of five studies agree, therefore the data are consistent); instead, we assessed the direction, magnitude, and statistical significance of all studies, and our meta-analysis results (when performed), to reach conclusions. We describe our logic when studies were not unanimous. We assessed the directness of the evidence ("direct" vs. "indirect") on the basis of the use of surrogate outcomes (e.g., platelet reactivity vs. clinical events as the outcomes of interest) or the need for indirect comparisons (e.g., when tests had not been directly compared in terms of predictive ability or utility for treatment decisionmaking and inference was based on observations across studies). We assessed the precision of the evidence as "precise" or "imprecise" on the basis of the degree of certainty surrounding each effect estimate. A precise estimate is one that allows for a clinically useful conclusion. An imprecise estimate is one for which the CI is wide enough to include clinically distinct conclusions (e.g., both clinically important superiority and inferiority—a situation in which the direction of effect is unknown) and that therefore precludes a conclusion. As a component of precision, the sparseness of the evidence was also assessed. We considered evidence to be sparse if it was from only one study with a small sample size. Because this review assessed many outcomes, the evaluation of overall strength of evidence was based on patient-relevant clinical outcomes, which we broadly defined as any outcomes that affect the patient's well-being.

We focused our assessment of the strength of evidence on studies reporting patient-relevant clinical outcomes (for Key Questions 1b–c, 2b–c, 3, and 4); we did not assess the strength of evidence for intermediate outcomes because they were ascertained using diverse methods (platelet reactivity measured with different assays) and their clinical importance is unclear at present. We also did not assess the strength of evidence of studies of analytic validity (Key Questions 1a and 2a) because technical test performance does not directly inform medical decisions (even though it is a prerequisite for the clinical use of tests).

We rated the strength of evidence for each comparison and outcome of interest according to four levels: high, moderate, low, and insufficient. Ratings reflect the investigators' level of confidence that the evidence reflects the true effect for the major comparisons of interest, and are defined as follows:

- *High:* There is high confidence that the evidence reflects the true effect. Further research is very unlikely to change our confidence in the estimate of effect. No important scientific disagreement exists across studies.

- *Moderate:* There is moderate confidence that the evidence reflects the true effect. Further research may change our confidence in the estimate of effect and may change the estimate. Little disagreement exists across studies.
- *Low:* There is low confidence that the evidence reflects the true effect. Further research is likely to change the confidence in the estimate of effect and is likely to change the estimate. Underlying studies may report conflicting results.
- *Insufficient:* Evidence is either unavailable or does not permit a conclusion. There are sparse or no data. (In general, the evidence is considered insufficient when only one study has been published, unless the study was particularly large, robust, and of good quality.)

These ratings provide a shorthand description of the strength of evidence supporting the major questions we addressed. However, they by necessity may oversimplify the many complex issues involved in the appraisal of a body of evidence. It is important to remember that the individual studies evaluated in formulating the composite rating differed in their design, reporting, and quality. The strengths and weaknesses of the individual reports, as described in detail in the text and tables, should also be taken into consideration.

Assessing Applicability

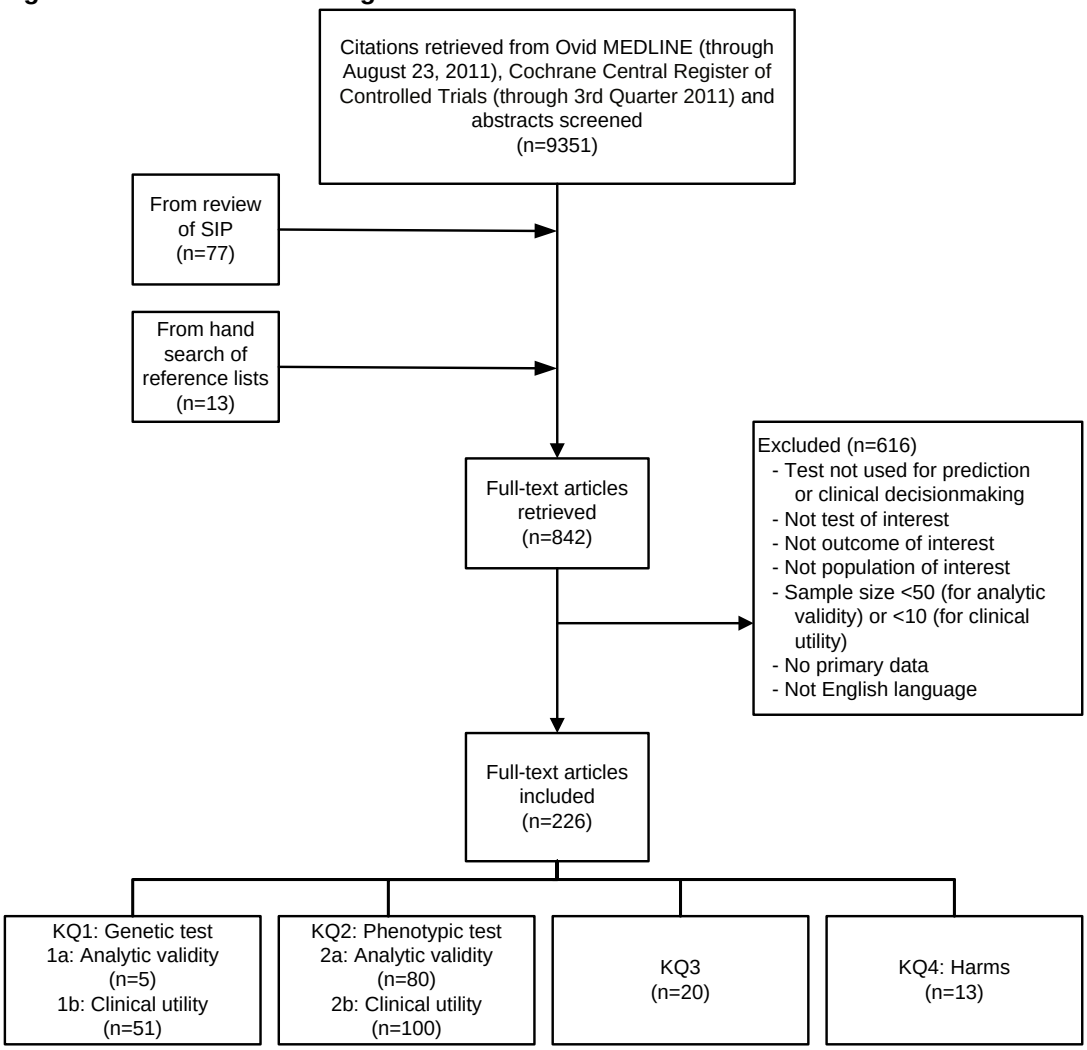
We assessed applicability of the study findings on the basis of the individual study eligibility criteria and baseline characteristics of the included populations, following recommendations in the AHRQ Methods Guide and recently updated recommendations for the EPC program.[70,71] We did not assess the applicability of studies on the analytic validity of the tests of interest (Key Questions 1a and 2a) because technical test performance does not directly inform medical decisions (it is, however, a prerequisite for the clinical use of tests).[72]

For studies of predictive ability (Key Questions 1b–c and 2b–c), we judged applicability on the basis of study eligibility criteria (e.g., narrow vs. broad range of demographics [men only vs. men and women]), the use of tests that are not widely available (tests developed "in-house" ["home-brew" assays]), and the assessment of outcomes that required laboratory measurement of platelet reactivity (rather than patient-relevant outcomes). We took a similar approach for studies considered relevant to test-and-treatment strategies (Key Questions 3 and 4). We address applicability along with other key issues relevant to the body of evidence pertaining to each Key Question and also provide comments on specific issues that, according to our best judgment, affected applicability.

Results

The literature search yielded 9441 citations (9351 from electronic databases; 77 from submission information packages; and 13 from hand-searching; **Figure 2**). Of these, 842 articles were reviewed in full text. After full text review, 226 were judged to have met the inclusion criteria for one or more of the four Key Questions. The most common reason for exclusion of articles was that the results of the genetic or phenotypic tests studied were not used for prediction of intermediate or clinical outcomes or physician/patient decisionmaking. Other common reasons for exclusion were that the tests, populations, or outcomes studied were not of interest (i.e., were not those specified in the eligibility criteria given in the Methods section); that the sample size was too small to yield useful estimates (n<50 for Key Question 1a or 2a about analytic validity or n<10 for Key Question 1b, 2b, or 3 about clinical utility or decisionmaking); that no primary data were reported; or that the full text was written in a language other than English. See **Appendix B** for a list of the excluded studies with the reason for exclusion. **Appendix C** contains a list of FDA documents that we reviewed. The summary tables for the 226 accepted studies are in **Appendices D** through **F**.

Figure 2: Literature Flow Diagram



KQ = Key Question; SIP = submission information package.

Key Question 1a: What Is The Analytic Validity (Technical Test Performance) of the Various Assays Used for CYP2C19 Genetic Testing?

Eligible Studies for Key Question 1a

We identified five studies[25,73-76] reporting information on the analytic validity of genotyping methods for detecting CYP2C19 variants. Information on the patient populations, assays evaluated, test timing, and study designs used is presented in **Appendix Table D1**. Studies reported limited information regarding the methods used to assess technical test performance, possibly reflecting their focus on clinical (rather than analytic) validity. Three of the studies were conducted by the same team of investigators and reported laboratory and clinical findings on patients identified at a single center in Germany (typically, these were participants in ongoing clinical trials); thus, at least partial overlap is likely. We also reviewed four FDA 510(k) summaries on genetic testing assays (see **Appendix C**).

All five studies included patients undergoing PCI for coronary artery disease and all assessed test–retest reliability (repeat testing of samples for the detection of CYP2C19 polymorphisms); no other aspects of analytic validity were evaluated. In three of the five studies, the proportion of retested samples was reported (20% in all three cases), corresponding to 305 samples (in each of two studies) and 497 samples (in the third study). In the remaining two studies, the proportion of retested samples was not reported (and could not be calculated).

Summary of Findings on Analytic Validity

The five clinical studies providing information on analytic validity for CYP2C19 genotyping presented results on test–retest reliability. Four of the five studies reported that the concordance rate on repeat genotyping was 100 percent. The fifth study reported that the rate was "higher than 98%." Two of the studies genotyped more than one variant; however, it was unclear whether agreement was assessed for all variants, and results were not reported for each variant separately.

FDA 510(k) summaries did not report analyses on samples from populations and gene of interest in our review. However, the documents provided evidence that genotyping methods have high test–retest reliability and indicated that rates of interassay agreement were high (based on fairly extensive studies of test-retest reliability and inter-assay agreement).

Risk of Bias and Completeness of Reporting

Studies reported limited information on the methods they used to assess analytic validity. This probably reflects the fact that the primary focus of all included publications was not the tests' analytic validity (but rather their clinical utility). Generally, studies provided adequate information on the genotyping methods used. However, they provided little information on the use of positive or negative control samples, the handling of uninterpretable results, and the test detection limits. No study reported information on the reproducibility of genotyping across operators or different instruments and none were conducted as part of interlaboratory standardization projects.

Appendix Figure D1 summarizes our assessment of study quality and reporting for studies of genetic testing for CYP2C19 variants. The assessment was based on 11 questions relevant to assay performance and reporting thereof. Additional information is provided in **Appendix Table D2**.

Key Question 1b: What is the Clinical Validity (Predictive Accuracy) of Genetic Testing for Predicting Intermediate and Clinical Outcomes in Patients Who Are Receiving Clopidogrel Therapy?

Eligible Studies for Key Question 1b

The studies included for this Key Question enrolled patients on clopidogrel-based antiplatelet therapy. Of the 56 studies addressing Key Question 1b (in 51 publications; 5 publications described 2 studies each), the vast majority (54 [96 percent]) were of patients with ischemic heart disease; one study enrolled patients with atrial fibrillation who were not candidates for vitamin K antagonist therapy; and one enrolled patients with cerebrovascular disease. In 43 studies (77 percent), 80 percent or more patients were undergoing PCI; 3 studies (5 percent) included patients undergoing angiography; and 10 (18 percent) included populations in which less than 80 percent of patients were undergoing a procedure or did not provide information on the proportion of patients undergoing revascularization. The majority of studies were of patients receiving dual antiplatelet therapy with clopidogrel in combination with aspirin. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix Tables D3** and **D4**.

The 56 studies had intermediate to large sample sizes (median number of enrolled individuals=281; 25th percentile=98; 75th percentile=1005; minimum=30; maximum=5148). They were conducted recently (median year of start of enrollment was 2006, with 75 percent beginning enrollment after 2004), reflecting the relatively recent widespread availability of genetic testing for CYP2C19 variants. Across studies, men represented the majority of enrolled patients (median proportion across studies was 76 percent; men were the majority sex in 50 of the 51 studies noting the sex of participants). In 52 studies providing information on patient age, the median of the mean or median age reported in each study was 64.7 years; mean or median age was equal to or older than 65 years in 24 of the studies. Patients had a relatively high burden of risk factors for coronary artery disease (which are associated with adverse cardiovascular outcomes): the median proportion of patients with dyslipidemia was 54 percent (in the 38 studies with data; 25th percentile=43 percent; 75th percentile=72 percent); hypertension, 67.9 percent (in 43 studies; 25th percentile=57 percent; 75th percentile=79 percent); diabetes mellitus, 26 percent (in 51 studies; 25th percentile=20 percent; 75th percentile=31 percent); and tobacco use, 28 percent (in 47 studies; 25th percentile=12 percent; 75th percentile=40 percent). Detailed information about patient characteristics and preexisting vascular disease is summarized in **Appendix Table D3**.

A total of 53 of the 56 studies (95 percent) had a longitudinal (cohort) design; 2 were case–control studies; and 1 was a case-cohort study. Of the longitudinal studies, 14 were genetic substudies consisting of prospectively followed clopidogrel-treated groups from randomized trials.[b]

Overall studies had moderate risk of bias (4 studies were rated as quality "A"; 49 studies were rated as quality "B"; and 3 were rated as quality "C"). We caution that this aggregate risk-of-bias rating can be misleading, especially in the presence of poor reporting. A more detailed discussion of risk of bias, focusing on the individual items assessed is presented later in this chapter.

[b] When appropriate, possible modification of the relative treatment effect by genotype status has been considered under Key Questions 3 and 4 of this report.

Genotyping Methods and Hardy–Weinberg Equilibrium

The 56 studies addressing Key Question 1b used a variety of genotyping methods for identifying CYP2C19 variants. The most common were TaqMan genotyping (21 studies; 38 percent), PCR-RFLP (7 studies; 13 percent), sequencing (4 studies; 7 percent), and real-time PCR (4 studies; 7 percent). In the majority of cases analyses were conducted on genetic material isolated from blood (49 studies; 88 percent). Among the 36 studies that reported the genotyping success rate, the median was 100 percent (minimum=82 percent; maximum=100 percent). All but one study genotyped samples for the CYP2C19 *2 allele; additional alleles were genotyped in a minority of studies and were rare in the sampled population. Detailed information on the genotyping methods used and the distribution of observed genotypes for each study are presented in **Appendix Table D5**.

Forty-four of the eligible studies provided information adequate to calculate goodness-of-fit P values for Hardy–Weinberg equilibrium (using an exact test). **Table 2** shows summary results across studies, and **Figure 3** shows a quantile–quantile plot of P values for deviations from Hardy–Weinberg equilibrium against the uniform distribution, for the *2 allele.

Table 2: P Values for Deviation from Hardy–Weinberg Equilibrium

CYP2C19 Allele	No. of Studies Reporting Genotype Distributions	Median P Value [25th, 75th percentile]	No. of Studies with P<0.05 (%)
*2	43	0.581 [0.263, 0.852]	3 (7%)
*3	17	1.00 [0.536, 1.000]	0 (0%)
*17	15	0.558 [0.088, 0.798]	1 (7%)
Other	11	1.000 [1.000,1.000]	0 (0%)
Total	86	0.742 [0.326, 1.000]	4 (5%)

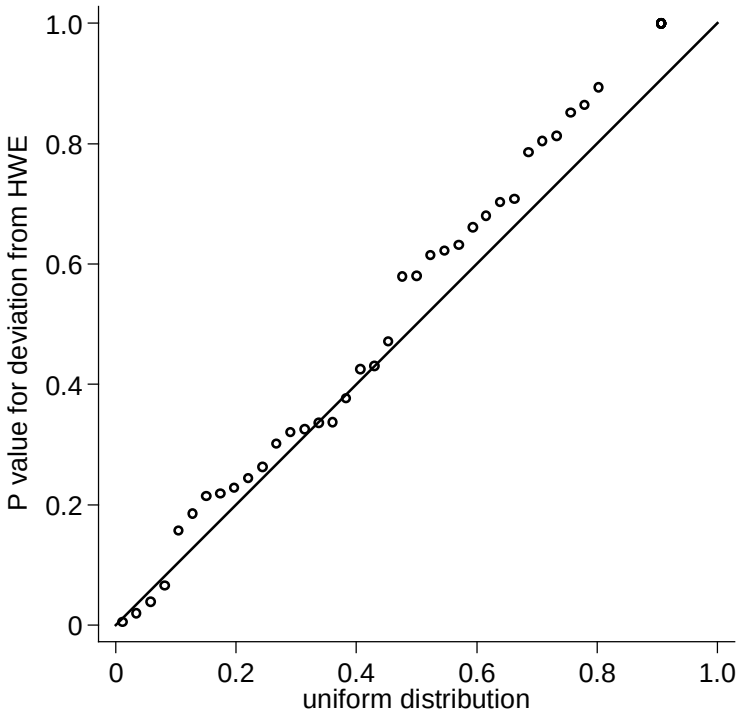
Studies not reporting the complete genotype distribution (n=12) have not been included in the table. The 44 studies included could have reported genotype distributions for more than one allele. For the two case–control studies and the one case-cohort study, we assessed violations for Hardy–Weinberg equilibrium (HWE) for the control group only. Variants other than *2, *3, and *17 were fairly uncommon, leading to P values for HWE violation equal to 1 in many cases.

Overall, statistically significant deviations were uncommon (n=4 across all genotyped alleles), and at the 0.05 alpha level, four or five significant results would be expected by chance alone. In addition, these results need to be interpreted with caution given that patients were selected for inclusion in the studies on the basis of the presence of ischemic heart disease (or other atherosclerotic vascular disease).

Of the 56 eligible studies, 28 provided information on the predictive value of genetic testing for CYP2C19 variants for predicting clinical outcomes, and 40 reported data appropriate for predicting on-clopidogrel platelet reactivity. In the following section we discuss studies according to the outcomes reported for each population of interest. Because the study designs and outcome definitions used across studies of ischemic coronary artery disease were considered sufficiently similar, we performed meta-analyses if at least three studies provided information for an outcome of interest. We synthesized findings across studies, on the assumption that their patient populations were nonoverlapping.[c] Subgroup and effect modification analyses for the same set of studies are presented in the next section (results for Key Question 1c).

[c] Please see the Methods section for additional details on how we assessed potential population overlap.

Figure 3: Quantile–Quantile Plot (Observed vs. Uniform Distribution) of P Values for Deviation From Hardy–Weinberg Violation in Studies Reporting the Genotype Distribution for the CYP2C19*2 Allele



HWE=Hardy–Weinberg equilibrium. P values are from an exact test.

Summary of Findings on the Predictive Value of CYP2C19 Variants for Patients on Clopidogrel-Based Antiplatelet Therapy

Table 3 summarizes our findings on the predictive value of CYP2C19 variants for clinical outcomes across studies reporting on nonoverlapping of clopidogrel-treated patient populations included in meta-analyses or synthesized quantitatively in patient populations with ischemic heart disease. (We performed meta-analyses when at least three studies reported on the comparison of interest and the occurrence of at least one outcome event). Only one study pertained to patients with atrial fibrillation, and one to patients with cerebrovascular disease undergoing neurointervention. Results from these studies are summarized separately below. As we discuss in subsequent sections, results regarding platelet reactivity (when used as an outcome) were not amenable to quantitative synthesis.

Table 3: Summary of Results on the Predictive Value of CYP2C19 Variants for Clinical Outcomes among Clopidogrel-Treated Patients with Ischemic Heart Disease

Exposure	Outcome	No. of Studies with Nonoverlapping Populations	Summary of Findings	P Value for Small-Study Effects
LOF vs. no LOF	All-cause mortality	5	RR=0.77 (95% CI 0.45, 1.29) P_Q=0.419; I^2=0%	0.057
	Cardiovascular mortality	4	RR=2.40 (95% CI 1.05, 5.53) P_Q=0.859; I^2=0%	0.644
	ACS	7	RR=1.22 (95% CI 0.80, 1.86) P_Q=0.764; I^2=0%	0.405
	Stent thrombosis	13	RR=1.56 (95% CI 1.15, 2.11) P_Q=0.667; I^2=0%	0.002
	Stroke	5	RR=1.92 (95% CI 0.34, 11.05) P_Q=0.171; I^2=38%	0.795
	MACE	15	RR=1.09 (95% CI 0.93, 1.29) P_Q=0.105; I^2=33%	0.024
	Bleeding events	4	RR=1.02 (95% CI 0.86, 1.21) P_Q=0.414; I^2=0%	0.007
	Other clinical outcomes	4 studies (5 outcomes)	Studies suggested that revascularization outcomes and "net clinical" benefit are more common among carriers of loss-of-function alleles.	NA
GOF vs. no GOF	All-cause mortality	3	RR=1.28 (95% CI 0.81, 2.02) P_Q=0.505; I^2=0%	0.351
	Cardiovascular mortality	0	NA	
	ACS	2	Both studies reported non–statistically significant results. Confidence intervals were wide; the two point estimates for the RR differed by >0.3.	NA
	Stent thrombosis	4	RR=0.92 (95% CI 0.55, 1.55) P_Q=0.795; I^2=0%	0.462
	Stroke	1	The study did not find a statistically significant association.	NA
	MACE	4	RR=0.80 (95% CI 0.70, 0.90) P_Q=0.992; I^2=0%	0.212
	Bleeding events	3	RR=1.46 (95% CI 0.99, 2.15) P_Q=0.049; I^2=62%	0.189
	Revascularization	0	NA	NA

ACS = acute coronary syndromes; CI = confidence interval; GOF = gain-of-function alleles; LOF = loss-of-function alleles; MACE = major adverse cardiovascular events; NA = not applicable; RR = relative risk.
Significant P values are in bold type.

Studies of Patients with Ischemic Heart Disease

Clinical Outcomes

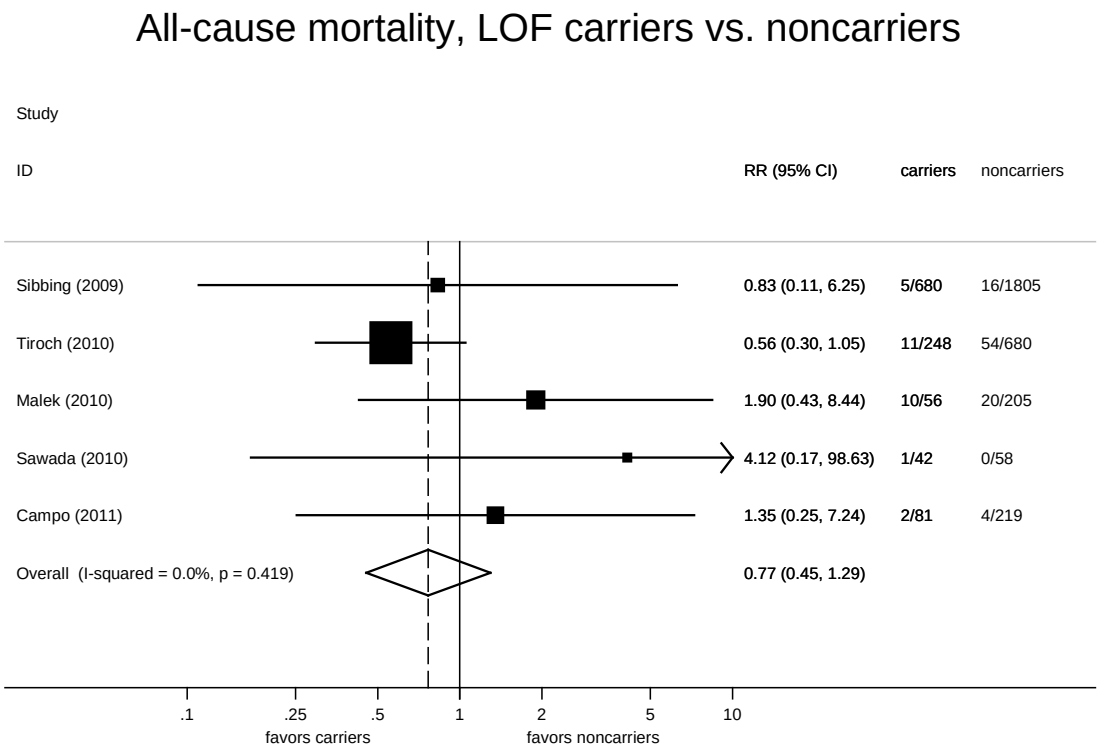
Twenty-seven of the 56 studies eligible for Key Question 1b reported information on the predictive value of CYP2C19 genotyping for predicting clinical outcomes in patients with ischemic heart disease. Eligible studies were fairly large (median number of genotyped individuals=684) and recent (median year enrollment started=2005).

All-Cause Mortality

Five studies with nonoverlapping populations of patients with ischemic heart disease reported information on the predictive value of loss-of-function alleles for all-cause mortality (and reported the occurrence of at least one outcome event).[73,77-80] The studies were large (minimum sample size=100) but represented a minority of all 27 studies reporting clinical

outcomes. The summary RR for all-cause mortality comparing carriers of any loss-of-function allele with noncarriers was 0.77 (95 percent CI 0.45, 1.29; P=0.321). Overall, there was little between-study heterogeneity (P_Q=0.419; I^2=0 percent). **Figure 4** presents the meta-analysis results, along with study-specific event rates.

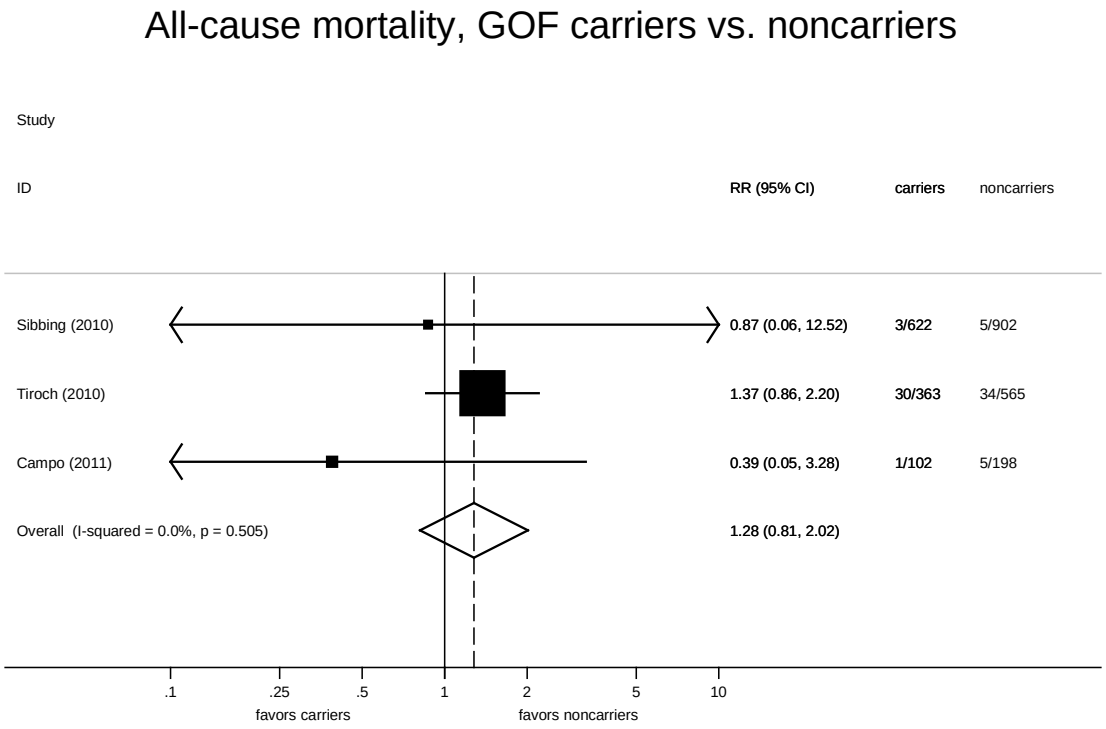
Figure 4: Meta-analysis of All-Cause Mortality Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles



CI = confidence interval; LOF = loss-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of all-cause mortality (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

The summary RR for all-cause mortality comparing carriers of any gain-of-function allele versus noncarriers (based on only three studies reporting relevant data[74,77,80]) was 1.28 (95 percent CI 0.81, 2.02; P=0.288). Overall, there was little between-study heterogeneity (P_Q=0.505; I^2=0 percent). **Figure 5** presents the meta-analysis results, along with study-specific event rates.

Figure 5: Meta-analysis of All-Cause Mortality Comparing Carriers and Noncarriers of Gain-of-Function CYP2C19 Alleles



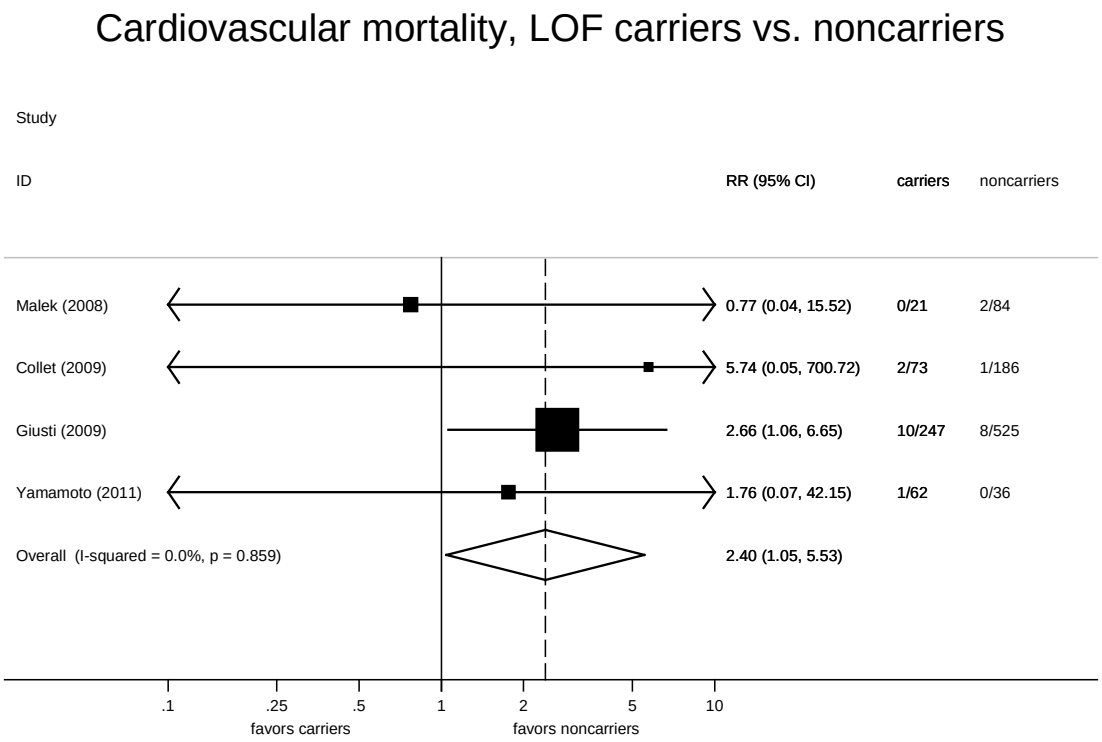
CI = confidence interval; GOF = gain-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of all-cause mortality (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Cardiovascular Mortality

Information on the predictive value of genetic testing for mortality due to cardiovascular causes was reported in four studies with nonoverlapping populations of patients with ischemic heart disease (in which at least one event was observed).[81-84] Studies were large (minimum sample size=105) but represented a minority of the 28 studies reporting clinical outcomes. The summary RR for cardiovascular mortality comparing carriers of any loss-of-function allele and noncarriers was 2.40 (95 percent CI 1.05, 5.53; P=0.039). Overall, there was little between-study heterogeneity (P_Q=0.859; I^2=0 percent). **Figure 6** presents the meta-analysis results, along with study-specific event rates.

No studies provided information on the impact of gain-of-function alleles on risk of cardiovascular mortality.

Figure 6: Meta-analysis of Cardiovascular Mortality Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles

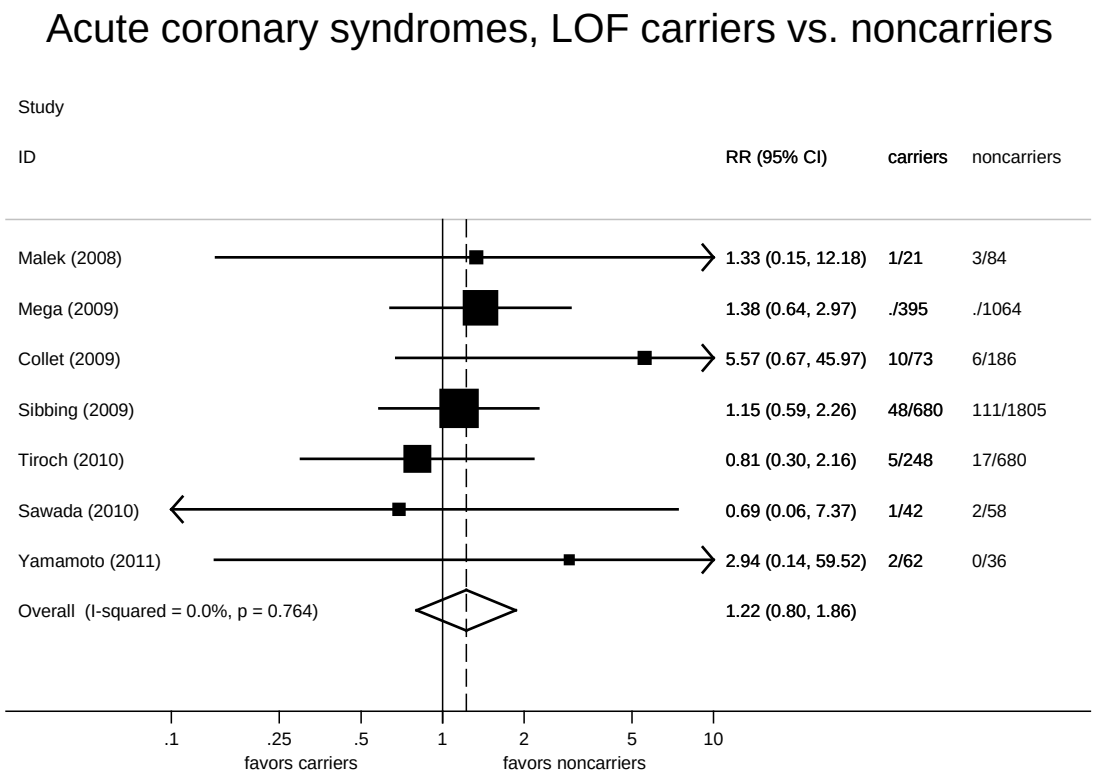


CI = confidence interval; LOF = loss-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of cardiovascular mortality (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Acute Coronary Syndromes

Information on the predictive value of genetic testing for acute coronary syndromes (including myocardial infarction) was reported in seven studies with nonoverlapping populations of patients with ischemic heart disease.[73,79-81,83-85] Studies were large (minimum sample size=100) but represented a minority of the 27 studies reporting clinical outcomes in patients with ischemic heart disease. The summary RR for acute coronary syndromes comparing carriers of any loss-of-function allele versus noncarriers was 1.22 (95 percent CI 0.80, 1.86; P=0.350). Overall, there was little between-study heterogeneity (P_Q=0.764; I^2=0 percent). **Figure 7** presents the meta-analysis results, along with study-specific event rates.

Figure 7: Meta-analysis of Acute Coronary Syndromes Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles



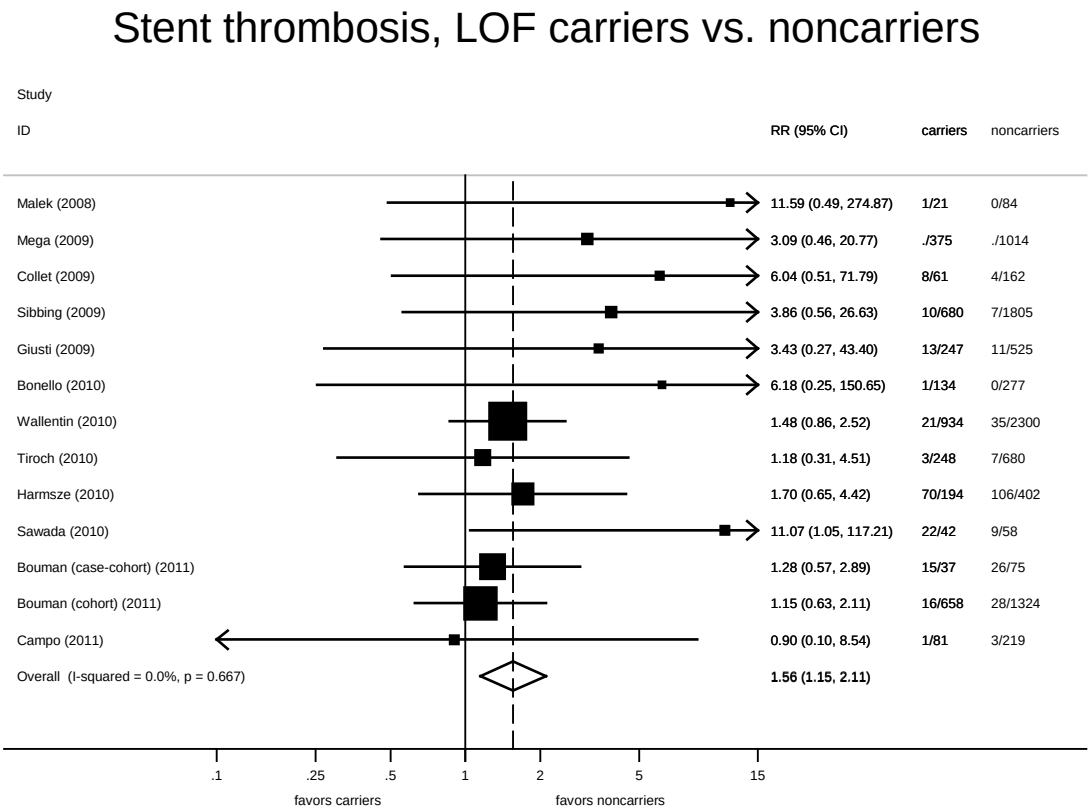
CI = confidence interval; LOF = loss-of-function; RR = relative risk.
One study (Mega et al. 2009[85]) did not report the number of observed events.
The solid squares (and horizontal lines) indicate the RR of acute coronary syndromes (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Only two studies[73,80] reported information on the impact of gain-of-function alleles on the risk of acute coronary syndromes. Both studies did not find a statistically significant association between carriage of gain-of-function alleles and acute coronary syndromes, but the CIs were wide (RR=1.05 [95 percent CI 0.34, 3.29] and RR=0.73 [95 percent CI 0.30, 1.76]), indicating substantial uncertainty in the estimates.

Stent Thrombosis

Information on the predictive value of loss-of-function alleles for stent thrombosis (definite or probable, based on study-specific definitions when available) was reported in 13 studies with nonoverlapping populations of patients with ischemic heart disease (in which at least one outcome event was observed).[73,77,79-83,85-90] Studies were large (minimum sample size=100; median sample size=596). The summary RR for stent thrombosis comparing carriers of any loss-of-function allele versus noncarriers was 1.56 (95 percent CI 1.15, 2.11; P=0.004). Overall, there was little between-study heterogeneity (P_Q=0.667; I^2=0 percent). **Figure 8** presents the meta-analysis results, along with study-specific event rates.

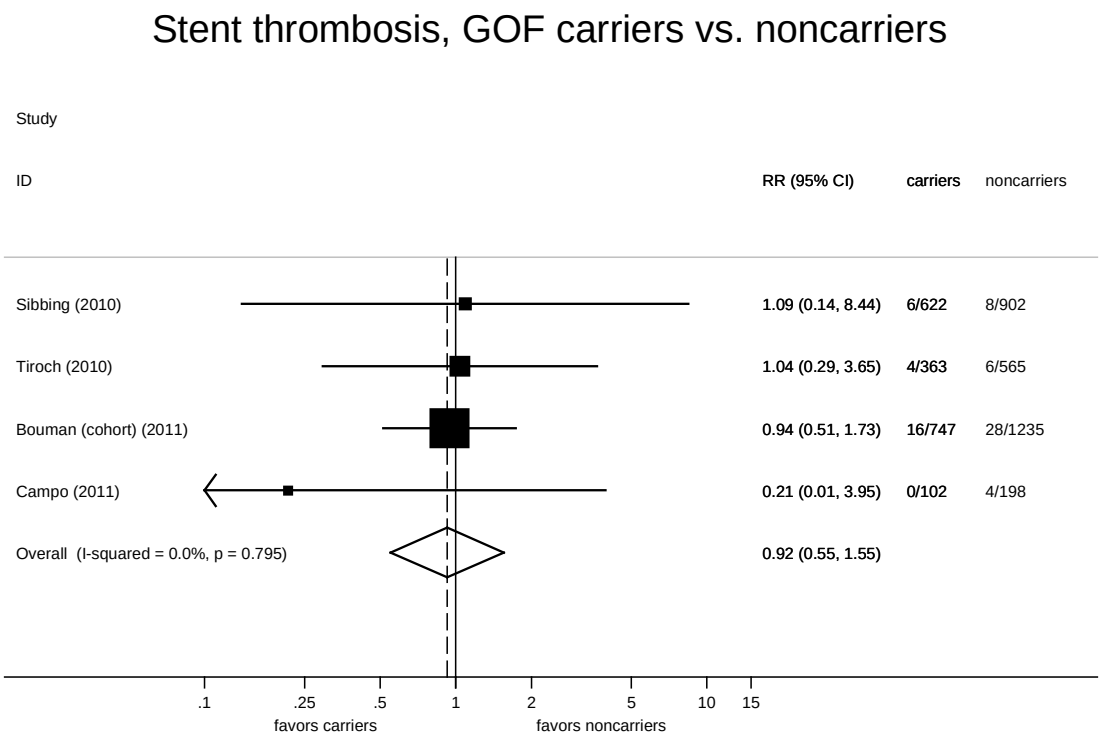
Figure 8: Meta-analysis of Stent Thrombosis Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles



CI = confidence interval; LOF = loss-of-function; RR = relative risk.
One study (Mega et al. 2009[85]) did not report the number of observed events.
The solid squares (and horizontal lines) indicate the RR of stent thrombosis (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Information on the predictive value of gain-of-function alleles for stent thrombosis (definite or probable) was reported in four studies with nonoverlapping populations of patients with ischemic heart disease.[73,77,79-81,87] Studies were large (minimum sample size=300); however, they represented a minority of the 13 studies reporting stent thrombosis. The summary RR for stent thrombosis comparing carriers of any gain-of-function allele versus noncarriers was 0.92 (95 percent CI 0.55, 1.55); P=0.766. Overall, there was little between-study heterogeneity (P_Q=0.795; I^2=0.0 percent). **Figure 9** presents the meta-analysis results, along with study-specific event rates.

Figure 9: Meta-analysis of Stent Thrombosis Comparing Carriers and Noncarriers of Gain-of-Function CYP2C19 Alleles

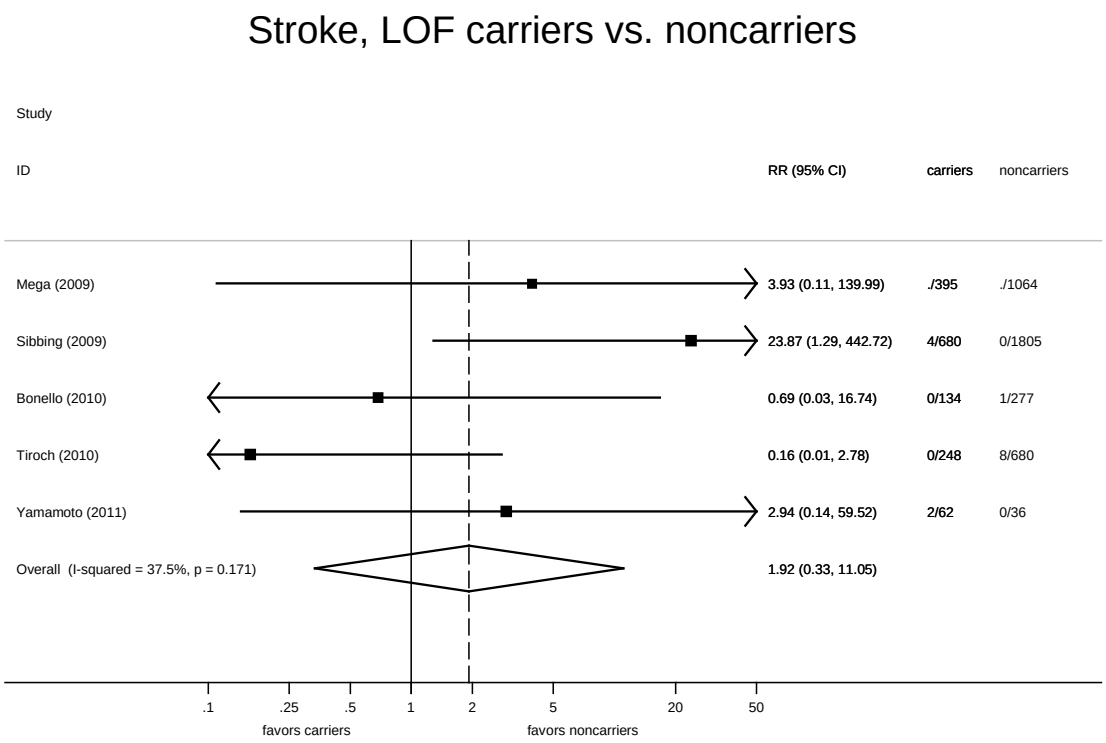


CI = confidence interval; GOF = gain-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of stent thrombosis (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Stroke

Information on the predictive value of loss-of-function alleles for strokewas reported in four studies with nonoverlapping populations of patients with ischemic heart disease.[73,80,84-86] Studies had heterogeneous sample sizes (minimum sample size=201; maximum=2485) and represented a minority of the 13 studies reporting clinical outcomes. The summary RR for stent thrombosis comparing carriers of any loss-of-function allele versus noncarriers was 1.92 (95 percent CI 0.34, 11.05); P=0.464. Overall, there was some evidence of moderate between-study heterogeneity (P_Q=0.171; I^2=37.5 percent). **Figure 10** presents the meta-analysis results, along with study-specific event rates.

Figure 10: Meta-analysis of Stroke Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles



CI = confidence interval; LOF = loss-of-function; RR = relative risk.
One study (Mega et al. 2009[85]) did not report the number of observed events.
The solid squares (and horizontal lines) indicate the RR of stroke (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Only a single study[80] reported information on the association between gain-of-function alleles (*17) and stroke risk. The study reported a non-statistically significant RR of 0.93 (95% CI 0.23, 3.88), suggesting that there is substantial uncertainty regarding the effect of gain-of-function alleles on stroke risk.

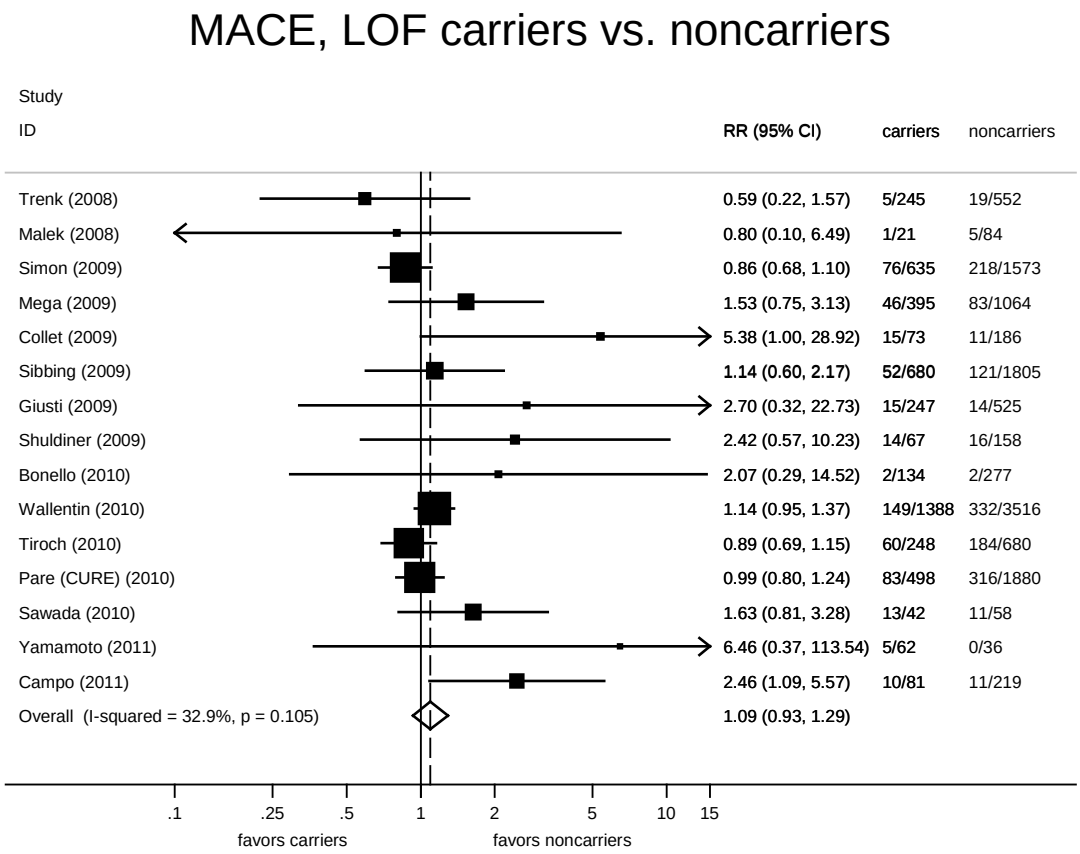
MACE

Composite clinical outcomes, often called MACE, were the most commonly reported clinical outcome across the eligible studies. Although definitions of these composite outcomes were somewhat variable, we reasoned that the most inclusive definition used by each study (i.e., the one including as many events as possible) would provide an estimate of the cardiovascular morbidity experienced by individuals in each study.[d] Details about the exposure and outcome definitions used in the eligible studies are presented in **Appendix D**.

[d] This is also common practice in meta-analyses in cardiovascular disease topics.

Of the studies reporting on MACE, 15 were considered to have included nonoverlapping patient populations and compared event rates among patients with at least one loss-of-function allele versus those with no loss-of function alleles.[25,74,77,79-86,90-93] Studies were large (minimum sample size=100; median sample size=772). The summary RR comparing carriers of any loss-of-function allele versus noncarriers was 1.09 (95 percent CI 0.93, 1.29; P=0.285). Overall, there was moderate between-study heterogeneity (P_Q=0.105; I^2=33 percent). **Figure 11** presents the meta-analysis results, along with study-specific event rates.

Figure 11: Meta-analysis of MACE Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles

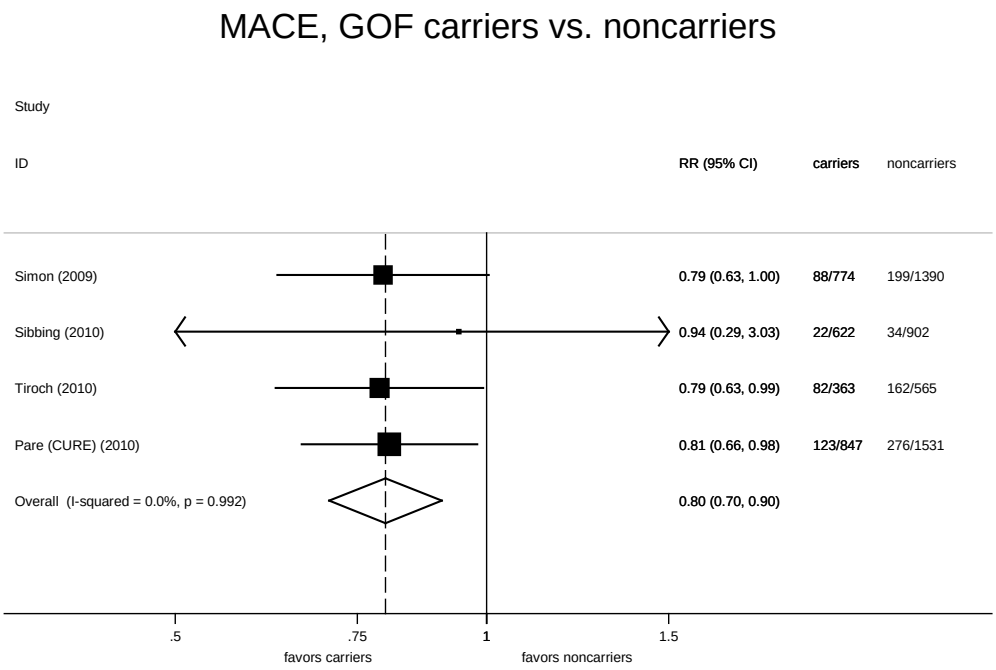


CI = confidence interval; LOF = loss-of-function; MACE = major adverse cardiovascular events; RR = relative risk. The solid squares (and horizontal lines) indicate the RR of MACE (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Information on the predictive value of gain-of-function alleles for major adverse cardiovascular events was reported in four studies with nonoverlapping populations of patients with ischemic heart disease.[74,80,91,92] Studies were large (minimum sample size=300); however, they represented a minority of the 15 studies reporting on MACE outcomes. The summary RR for all cause mortality comparing carriers of any gain-of-function allele versus noncarriers was

0.80 (95 percent CI 0.70, 0.90; P<0.001). Overall, there was little between-study heterogeneity (P_Q=0.992; I^2=0 percent). **Figure 12** presents the meta-analysis results, along with study-specific event rates.

Figure 12: Meta-analysis of MACE Comparing Carriers and Noncarriers of Gain-of-Function CYP2C19 Alleles

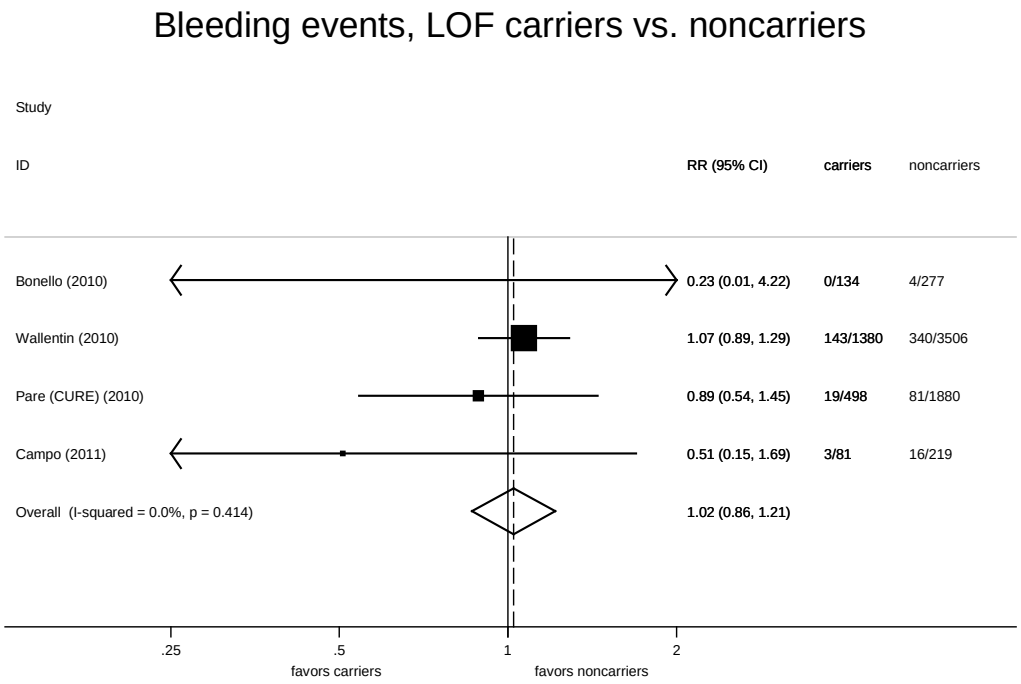


CI = confidence interval; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; GOF = gain-of-function; MACE = major adverse cardiovascular events; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of MACE (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Bleeding Events

Of the studies reporting on bleeding events, four were considered to have included nonoverlapping patient populations and compared event rates among patients with at least one loss-of-function allele versus those with no loss-of function alleles.[77,86,90,91] Studies were large (minimum sample size=300). The summary RR comparing carriers of any loss-of-function allele versus noncarriers was 1.02 (95 percent CI 0.86, 1.21; P=0.41). Overall, there was little between-study heterogeneity (P_Q=0.414; I^2=0 percent). **Figure 13** presents the meta-analysis results, along with study-specific event rates.

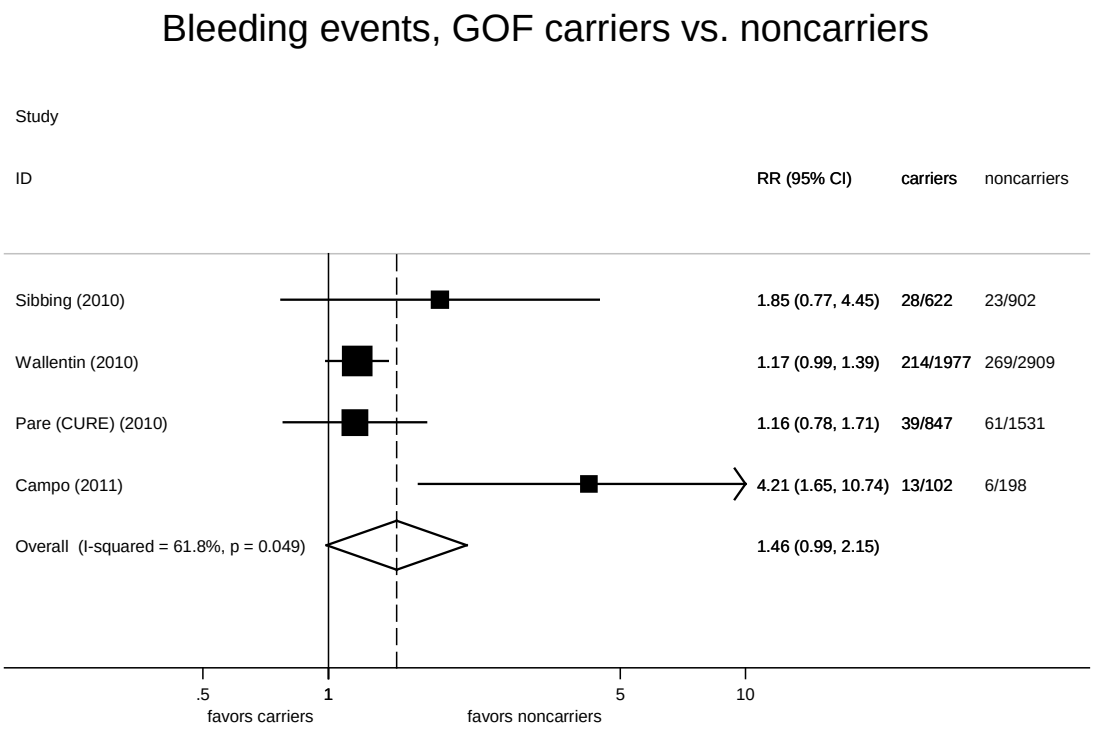
Figure 13: Meta-analysis of Bleeding Events Comparing Carriers and Noncarriers of Loss-of-Function CYP2C19 Alleles



CI = confidence interval; LOF = loss-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of bleeding events (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Information on the predictive value of gain-of-function alleles for bleeding events was reported in four studies with nonoverlapping populations of patients with ischemic heart disease. Studies were large (minimum sample size=300); however, they represented a minority of the 28 studies reporting clinical outcomes. The summary RR for all bleeding events comparing carriers of any loss-of-function allele versus noncarriers was 1.46 (95 percent CI 0.99, 2.15); P=0.057. Overall, there was moderate (and statistically significant) between-study heterogeneity (P_Q=0.049; I^2=62 percent). **Figure 14** presents the meta-analysis results, along with study-specific event rates.

Figure 14: Meta-analysis of Bleeding Events Comparing Carriers and Noncarriers of Gain-of-Function CYP2C19 Alleles



CI = confidence interval; GOF = gain-of-function; RR = relative risk.
The solid squares (and horizontal lines) indicate the RR of bleeding events (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Other clinical outcomes

Four studies reported information on five additional clinical outcomes (1 on urgent revascularization of any vessel and on a composite of "ischemic endpoints related to stent thrombosis" [94]; 1 on urgent revascularization (no additional details provided) [86]; 1 on target vessel revascularization throughout the study period[79]; and one on a composite of benefits and harms, termed "net clinical benefit" [95]). All studies assessed the effect of loss-of-function alleles. Generally event rates were higher among carriers of loss-of-function alleles (results were statistically significant only for the outcome of "ischemic endpoints related to stent thrombosis" [94], indicating increased rate of adverse outcomes among carriers of loss-of-function alleles). These outcomes were not considered to heterogeneous for further quantitative synthesis; study specific results have been summarized in **Appendix D**.

Intermediate Outcome: Platelet Reactivity

Forty studies used the results of CYP2C19 genetic testing to predict intermediate outcomes of platelet reactivity, either as a continuous variable or according to a threshold of reactivity (e.g., high vs. low). The assays used to measure platelet reactivity are described in detail later in the Results section (see Key Question 2a and **Appendix D**). Several studies used more than one assay as a method for outcome ascertainment, with platelet reactivity measured with light-

transmission aggregometry (LTA) in 19 studies (48 percent), the VASP assay in 9 (23 percent), VerifyNow P2Y12 in 16 (40 percent), and other assays in 7 studies (18 percent). All studies used ADP as the agonist of interest. Detailed information about the assays used and study-level results of platelet reactivity as a continuous outcome are presented in **Appendix D**.

Platelet Reactivity as a Continuous Outcome

Thirty-four studies reported on the effect of genotype on platelet reactivity as a continuous outcome. Reactivity was commonly assessed using light-transmissionLTA, the VASP assay, or VerifyNow P2Y12.

Studies generally showed that the mean or median reactivity was higher among clopidogrel-treated patients with one or two loss-of-function alleles compared to those with no loss-of-function alleles. The followup periods differed across the studies (from a few hours to more than 1 year after clopidogrel loading) and measurements were obtained using different assays and different calculation methods (e.g., absolute within-patient change from baseline, percent change within patient, or absolute value at last followup). Furthermore, the genotype groupings employed were also variable. Finally, the studies used different statistical tests and made different parametric assumptions for their comparisons. Because of the extensive differences among studies and the often incomplete reporting of numerical information we did not perform meta-analyses for studies using continuous measurements of reactivity as the outcome of interest.

Platelet Reactivity as a Categorical Outcome

Twenty-two studies reported on the effect of genotype on platelet reactivity as a categorical outcome. Reactivity was commonly assessed using light-transmissionLTA, the VASP assay, or VerifyNow P2Y12.

Studies generally showed that platelet reactivity above the threshold used (or in higher quantiles compared to lower quantiles of reactivity) was more common in clopidogrel-treated patients with one or two loss-of-function alleles than those with no loss-of-function alleles. As was true for studies assessing reactivity as a continuous outcome, results in studies of categories of reactivity were reported with different followup periods and measurements were obtained using different assays and different calculation methods (i.e., by discretizing absolute within-patient change from baseline, percent change within patient, or absolute reactivity values at last followup). Furthermore, genotype grouping was also variable. Because of these extensive differences among studies, we did not perform meta-analyses for studies using categorical measurements of reactivity as the outcome of interest.

Patient Populations Other Than Those with Ischemic Heart Disease

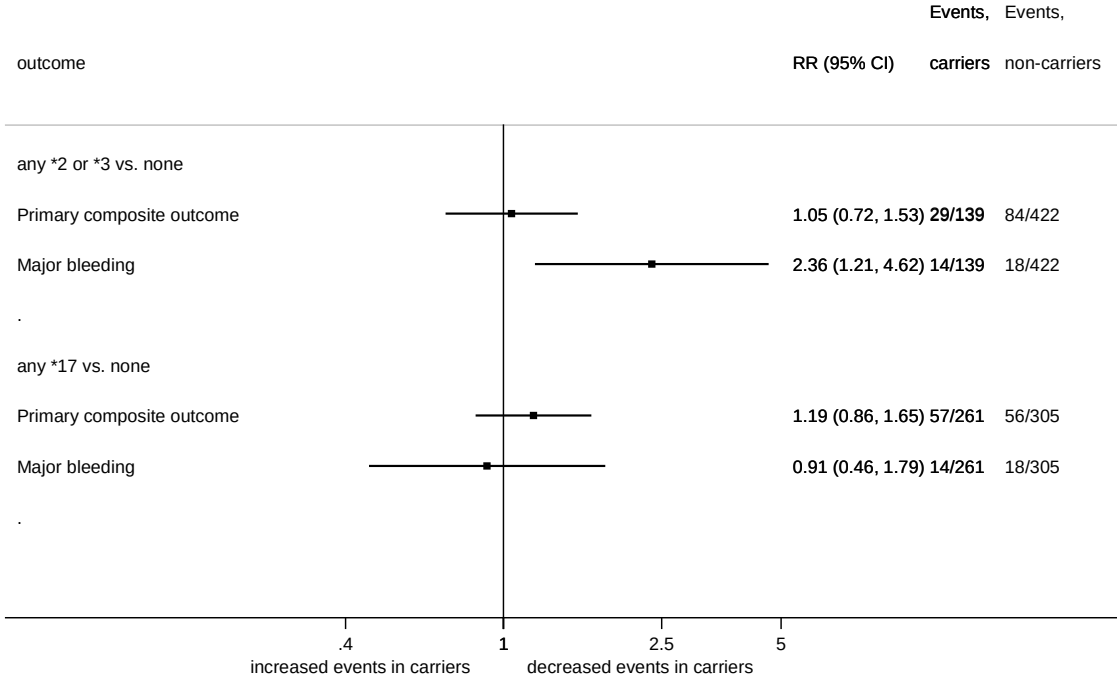
Only two studies reported results on the predictive value of CYP2C19 genotyping for clinical or intermediate outcomes in non–ischemic heart disease populations (one of patients with atrial fibrillation at risk for stroke and one of patients with cerebrovascular disease). Detailed information on these studies is presented in **Appendix Tables D3** and **D4**.

Briefly, the first study[91] reported information from the clopidogrel-treated group of the ACTIVE A randomized trial of patients with atrial fibrillation and at least one additional risk factor for stroke who were not candidates for vitamin K antagonist therapy. The study presented genotyping results for a total of 570 clopidogrel-treated patients. This study group received dual-antiplatelet therapy with aspirin and clopidogrel (75 mg/day) and was followed for 3 to 12

months. The main study results reported information on patients of European or Latin American ancestry; patients of different ethnic/racial backgrounds were excluded on the basis of concerns about population stratification.

Comparing the primary composite outcome (any major vascular event [stroke, systemic embolism outside the central nervous system, myocardial infarction, or death from vascular causes) between clopidogrel-treated patients with at least one loss-of-function allele (*2 or *3) versus those with no loss-of-function allele yielded a log-rank P value of 0.87, indicating that genotype group was not statistically significantly associated with this outcome. For major bleeding events, the study reported a log-rank P value of 0.01, indicating that the rate of major bleeding events was higher among patients carrying no loss-of-function alleles than among those with such alleles. The rates of the primary composite outcome did not differ significantly between patients with at least one gain-of-function allele (*17) and those with none (log-rank P=0.34; nor did the rates of major bleeding, log-rank P=0.29). For purposes of illustration, we present a forest plot comparing the risk of the primary composite outcome and the risk of major bleeding between genotype groups (**Figure 15**).

Figure 15: Event Rates and Relative Risks Across Genotype Groups of Clopidogrel-Treated Patients in the Genetic Substudy of the ACTIVE A Trial



ACTIVE A Trial = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A trial; CI = confidence interval; RR = relative risk.

The solid squares (and horizontal lines) indicate the RR (and the corresponding 95% CI) for individual studies. The affected numbers of carriers or noncarriers are shown to the right of the plot. The size of the squares is proportional to the weight of each study in the meta-analysis. In the ACTIVE A trial,[91] the primary composite outcome was any major vascular event (stroke, systemic embolism outside the central nervous system, myocardial infarction, or death from vascular causes). Major bleeding was defined as any overt bleeding requiring transfusion of at least 2 units of blood or any overt bleeding meeting the criteria for severe hemorrhage.

The second study was a small (n=166) retrospective study of patients receiving clopidogrel for cerebrovascular disease (cerebral infarction in 142 and intra- or extracranial artery stenosis in 24) for at least 6 months. The authors reported information regarding the ability of CYP2C19 genotype (regarding *2 and *3 alleles) to predict on-clopidogrel platelet reactivity measured with the VerifyNow P2Y12 assay. Platelet reactivity was least among extensive metabolizers (no *2 or *3 allele), intermediate among intermediate metabolizers (one *2 or *3 allele), and greatest among poor metabolizers (two loss-of-function alleles, either two *2 or two *3 or one of each) (Bonferroni corrected P<0.001 for pairwise comparisons).

Risk of Bias for Studies Reviewed for Key Question 1b

The detailed assessment of 11 risk-of-bias items for studies evaluating the predictive ability of genetic testing regarding clinical outcomes or platelet reactivity is presented in **Appendix Table D16**. Because of the large number of eligible studies, we did not generate a risk of bias graph showing individual items results for each study. Instead, we provide averages across studies for each item assessed (**Appendix Figure D2**). Briefly, most studies used a longitudinal design (not case–control) and no studies had substantial loss-to-followup. Inappropriate exclusions were uncommon however information on blinding was often not reported (particularly for the index test) or not used. Using the arbitrary cutoff values based on the number of adequately addressed risk of bias items 4 studies were rated as quality "A," 44 studies were rated as quality "B," and 3 were rated as quality "C."

For outcomes for which we performed meta-analysis, we assessed differences between more precise (larger) and less precise (smaller) studies using the Egger regression-based test for small-study effects. Overall, we found a statistically significant difference between smaller and larger studies on the effect size for stent thrombosis, MACE, and bleeding events for loss-of-function carriers (see "P Value" column in **Table 3**). Although publication bias (i.e., selective publication of positive or extreme findings) is a potential explanation for these associations, other factors, such as "true" heterogeneity between smaller and larger studies, chance, or other sources of bias could explain these results.

Key Question 1c: What is the Impact of Modifying Factors on the Association Between Genetic Test Results and Clinical Outcomes?

We reviewed studies to identify any evidence that patient- or system-level factors or test characteristics could modify the predictive ability of genetic testing for CYP2C19 variants. Evidence of effect modification by person-level characteristics (e.g., age, sex, disease severity) is best obtained from comparisons within a given study (i.e., among subgroups of the patient population that were selected using the same criteria and managed in a similar way). Additional evidence on effect modification can be derived from exploratory comparisons across studies, by assessing study-level characteristics (e.g., the setting of care or study design characteristics) or characteristics of the overall patient population (e.g., populations selected on the basis of ancestry).[e] In the following sections we discuss each of these sources of information regarding effect modification separately.

Effect Modification Within Studies

In total, 10 studies (see **Table 4**) reported information on modification of the predictive effect of the genetic test by various factors. Five of the studies used platelet reactivity as the outcome of interest and five reported information on clinical outcomes (major adverse cardiovascular events or bleeding events). We caution that, because only a small subset of the eligible studies provided information adequate to statistically assess effect modification, selective reporting is highly likely.

The 10 studies assessed a diverse set of potential modifiers. Three studied effect modification by proton-pump inhibitors[96-98]; two others assessed gene–gene interactions (between CYP2C19*2 and *3 variant in one[89] and between CYP2C19*2 and an ABCB1 polymorphism in another[92]). The following modifiers were also evaluated: indication for clopidogrel use (acute coronary syndrome vs. stable angina),[99] whether patients required a loading dose or not (because they were on chronic clopidogrel therapy),[89] the duration of clopidogrel therapy,[84] smoking status (number of cigarettes per day),[100] body-mass index ($\geq$25 kg/m^2 vs. <25 kg/m^2),[97] ancestry (European vs. Latin American),[101] and stent type (bare metal vs. drug eluting)[93] (Two studies each assessed two potential effect modifiers using platelet reactivity as the outcome.[89,97])

Only one of the effects assessed was statistically significant (a multiplicative interaction between the *2 and *3 CYP2C19 alleles on on-clopidogrel platelet reactivity[89]). Overall, the reported findings do not provide sufficient evidence to support (or exclude) a differential effect of CYP2C19 variants across any of the factors assessed in the studies we reviewed.

[e] We refer to the information obtained from cross-study comparisons as "exploratory" because comparisons for a given characteristics across studies may be confounded (by other study features). Our ability to statistically "control" for such differences is typically limited by the relatively small number of available studies available for meta-analysis.

Table 4: Studies Reporting Information on Effect Modification

Author Year Country PMID Study Name (when available)	Patient Population	Antiplatelet Regimen	Effect Modifier	Summary of Findings[f]
Fernando[96] 2011 21696537 Australia NR	Patients with ACS treated with PCI with stent implantation; all patients were participants in a randomized cross-over trial of esomeprazole 40 mg vs. placebo	Aspirin (100 mg/d) and clopidogrel (75 mg/d)	Esomeprazole (40 mg/d) [the study drug]	After 6 w of exposure to each study drug, extensive metabolizers (those with no LOF alleles, i.e., no CYP2C19*2 allele) showed a significant mean reduction from baseline (−8% for PRI by VASP assay, −8.5 AUC units by aggregometry, −37 PRU by VerifyNow; P<0.01 for all comparisons) in platelet reactivity with esomeprazole vs. placebo. In poor or intermediate metabolizers (with at least one CYP2C19*2 allele) the mean reduction was nonsignificant (−8.73% for PRI, P=0.157; −3 AUC units for aggregometry, P=0.566; 39 PRU by VerifyNow, P=0.139) with esomeprazole vs. placebo.
Harmsze[99] 2010 19934793 Netherlands NR	Patients with CAD undergoing PCI with stent implantation	Clopidogrel "maintenance therapy" and aspirin (80 mg/d)	Indication for PCI with stent placement: ACS or stable angina	ORs for stent thrombosis: *For patients undergoing PCI for ACS* Carrier of *2 vs. not, OR=2.0 (95% CI 1.1, 4.5) Carrier of *3 vs. not, OR=2.9 (95% CI, 1.0, 9.3) *For patients undergoing PCI for stable angina* Carrier of *2 vs. not, OR=1.7 (95% CI 0.9, 4.1) Carrier of *3 vs. not, OR=1.2 (95% CI, 0.4, 6.5) Interaction P value for the effect of *2 over PCI indication=0.97 Interaction P value for the effect of *3 over PCI indication=0.18
Harmsze[89] 2010 20833683 Netherlands NR	Patients undergoing elective stent implantation	All patients received aspirin (80–100 mg/d); 297 patients were on chronic clopidogrel (maintenance dose of 75 mg/d) and 131 patients received a clopidogrel loading dose of 300 mg upon study entry	Clopidogrel treatment status (maintenance treatment vs. loading dose); assessment of gene–gene interaction	Mean difference between carriers and noncarriers of CYP2C19*2 allele stratified by clopidogrel treatment group, using different assays (unadjusted results): *Clopidogrel maintenance treatment* LTA ADP 5 μmol/L: 6.7 (95% CI 3.6, 9.8) LTA ADP 20 μmol/L: 6.3 (95% CI 3.4, 9.3) VerifyNow PRU: 35.1 (95% CI 17.2, 53.0) *Clopidogrel loading dose* LTA ADP 5 μmol/L: 7.8 (95% CI 3.9, 12.6) LTA ADP 20 μmol/L: 7.4 (95% CI 3.3, 11.6) VerifyNow PRU: 37.5 (95% CI 16.5, 58.5) z-score P values for effect modification (calculated): nonsignificant for all comparisons OR for *2 carriers vs. noncarriers for predicting poor response (>70% aggregation on LTA ADP 20 μmol/L) to antiplatelet treatment (unadjusted results): *Maintenance treatment, LTA ADP 20 μmol/L* OR=3.7 (95% CI 2.0, 6.9) *Loading, LTA ADP 20 μmol/L* OR=3.7 (95% CI, 2.0, 6.9) z-score P value for interaction: >0.99 Multiplicative interaction between *2 and *3 variants

[f] The description "calculated values" indicates that the results are based on statistical procedures we conducted in cases where studies provided sufficient statistics for the assessment of effect modification but did not assess its statistical significance.

				for poor response: *LTA ADP 20 μmol/L* 0.1 (95% CI 0.004, 0.79); P=0.033 *VerifyNow PRU* 0.1 (95% CI 0.002, 0.7); P=0.035
Liu[100] 2010 21163112 China NR	Patients undergoing elective stent implantation for stable angina	Clopidogrel (300 mg loading dose and 75 mg/d maintenance dose) and aspirin (300 mg/d)	Smoking status (≥10 cigarettes/d)	P=0.671 for interaction between smoking status and *2 carrier status for predicting poor response by LTA (≤10% reduction in baseline aggregation at 24 h) in a logistic regression model
Maeda[97] 2011 21178986 Japan NR	Patients with CAD undergoing PCI	Aspirin (100 mg/d) and clopidogrel (75 mg/d) for >4 w	PPI treatment vs. not; BMI≥25 kg/m^2	CYP2C19 genotype effect (mean residual % on-clopidogrel reactivity) stratified by PPI status: *On PPI* Two LOF alleles: n=2, mean=36.0, SD=8.5 One LOF allele: n=13, mean=27.5, SD=9.9 No LOF allele: n=14, mean=22.7, SD=7.6 *Not on PPI* Two LOF alleles: n=14, mean=33.5, SD=8.0 One LOF allele: n=33, mean=25.7, SD=9.0 No LOF allele: n=25, mean=17.7, SD=8.4 Interaction P values (calculated with inverse variance-weighted least-squares regression): for one LOF allele × PPI=0.466 for two LOF alleles × PPI=0.724 CYP2C19 genotype effect (mean residual % on-clopidogrel reactivity) stratified by BMI status: *BMI≥25 kg/m^2* Two LOF alleles: n=4, mean=36.0, SD=8.7 One LOF allele: n=16, mean=25.9, SD=9.8 No LOF allele: n=9, mean=24.4, SD=7.4 *BMI<25 kg/m^2* Two LOF alleles: n=14, mean=33.1, SD=4.3 One LOF allele: n=14, mean=26.4, SD=8.5 No LOF allele: n=14, mean=17.1, SD=8.9 Interaction P values (calculated with inverse variance-weighted least-squares regression): for one LOF allele × PPI=0.071 for two LOF alleles × PPI=0.347
Pare[101] 2010 20979470 Multiple countries CURE	Patients with NSTE ACS in the clopidogrel-treated arm of the CURE randomized trial	Aspirin and clopidogrel (75 mg/d)	Race/ethnicity (European ancestry vs. Latin American Ancestry)	Interaction P value for the predictive value of genotype group (intermediate/poor metabolizer vs. extensive/ultra metabolizer) between ancestry groups (calculated with chi-square test for heterogeneity): *For the first primary composite outcome* (cardiovascular death, nonfatal MI, or stroke)=0.682 *For another first primary composite outcome* (cardiovascular death, nonfatalMI, stroke, recurrent ischemia, or hospitalization for unstable angina)=0.722 *For major bleeding*=0.678
Simon[98] 2011 21262992 France FAST-MI	Patients admitted to intensive care unit for definite AMI within 48 h after symptom onset with available DNA; patients had to be PPI-naïve at study entry for the main analysis	Clopidogrel-based treatment	PPI therapy (yes vs. no)	Estimate for PPI group vs. no-PPI group (adjusted for GRACE score, sex, and cardiovascular risk factors) *OR for in-hospital death, MI, or stroke* No variant allele, OR=0.68 (95% CI 0.37, 1.25) One variant allele, OR=0.34 (95% CI 0.11, 1.08) Two variant alleles, OR=1.05 (95% CI 0.03, 34.6) Interaction P value across groups=0.547 [calculated

				with chi-square test for heterogeneity] *HR for death, MI, or stroke in hospital survivors receiving clopidogrel at 1 year* No variant allele, 1.13 (95% CI 0.74, 1.74 One variant allele, 1.02 (95% CI 0.44, 2.40) Two variant alleles, 0.25 (95% CI 0.02, 3.58) Interaction P value across groups=0.527 calculated with chi-square test for heterogeneity] Overall interaction effect: For the short-term outcome P=0.57 For the 1-year outcome P=0.64
Simon[92] 2009 19106083 France FAST-MI	Patients admitted to intensive care unit for definite AMI within 48 h after symptom onset with available DNA	Clopidogrel-based treatment (mean clopidogrel loading dose 300 mg; mean maintenance dose 75 mg/d)	ABCB1 genotype (rs1045642) [carrier of at least one T allele vs. none]	P=0.99 for interaction between CYP2C19 and ABCB1 for the composite outcome of death, nonfatal MI, or stroke at 1 yr
Trenk[93] 2008 18482659 Germany EXCELSIOR	Patients undergoing elective PCI with stent placement	Clopidogrel (600 mg loading dose) and aspirin (100 mg) for ≥5 d before the procedure	Stent type (BMS vs. DES)	OR for CYP2C19*2 carriers vs. noncarriers for death or MI at 1 yr *Patients with ≥ 1 DES* OR=1.60 (95% CI 0.35, 7.32) *Patients with BMS only* OR=0.30 (95% CI 0.07, 1.34) Interaction P value=0.118 [calculated with chi-square test for heterogeneity]
Yamamoto[84] 2011 21168310 Japan NR	Patients undergoing cardiac catheterization at time of diagnosis of stable CAD	Aspirin (100 mg/d) and clopidogrel (75 mg/d)	Days on clopidogrel-based therapy (<7 vs. ≥7)	CYP2C19 genotype effect (on-clopidogrel reactivity in AU*min by LTA ADP 20 mol/L) stratified by days on treatment: *<7 d* Two LOF alleles: n=12, mean=3186, SD=1595 One LOF allele: n=32, mean=4655, SD=1380 No LOF allele: n=25, mean=5663, SD=1385 *≥7 d* Two LOF alleles: n=13, mean=3007, SD=1541 One LOF allele: n=19, mean=3490, SD=1392 No LOF allele: n=22, mean=4674, SD=824 Interaction P value [calculated with inverse variance-weighted least-squares regression] For one LOF allele × PPI=0.106 For two LOF alleles × PPI=0.212

ACS = acute coronary syndromes; ADP = adenosine diphosphate; AMI = acute myocardial infarction; AU = aggregation units; AUC = area under the curve; BMI = body-mass index; BMS = bare-metal stent; CAD = coronary artery disease; CI = confidence interval; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; d = day(s); DES = drug-eluting stent; EXCELSIOR = Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate; FAST-MI = French Registry on Acute ST-elevation and non ST-elevation Myocardial Infarction; GOF = gain-of-function; GRACE = Global Registry of Acute Coronary Events; h = hours; LOF = loss-of-function; LTA = light-transmission aggregometry; MI = myocardial infarction; NA = not applicable; NR = not reported; NSTE = non–ST-elevation; OR = odds ratio; PCI = percutaneous coronary intervention; PPI = proton-pump inhibitor; PRI = platelet reactivity index; PRU = platelet reactivity units; SD = standard deviation; VASP = vasodilator-stimulated phosphoprotein; w = weeks.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

Effect Modification Across Studies

On the basis of the availability of data from studies considered eligible for Key Question 1b, we assessed the following characteristics as potential modifiers of the predictive effect of genetic testing for CYP2C19 by performing comparisons across studies using subgroup analysis: disease subtype (acute coronary syndromes vs. mixed coronary artery disease populations), setting of care (PCI vs. other), race or ethnicity (white vs. East Asian), duration of followup (≤30 days vs. >30 days); and year when enrollment was started (continuous variable). Meta-regressions were performed for only the two outcomes that were reported in 10 or more studies—major adverse cardiovascular events and stent thrombosis—and for only carriers versus noncarriers of CYP2C19 loss-of-function alleles.

Table 5 summarizes the findings of our meta-regression analyses for the outcome of stent thrombosis and **Table 6** summarizes findings for the composite endpoint of MACE.

Table 5: Meta-regression Results for Study-Level Effect Modifiers of the Predictive Value of Genetic Testing for CYP2C19 Variants in Patients with Ischemic Heart Disease: Stent Thrombosis

Effect Modifier	Groups Compared	No. of Studies	RR (95% CI) Within Subgroup	rRR (95% CI); P Value
All studies	NA	13	1.56 (1.15, 2.11)	NA
ACS vs. ischemic heart disease	Other ischemic heart disease or mixed	6	1.76 (1.02, 3.02)	0.84 (0.44, 1.61); 0.595
	ACS	7	1.47 (1.02, 2.13)	
Race or ethnicity	Whites	11	1.47 (1.08, 2.01)	7.51 (0.69, 81.15); 0.097
	East Asians	1	11.07 (1.05, 117.21)	
Followup	Longitudinal	11	1.59 (1.12, 2.26)	0.91 (0.45, 1.84); 0.785
	Case–control or case-cohort	2	1.44 (0.78, 2.68)	
Duration of followup	>30 d	11	1.50 (1.10, 2.05)	2.57 (0.36, 18.18); 0.344
	≤30 d	1	3.86 (0.56, 26.63)	
Enrollment start year	(continuous variable)	12	NA	0.93 (0.80, 1.08); 0.324

ACS = acute coronary syndromes; CI = confidence interval; d = day(s); NA = not applicable; RR = relative risk; rRR = relative RR (between groups).
Results across all studies are provided for comparison. For stent thrombosis, all patients had to undergo percutaneous coronary intervention, so results by setting of care are not presented. One study did not report the year enrollment was started.

Table 6: Meta-regression Results for Study-Level Effect Modifiers of the Predictive Value of Genetic Testing for CYP2C19 Variants n Patients with Ischemic Heart Disease: MACE

Effect Modifier	Groups Compared	No. of Studies	RR (95% CI) Within Subgroup	rRR (95% CI); P Value
All studies	NA	15	1.09 (0.93, 1.29)	NA
ACS vs. ischemic heart disease	Other ischemic heart disease or mixed	7	1.49 (0.98, 2.26)	0.69 (0.45, 1.05); 0.084
	ACS	8	1.01 (0.87, 1.17)	
PCI vs. other clinical settings	Other	5	1.04 (0.83, 1.29)	1.15 (0.78, 1.69); 0.470
	PCI	10	1.24 (0.92, 1.66)	
Race or ethnicity	Whites	11	1.02 (0.89, 1.17)	1.73 (0.83, 3.60); 0.141
	East Asians	2	1.76 (0.89, 3.47)	
Duration of follow-up	>30 d	13	1.10 (0.92, 1.32)	1.07 (0.46, 2.46); 0.882
	≤30 d	1	1.14 (0.60, 2.17)	
Enrollment start year	(continuous variable)	15	NA	1.01 (0.95, 1.08); 0.646

ACS = acute coronary syndromes; CI = confidence interval; d = days; NA = not applicable; PCI = percutaneous coronary intervention; RR = relative risk; rRR = relative RR (between groups).
Results across all studies are provided for comparison. All eligible studies were longitudinal (cohort studies), so results by study design are not presented.

Considering both within-study and across studies analyses, there is insufficient information to support or exclude the presence of substantial modification of the predictive effect of CYP2C19 variants by any of the investigated factors.

Key Question 2a: What is the Analytic Validity (Technical Test Performance) of the Various Assays Used in Phenotypic Testing of Platelet Reactivity?

Eligible Studies for Key Question 2a

We identified 80 studies reporting information on the analytic validity of assays for measuring platelet reactivity; details about the design and results of individual studies are provided in **Appendix Tables E1–E4**. We also reviewed 20 FDA 510(k) summaries on phenotypic testing assays (see **Appendix C**). Studies had intermediate sample sizes (median sample size=120; 25th percentile=93; 75th percentile=296) and were published between 2002 and 2011 (median publication year=2009). Twelve studies (15 percent) were conducted in the United States, 51 (64 percent) in Europe, and 17 (21 percent) elsewhere. All studies enrolled patients with ischemic cardiovascular disease. The five most commonly assessed assays (with some studies assessing more than one) wereLTA (in 56 studies; 70 percent), the VASP assay (in 29 studies, 36 percent), the VerifyNow P2Y12 assay (in 32 studies, 40 percent), PFA-100 (in 7 studies; 9 percent), and thromboelastography (in 6 studies; 8 percent). The vast majority of studies used ADP as the agonist to stimulate platelets (in order to assess reactivity).

None of the 20 FDA 510(k) summaries on phenotypic tests of platelet reactivity reported relevant analyses (either no data were reported or the population or agonist used in test was not of interest, the analytic validity results were not reported for clopidogrel [rather, for a comedication], or the sample size was <10).

We organized studies reporting information relevant to analytic validity into four groups: analytic performance, interassay agreement, test reliability and assay variation, and correlations between assays applied to the same sample. No other aspect of analytic validity was evaluated in studies considered eligible for this review. **Table 7** summarizes the information provided by each study type and the number of relevant publications included in our report. In subsequent sections we summarize key findings pertaining to each of these outcomes.

Overall there appeared to be low to moderate agreement between assays. Agreement was generally greater between measurements obtained with the same assay using different agonist concentrations than between different assays. Studies were generally of low methodological quality and poorly reported. Only 1 of the 11 items capturing risk of bias and completeness of reporting ("providing adequate details on the testing method" to allow for replication of test results) was satisfactorily addressed across studies. Detailed results regarding the quality items are presented at the end of this section.

Figure 16 presents an evidence map (a weighted scatter plot of tests and outcomes considered in the eligible studies) of studies assessing pairs of tests for analytic accuracy, agreement, or correlation (studies of these outcomes need to assess at least two tests). Only a minority of these studies reported information on analytic performance or test agreement, whereas a large number provided correlation results. Most of the studies reported test agreement or correlations between measurements obtained with LTA versus the VASP assay, VerifyNow, or the Multiplate analyzer; comparisons between measurements performed using the same assay with different agonist concentrations were also commonly reported.

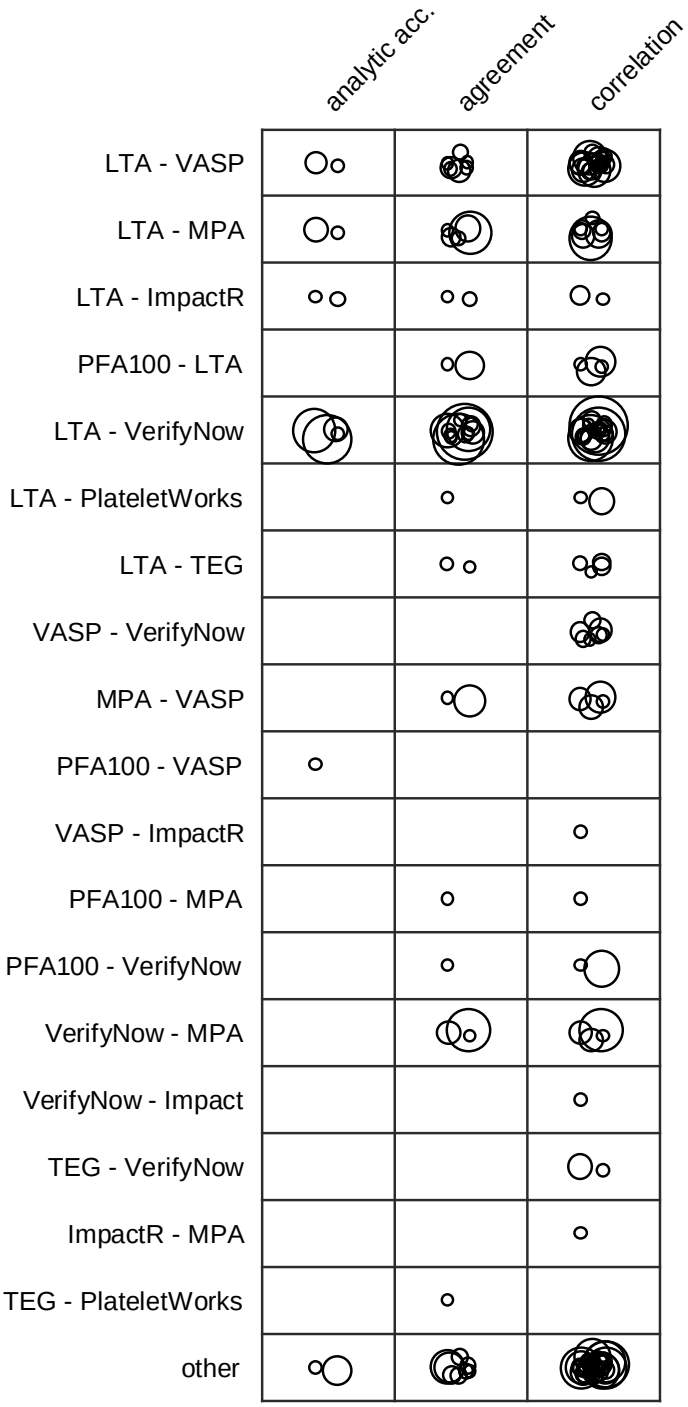
Table 7: Studies Reporting Information Relevant to Analytic Validity

Analytic Validity Category	Relevant Information	Assays Evaluated (no. of studies; % of total studies)
Analytic performance (analytic accuracy, sensitivity, specificity)	These studies used a reference standard test against which the analytic performance of one or more index tests is assessed. In theory, the reference standard test can also be considered to have non-negligible measurement error (i.e., to be a "tarnished" reference standard); however, in all the studies we reviewed, the analytic reference standards were considered to be "gold standards" (i.e., to have no measurement error).	Total=10 studies LTA (7; 70%) VASP assay (4; 40%) VerifyNow P2Y12 (5; 50%) Multiplate analyzer (2; 20%) PFA-100 (3; 30%) TEG (1; 10%) Plateletworks (0; 0%) Others (3; 30%)
Interassay agreement	These studies assessed the amount of agreement (concordance) between measurements of the same sample obtained with different assays. Agreement can be assessed between continuous measurements or using nominal or ordinal classifications (e.g., by discretizing continuous measurements by applying a threshold).	Total=33 studies LTA (26; 79%) VASP assay (13; 39%) VerifyNow P2Y12 (19; 58%) Multiplate analyzer (8; 24%) PFA-100 (5; 15%) TEG (2; 6%) Plateletworks (2; 6%) Others (6; 18%)
Assay reliability (variability)	These studies assessed the reliability of assays during repeat testing and provided estimates of the variation of results when testing was repeated across different batches of analytes or portions of the same sample. The studies we reviewed typically expressed results as coefficients of variation.[g]	Total=34 studies LTA (22; 65%) VASP assay (13; 38%) VerifyNow P2Y12 (8; 24%) Multiplate analyzer (3; 9%) PFA-100 (4; 12%) TEG (1; 3%) Plateletworks (0; 0%) Others (6; 18%)
Correlation between measurements obtained by different assays	Many of these studies reported correlations (typically Spearman or Pearson) between measurements obtained from the same sample. (These correlations were reported to support claims regarding interassay agreement; we discuss in the text why some methods for calculating correlations are not appropriate for this purpose.)	Total=56 studies LTA (45; 80%) VASP assay (25; 45%) VerifyNow P2Y12 (27; 48%) Multiplate analyzer (10; 18%) PFA-100 (5; 9%) TEG (5; 9%) Plateletworks (1; 5%) Others (15; 27%)

LTA = light-transmission aggregometry; PFA = Platelet Function Analyzer; TEG = thromboelastography; VASP = vasodilator-stimulated phosphoprotein assay using flow cytometry. "Other" denotes less commonly assessed assay types. See **Appendix Tables E1-E4** for a complete list of assays used in the included studies. Studies could have used more than one assay.

[g] The coefficient of variation is generally defined as the ratio of a measure of dispersion over a measure of central tendency. [102]The most commonly used definition is the ratio of the estimated standard deviation (SD) over the sample mean ($\bar{x}$): $CoV = \frac{SD}{\bar{x}}$.

Figure 16: Evidence Map of Studies Reporting Information on the Analytic Validity of Phenotypic Assays.



Acc = accuracy; LTA = light-transmission aggregometry; MPA = Multiplate analyzer; PFA = Platelet Function Analyzer; TEG = thromboelastography; VASP = vasodilator-stimulated phosphoprotein. "Other" denotes pairs of assays where at least one of the assays was used in fewer than five studies or comparisons between measurements obtained with the same assay (e.g., using different agonist concentrations). Each circle represents a study; the size of the circle increases with increasing sample size, and location of circles within boxes is random. The assays used are described in detail in **Appendix E**.

Findings on the Analytic Validity of Platelet Reactivity Assays

Analytic Performance

Only a small number of studies (n=10) provided information on analytic performance. In these studies, LTA (six studies) or the VASP assay (two studies) was considered as "gold standard" tests. (In one of the two remaining studies, the reference standard test was platelet-receptor expression after ADP stimulation, assessed by flow cytometry; in the other study, the reference standard was not explicitly reported). Reported analytic sensitivities ranged between 0.35 and 1.00; reported specificities ranged between 0.42 and 0.95. In studies reporting results across multiple cutoff values, a tradeoff between sensitivity and specificity was apparent (as expected). Overall, results were indicative of poor agreement between classifications (e.g., "high reactivity" vs. "low reactivity") obtained by the pairs of tests assessed when one of the tests was considered a gold standard. We did not perform meta-analyses of test performance metrics because only a few studies were available for each index test–reference standard pair and because studies used different thresholds to define positive and negative results. Detailed information on the populations included, assays used, analyses performed, and study results are presented in **Appendix Table E1**.

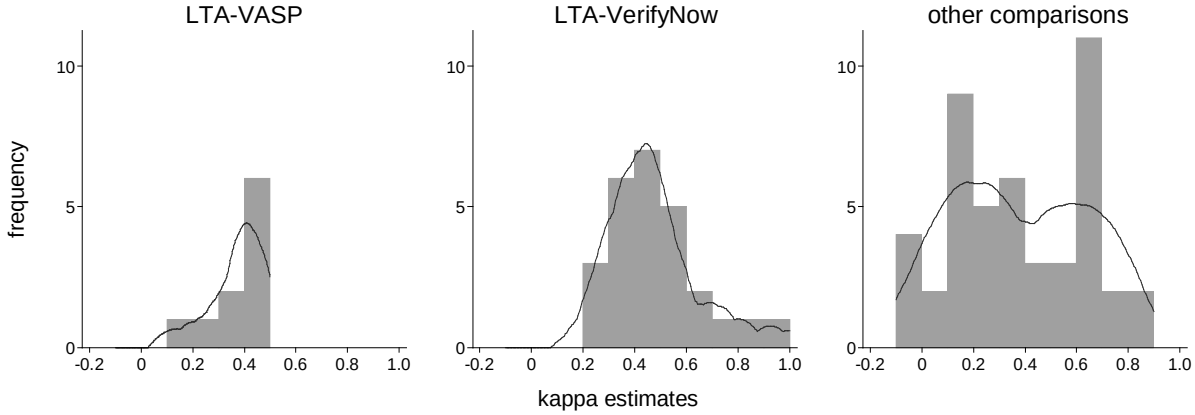
Interassay Agreement

Thirty-three studies provided information on interassay agreement. The most commonly assessed pairs of tests were LTA and VerifyNow (13 studies), LTA and the VASP assay (7 studies), and LTA and the Multiplate analyzer (5 studies). Information on the included populations, assays used, thresholds applied, and study results are presented in **Appendix Table E1**.

Twelve studies evaluated interassay agreement on the basis of continuous measurements by various assays (using valid methods, such as Bland–Altman analyses[103] or Lin's concordance correlation coefficient[104]—i.e., major axis regression). Twenty-seven of the studies reported agreement on the basis of dichotomizing platelet reactivity values using various thresholds; 24 of these reported or allowed for the calculation of kappa statistics (83 estimates in total). Across all comparisons reported, the median kappa value was 0.42 (25th percentile=0.25; 75th percentile=0.55). Only four (5 percent) of the reported kappa values were higher than 0.8 (a commonly used threshold for "excellent" agreement[105]), 16 (16 percent) of the values were between 0.6 and 0.8 (considered indicative of "substantial" agreement), 22 (27 percent) were between 0.4 and 0.6 (considered to indicate "moderate" agreement), and 41 (49 percent) were lower than 0.4 (indicating "poor to fair" agreement). Overall these results indicate that disagreements are relatively common between measurements obtained by different assays or by using different agonist concentrations within the same assays.

Figure 17 presents histograms of reported kappa values (chance corrected agreement statistics), suggesting that overall, agreement was somewhat lower between LTA and the VASP assay than between LTA and the VerifyNow P2Y12 assay. Quantitative synthesis of kappa statistics was not performed because only a few studies were available for each index test–reference standard pair and because studies used different thresholds to define positive and negative results.

Figure 17: Histograms of Kappa Values Between Methods for Measurement of Platelet Reactivity



LTA=light-transmission aggregometry; VASP=vasodilator-stimulated phosphoprotein.
"Frequency" refers to the number of studies. Black solid lines are kernel densities fitted over the distribution of kappa values. Each panel shows at least 10 reported kappa values.

Assay Reliability

Thirty-four studies reported information on assay variability. The information reported about the experimental designs used to obtain the reported results was limited (e.g., >90 percent of the studies did not report the number of replicate samples). Variability or coefficient of variation results were less than 10 percent in all but two studies. All reported assay reliability results are presented in **Appendix Table E2**. These results need to be interpreted with caution, give the poor reporting of study methods and the fact that multiple studies were published by a limited number of investigative teams (there was no way to ascertain whether the studied populations are the same or similar).

Correlation Between Measurements Used as a Method to Assess Agreement

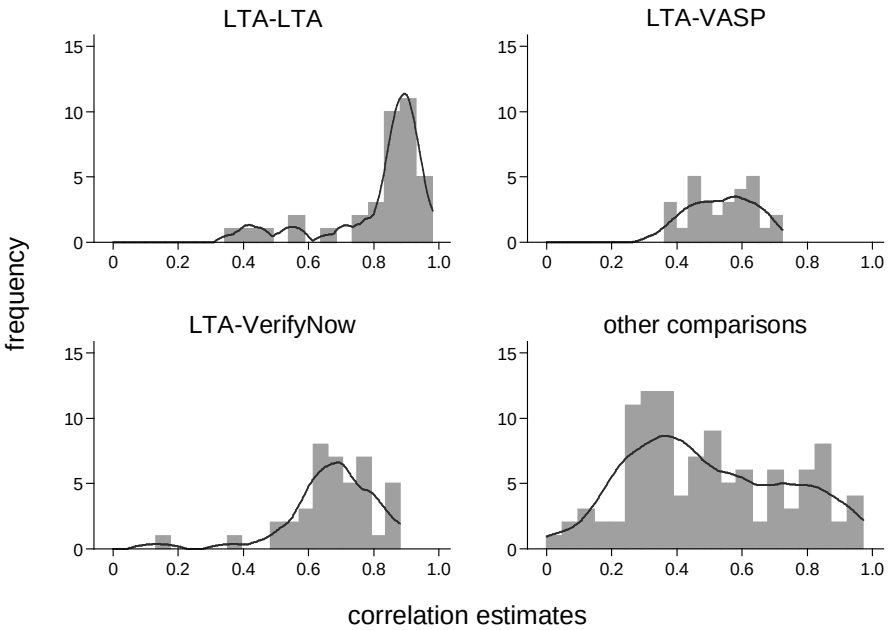
It is accepted that the Pearson or Spearman correlation coefficients are poor metrics for assessing agreement between measurements.[h] Correlation values are informative only in extreme cases: for example, a Pearson correlation coefficient very close to 1 (or −1) indicates that the values of one assay can be linearly transformed to those of the other. More appropriate methods for assessing agreement between continuous measurements are available, such as the Bland–Altman approach[103] and Lin's concordance correlation coefficient.[104] Studies using the Bland–Altman method have been discussed above (see Interassay Agreement).

Of the 56 studies reporting correlation values, only one used Lin's concordance correlation coefficient,[106] reporting a high correlation (ρ=0.97) between observed and estimated platelet inhibition for the VerifyNow assay (comparing the change between pre- and post-treatment measurements with the change between post-treatment measurements and those from the TRAP

[h] Briefly, these coefficients are suboptimal for assessing agreement because: (1) correlations measure association and not agreement; (2) correlations are independent of scale of measurement, whereas agreement is not; (3) significant correlation results are likely when the range of measurements is wide, regardless of agreement; (4) the test for the null hypothesis of no correlation is irrelevant to the question of agreement; and (5) even high correlation values (>0.95) do not imply good agreement. For additional discussion, see Bland and Altman 1986[103].

channel of the device). The remaining studies reported Spearman or Pearson correlation coefficients, used linear regression (which in the simple bivariate case of two measurements is equivalent to the Pearson correlation), or did not report the calculation method employed. Detailed results from each comparison reported in the reviewed studies are presented in **Appendix Table E3**. Given that the majority of correlation estimates reported had an absolute value lower than 0.9 (93 percent of the 215 values reported) the results indicate that the association between measurements obtained using different methods is relatively poor. However, given the inappropriateness of the methods used (for the purpose of assessing agreement), even high correlation values would not be considered indicative of good agreement. **Figure 18** presents histograms of reported correlation values.

Figure 18: Histogram of Reported Correlation Values Between Methods for Measurement of Platelet Reactivity



LTA = light-transmission aggregometry; VASP = vasodilator-stimulated phosphoprotein.
"LTA-LTA" denotes comparisons between LTA assays using different agonists or different agonist concentrations.
"Frequency" refers to the number of studies. Black solid lines are kernel densities fitted over the distribution of correlation values. Each panel shows at least 20 reported correlation values.

Risk of Bias and Completeness of Reporting

Studies reporting on assay variability were extremely poorly reported (in all but three studies, no information was provided on the study design, selection criteria, or measurement methods used; in over 90 percent of the studies, the number of replicate samples used for analyses was not reported). Thus it was impossible to assess the risk of bias of these studies. In addition, as mentioned in the preceding section, correlation estimates are practically uninformative for assessing agreement between assays. For these reasons, we limited the assessment of study quality to the 35 studies that reported information on analytic test performance or interassay agreement.

Appendix Figure E1 summarizes our assessment of 11 items related to risk of bias and completeness of reporting of studies presenting information on test agreement or analytic performance (including the single study reporting Lin's concordance correlation coefficient[106]). Additional information for each study is presented in **Appendix Table E4**. All but 1 of the 35 studies (a study published as a letter to the editor[107]) provided adequate descriptions of the assays used. Information on the use of positive or negative controls was poorly reported. About half the studies did not report the criteria for positive, negative, or indeterminate results; no study reported the limits of detection or assay linearity range. Only a minority of studies assessed reproducibility of results on repeat testing, and no study assessed reproducibility across operators, instruments, reagent lots, or over time. Only one study was conducted as part of a multilaboratory collaboration[108]; however, it did not provide details on the standardization procedures followed.

Key Question 2b: What Is the Clinical Validity (Predictive Accuracy) of Phenotypic Testing for Predicting Intermediate and Clinical Outcomes in Patients Who Are Receiving Clopidogrel Therapy?

Eligible Studies

The studies included in our review all involved administration of clopidogrel, although the populations varied in their indications for use of the drug. Of the 100 studies addressing Key Question 2b, the vast majority (95 studies) were of patients with ischemic heart disease; 4 studies enrolled patients with cerebrovascular disease, and 1 study enrolled patients with peripheral artery disease. Among studies with ischemic heart disease, 35 enrolled patients with acute coronary syndromes; the remaining either included patients with chronic stable coronary artery disease (58 studies) or mixed populations with chronic and acute presentations (7 studies). Of the 95 studies enrolling patients with ischemic heart disease, 89 (94 percent) included predominantly (>80 percent of the included population) patients undergoing PCI; 2 (2 percent), patients undergoing angiography; 2 (2 percent), patients undergoing CABG surgery; and 2 (2 percent), patients being treated with medical therapy without undergoing a procedure. The majority of studies were of patients receiving dual antiplatelet therapy with clopidogrel in combination with aspirin; when loading doses were used, the aspirin loading dose ranged between 250 and 500 mg and the clopidogrel loading dose ranged between 300 mg and 900 mg. Maintenance doses were between 81 mg and 325 mg for aspirin and 75 mg and 150 mg for clopidogrel. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented under subsequent sections addressing each test for platelet reactivity separately.

Overall, studies had intermediate to large sample sizes (median=221, 25th percentile=98, 75th percentile=602, minimum=15, maximum=2533). They had been conducted in recent years (median year of enrollment start was 2005). In 81of the 93 studies providing relevant information the median of the mean or median age was 63 years (range 54.3 to 70 years). Patients had a relatively high burden of risk factors for coronary artery disease (which are associated with adverse cardiovascular outcomes): the median proportion of patients with dyslipidemia was 61 percent (in 77 studies; range 11 - 100 percent); with hypertension, 70 percent (in 84 studies; range 18 - 92 percent); with diabetes mellitus, 29 percent (in 88 studies; range 8 -100 percent); who used tobacco, 30 percent (in 77 studies; range 6 - 73 percent). All studies were longitudinal cohort studies (as per our inclusion criteria for this Key Question).

Studies reported information on a variety of tests for measuring platelet reactivity. The predictive ability of a single platelet reactivity assay was evaluated in 84 of the 100 studies, whereas 16 studies evaluated two or more assays (13 studies evaluated two assays, 2 studies evaluated three assays, and 1 study evaluated four assays). The assays used included those based on the principle of LTA (46 studies; 46 percent), the VerifyNow P2Y12 assay (28 studies; 28 percent), the VASP assay (14 studies; 14 percent), the Multiplate analyzer assay (12 studies; 12 percent), the PFA-100 assay (8 studies; 8 percent), thromboelastography (5 studies; 5 percent), and other tests (7 studies; 7 percent). Detailed information on each test is presented separately under the discussion of individual tests for platelet reactivity.

Overall, studies were of poor to moderate quality. Based on our assessment of 11 items derived from the QUADAS-2 instrument and using arbitrary thresholds based on the number of items considered as adequately addressed, 21 of the studies were rated “A,” 67 were rated “B,” and 12 were rated “C.” We caution that this aggregate risk-of-bias rating can be misleading,

especially in the presence of poor reporting. A more detailed discussion of risk of bias, focusing on the individual items assessed, is presented later in this chapter.

In the following sections we discuss studies by outcomes reported, for each individual test, grouped by the patient populations enrolled. When study designs, assays, and outcome definitions were considered sufficiently similar across three or more studies, we performed meta-analyses to synthesize findings (using data from nonoverlapping populations).[i] Based on the results of our review on the analytic validity of assays for measuring platelet reactivity (presented under Key Question 2a of this report), we did not combine information across different assays or different agonist concentrations.

Results from subgroup and effect-modification analyses for studies relevant to Key Question 2b are presented under Key Question 2c. **Table 8** summarizes information on the patient populations and outcomes assessed in the studies considered eligible for Key Question 2b.

Table 8: Populations and Outcomes in Studies for Key Question 2b, According to Test Used**

Test Used Populations (no. of studies)	All-Cause Death	CV Death	ACS	ST	Stroke	Bleeding	MACE	Other clinical outcomes	Platelet Reactivity
LTA (n=46) IHD=45; PAD=1	IHD=10	IHD=8	IHD=17	IHD=18	IHD=11	IHD=6	IHD=33	IHD=7	IHD=9 PAD=1
VerifyNow P2Y12 (n=28) IHD=25; CVD=3	IHD=5	IHD=6	IHD=14	IHD=9	IHD=5	IHD=8	IHD=17	IHD=3 CVD=3	IHD=3
VASP (n=14) IHD=14	IHD=3	IHD=6	IHD=6	IHD=7	IHD=1	No studies	IHD=7	IHD=4	IHD=6
Multiplate analyzer (n=12) IHD=11; CVD=1	IHD=4	IHD=4	IHD=7	IHD=7	IHD=3	IHD=5	IHD=9 CVD=1	IHD=4	IHD=1
TEG (n=5) IHD=5	IHD=1	No studies	IHD=1	No studies	No studies	IHD=2	IHD=4	No studies	No studies
PFA-100 (n=8) IHD=8	IHD=2	IHD=2	IHD=5	IHD=2	IHD=1	IHD=1	IHD=7	IHD=2	IHD=1
Other (n=7) IHD=7	IHD=2	No studies	IHD=2	IHD=1	IHD=1	IHD=2	IHD=4	IHD=2	IHD=3

ACS = acute coronary syndromes; CV = cardiovascular; CVD = cerebrovascular disease; IHD = ischemic heart disease, LTA = light-transmission aggregometry, MACE = major adverse cardiovascular events; PAD = peripheral arterial disease; PFA = Platelet Function Analyzer, ST = stent thrombosis, TEG = thromboelastography, VASP = vasodilator-stimulated phosphoprotein.
*Numbers indicate the number of available studies for each test–outcome combination in the population specified. (Studies could have involved more than one combination.) The ratings in brackets reflect our assessment of the strength of evidence for each test–outcome association.

LTA

Forty-six studies reported information on the predictive ability of platelet reactivity measured by LTA. Of these, 45 included patients with ischemic heart disease and 1 included patients with peripheral arterial disease. None of the studies included patients with cerebrovascular disease or other populations who require antiplatelet therapy. Detailed information on selection criteria, settings, and treatment strategies (including adjunctive treatments) used in these studies is presented in **Appendix E**.

[i] Please see the Methods section for additional details on how we assessed potential population overlap.

Overall, studies using LTA to measure platelet reactivity employed variable metrics, such as percent change of reactivity within a patient (termed "inhibition of platelet aggregation") or absolute values of on-clopidogrel reactivity at a single timepoint (termed "residual platelet reactivity"). Furthermore, the cutoff values for defining increased reactivity varied across studies, even among studies using the same metric. We decided not to perform meta-analyses for studies assessing platelet reactivity using LTA because for all outcomes considered, the heterogeneity in metrics and cutoff values would result in the inclusion of only a select subset of studies for each comparison. Instead, we narratively summarized study results and performed qualitative synthesis for each reported outcome.

Ischemic Heart Disease

Forty-five studies included patients with ischemic heart disease and reported information on the predictive value of platelet reactivity measured by LTA.[82,93,109-151] Of these, 40 studies assessed the value of the test for predicting clinical outcomes; and 9, for predicting platelet reactivity during followup (4 studies reported both clinical and intermediate outcomes). Thirty-two of the 45 studies enrolled patients with chronic stable coronary artery disease, 11 enrolled patients with acute coronary syndromes, and 2 studies enrolled mixed populations with chronic and acute presentations.

Clinical Outcomes

All-Cause Mortality

Ten studies reported information on the ability of platelet reactivity measured by LTA to predict all-cause mortality.[93,112,115,116,118,125,131,135,138,140] Most studies used ADP as the agonist to assess reactivity. One study used ADP in combination with arachidonic acid (AA) to assess the response to both clopidogrel and aspirin.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in five studies; derived from the observed study data in two studies; and not explicitly reported in three studies. Studies had variable sample sizes, ranging from 100 to 1058 enrolled patients. Of the 10 studies reporting relevant information on all-cause mortality, 7 did not report statistically significant results (5 studies reported higher rates of death in patients with higher reactivity and 2 did not report any deaths); additionally, 3 studies reported statistically significantly higher risk of deaths due to any cause in patients with high reactivity compared to those with low reactivity. In summary, reviewed studies suggest an association between increased platelet reactivity by LTA and all-cause mortality in patients with ischemic heart disease; however, results were inconclusive as heterogeneity between the studies prevents pooling of results.

Cardiovascular Mortality

Eight studies reported information on the ability of platelet reactivity measured by LTA to predict cardiovascular mortality.[117,125,128,130,141,143,149,150] All studies used ADP as the agonist for measuring platelet reactivity. One study used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[130] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in six studies, derived from the observed study data in one study,[117] and not explicitly reported in one study.[128] Studies had variable sample sizes, ranging from 106 to 1058 patients. Of the eight studies reporting relevant information, five reported a statistically significantly higher risk of death among patients with high platelet reactivity and three found no significant association when comparing patients with

high reactivity versus those with low reactivity. In summary, reviewed studies suggest an association between increased platelet reactivity by LTA and cardiovascular death in patients with ischemic heart disease; however, results were inconclusive, as heterogeneity between the studies prevented our pooling the results.

Acute Coronary Syndromes

Seventeen studies reported information on the ability of platelet reactivity measured by LTA to predict acute coronary syndromes.[93,112,115-118,125,128,131,135,140,143,148-152] All studies used ADP as the agonist to assess platelet reactivity. One study used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in 10 studies, derived from the observed study data in 3 studies, and were not explicitly reported in 4 studies. Studies had variable sample sizes, enrolling 26 to 1058 patients. Of the 16 studies reporting relevant information, 8 reported statistically significant associations between increased reactivity and myocardial infarction and eight did not reach statistical significance. In summary, results were inconclusive regarding the association between increased platelet reactivity as measured by LTA and acute coronary syndrome risk in patients with ischemic heart disease.

Stent Thrombosis

Eighteen studies reported information on the ability of platelet reactivity measured by LTA to predict stent thrombosis.[82,93,113,115-117,123,128-131,135,141,143,145,146,149,150] Three publications report data from the same population.[82,129,130] The longest available followup was considered for these studies. All studies used ADP as the agonist; two studies used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[116,130] Thresholds for defining high platelet reactivity were based on prior literature (or other sources external to the study) in eight studies, were derived from the observed study data in four studies, and were not explicitly reported in six studies. The numbers of patients included in the studies ranged from 105 to 1058. Of the 16 studies reporting relevant information, 12 reported statistically significant associations between increased reactivity and stent thrombosis and 4 did not report statistically significant results. Taken together, studies suggested an association between increased platelet reactivity (as measured by LTA) and increased risk of stent thrombosis in patients with ischemic heart disease.

MACE

Thirty-three studies reported information on the ability of platelet reactivity measured by LTA to predict composites of major adverse cardiovascular events for clopidogrel-treated patients with ischemic heart disease. Three studies reported data from the same population.[82,129,130] The longest followup was considered for these studies. All studies used ADP as the agonist to measure platelet reactivity; two studies used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[116,130] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in 14 studies, were derived from the observed study data in 12 studies, and were not explicitly reported in 7 studies. Studies had variable sample sizes, ranging from 100 to 1335 enrolled patients. Of the 30 studies reporting relevant information, 27 reported statistically significant associations between increased platelet reactivity and risk of MACE; only 3 did not find a statistically significant association. In summary, the majority of reviewed studies suggested an association between increased platelet reactivity by LTA and MACE in patients with ischemic heart disease.

Stroke

Eleven studies reported information on the ability of platelet reactivity measured by LTA to predict stroke.[112,115,116,125,128,131,135,140,141,143,150] All studies used ADP as the agonist to measure reactivity. One study also used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in eight studies, were derived from the observed study data in two studies, and were not explicitly reported in one study.[128] Sample sizes ranged from 100 to 1058. Of the 11 studies reporting relevant information on stroke, 9 did not report statistically significant results. One study reported that clopidogrel nonresponders had higher stroke risk than patients who were low responders or normal responders.[143] One study reported a significantly higher stroke risk in patients who were either clopidogrel nonresponders or clopidogrel and aspirin nonresponders than responders to both drugs or to aspirin alone.[116] In summary, reviewed studies did not suggest an association between increased platelet reactivity as measured by LTA and stroke in patients with ischemic heart disease; however, results were inconclusive, as heterogeneity in reactivity measurements prevented our conducting quantitative synthesis and study specific estimates had wide CIs, indicating considerable uncertainty.

Bleeding Events

Six studies reported information on the ability of platelet reactivity measured by LTA to predict bleeding events.[112,120,123,138,141,143] All studies used ADP as the agonist for measuring reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four studies and were not explicitly reported in two studies. Sample sizes ranged from 100 to 1058patients. Of the six studies reporting relevant information on bleeding events, five did not report statistically significant associations; one study reported significantly higher bleeding rate in "hyper-responders" (i.e., patients with ADP-induced aggregation <40%).[123] In summary, the studies did not suggest an association between increased platelet reactivity as measured by LTA and bleeding events in patients with ischemic heart disease; however, results were considered inconclusive because of between-study heterogeneity in reactivity measurements that precluded quantitative synthesis.

Other Clinical Outcomes

Seven studies reported information on the ability of platelet reactivity measured by LTA to predict other clinical outcomes.[93,112,118,131,135,143,149] Six studies reported target-vessel revascularization as an outcome,[93,112,118,131,135,149] two reported non–target-vessel revascularization as an outcome,[112,135], one reported on recurrent ischemia,[143] and two reported on rehospitalization for ischemia.[112,135] All studies used ADP as the assay to measure platelet reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in five studies, were derived from the observed study data in one study,[135] and were not explicitly reported in one study.[118] Sample sizes were variable, ranging from 96 to 802 patients. Of the six studies reporting information on target-vessel revascularization, four did not report statistically significant associations and two reported significantly higher event rates of target-vessel revascularization among patients with increased platelet reactivity. Two studies on non–target-vessel revascularization and two studies on recurrent ischemia also reported significantly higher event rates among patients with increased platelet reactivity. In summary, reviewed studies did not provide sufficient evidence for an association between increased platelet reactivity as measured by LTA and target-vessel revascularization, non–target-vessel revascularization, or recurrent ischemia.

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

Six studies[109,111,119,143,144,147] reported on the predictive value of baseline on-clopidogrel platelet reactivity measured by LTA on platelet reactivity as a continuous outcome assessed during subsequent followup timepoints. In these studies reactivity was assessed using LTA in four studies (72 percent); the VASP assay in one (14 percent); and flow cytometry in one (14 percent).

Four of the studies enrolled patients with acute coronary syndromes,[111,119,144,147] whereas two studies[109,143] enrolled patients with chronic ischemic heart disease. Five studies were conducted in the setting of interventional cardiac procedures (four with PCI and two with PCI with stenting); one study treated patients with medical therapy.

Studies generally showed that the mean or median reactivity was higher among clopidogrel-treated patients who were classified either as nonresponders or as slow responders at baseline, as compared to responders. Results were reported for different followup periods (ranging from a few hours post clopidogrel loading to a followup of 1 year) and measurements were obtained using different assays and different calculation methods (percent change within a patient or absolute value at last followup). All of the six studies reported that platelet reactivity during followup was statistically significantly higher among patients with high baseline reactivity compared to those with low or normal baseline reactivity. Because of the extensive between-study differences we did not perform meta-analyses for studies using reactivity as the outcome of interest.

Reactivity as a Categorical Outcome

Three studies[132,137,139] reported information on the association between baseline reactivity measured by LTA and platelet reactivity during followup reported as a categorical outcome. In two studies reactivity (during followup) was assessed using LTA only[132,139]; in one study it was assessed using 3 different assays (LTA, VASP and VerifyNow P2Y12).[137] Cutoff values for assessing reactivity were variable (among studies using the same assay). Two studies explicitly provided the rationale for selecting a specific cutoff.[137,139] In both, the cutoff value was based on prior literature (i.e., information external to the dataset where the cutoff value was applied). All studies enrolled patients with chronic ischemic heart disease. Two studies were conducted in the setting of interventional cardiac procedures,[137,139] whereas one included patients managed medically.[132] One of the studies reported data in graphical form which was not amenable to quantitative analysis.[139] When 20 μmol/L ADP was used as an agonist at baseline measurement, response status was not significantly associated with platelet reactivity after 30 days of followup (using LTA or VerifyNow). Increased baseline reactivity by LTA was significantly associated with platelet reactivity during followup measured by the VASP assay. When 5 μmol/L ADP was used as an agonist at baseline measurement, platelet reactivity by LTA was significantly associated with reactivity after 30 days of followup.

In summary, while studies suggested an association between increased platelet reactivity at baseline (measured by LTA) and subsequent platelet reactivity measurements (with different assays), the results are inconclusive due to sparseness of the data.

Patients with Peripheral Arterial Disease

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Categorical Outcome

One study[153] reported on the effect using LTA on platelet reactivity as a categorical outcome for clopidogrel-treated patients with peripheral artery disease. In this study, both baseline and follow up measurements were performed with LTA. The cutoff value used to assess nonresponsiveness was based on prior literature (i.e., information external to the dataset where the cutoff value was applied). The study showed that baseline LTA reactivity was statistically significantly associated with the of responding patients after a median followup of 17.5 months.

VerifyNow P2Y12

Twenty-eight studies reported information on the predictive ability of the VerifyNow P2Y12 assay. Of these, 25 studies included patients with ischemic heart disease and three studies included patients with cerebrovascular disease. None of the studies included patients with peripheral arterial disease or other populations receiving clopidogrel-based antiplatelet therapy. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E**.

Patients with Ischemic Heart Disease

Twenty-five studies included patients with ischemic heart disease and reported information on the predictive value of the VerifyNow P2Y12 assay (this assay uses ADP as the agonist to assess reactivity).[77,115,116,141,154-174] Of these, 24 assessed the value of the test for predicting clinical outcomes and three for predicting platelet reactivity during followup (two studies reported both clinical and platelet reactivity outcomes). Of the 25 studies, 16 enrolled patients with chronic stable coronary artery disease, eight enrolled patients with acute coronary syndromes, and one enrolled mixed populations with chronic and acute presentations.

Clinical Outcomes

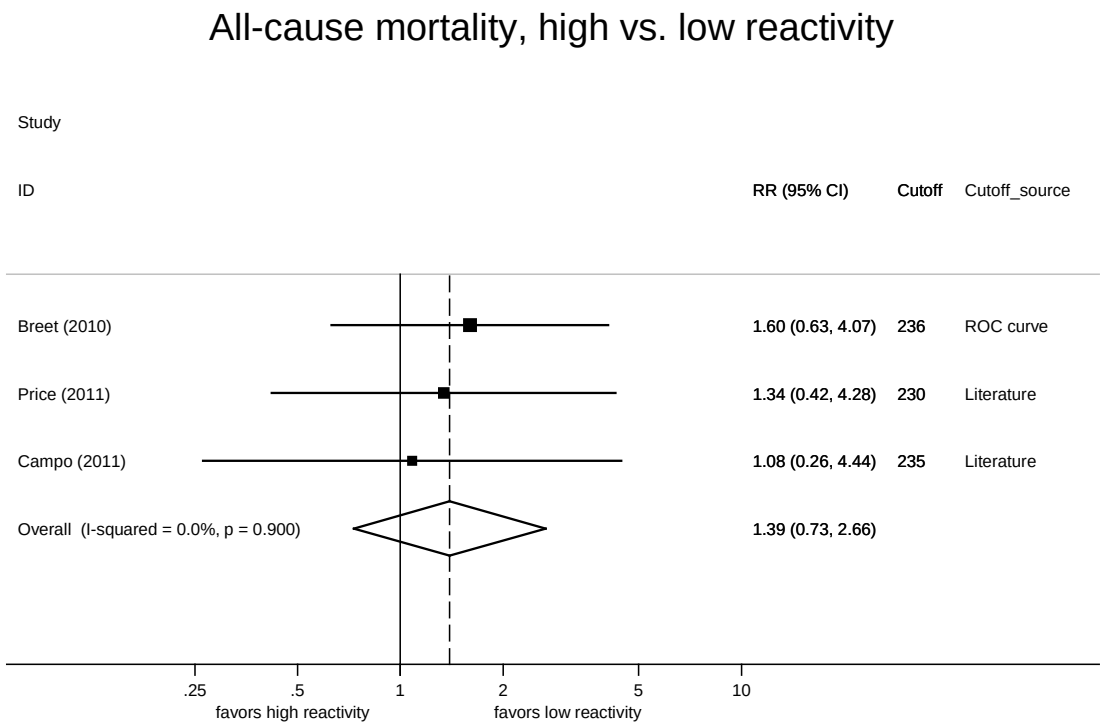
All-Cause Mortality

Five studies reported information on the ability of the VerifyNow P2Y12 assay to predict all-cause mortality for patients receiving clopidogrel-based treatment.[77,115,116,154,170] One study used a combination of ADP and AA (in different assays) to define a population of combined clopidogrel and aspirin responders.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three of the studies and derived from the observed study data in two of the studies (two of the studies used both approaches). Studies were generally large; sample sizes ranged from 380 to 1691 patients. All five studies did not report a statistically significant association between platelet reactivity (measured by the VerifyNow assay) and all-cause mortality.

Figure 19 presents a meta-analysis of the three studies that used ADP as the agonist and defined high platelet reactivity based on platelet reactivity units. The summary RR was 1.39 (95% CI 0.73, 2.66); P=0.313, indicating no significant association between high platelet reactivity and all-cause mortality. There was little evidence of between study heterogeneity

(P_Q=0.900; I^2=0 percent). The two studies not included in the meta-analysis did not report a significant association between higher platelet reactivity and increased all-cause mortality.

Figure 19: Meta-analysis of All-Cause Mortality Comparing Patients with High vs. Low Reactivity Measured Using the VerifyNow P2Y12 Assay



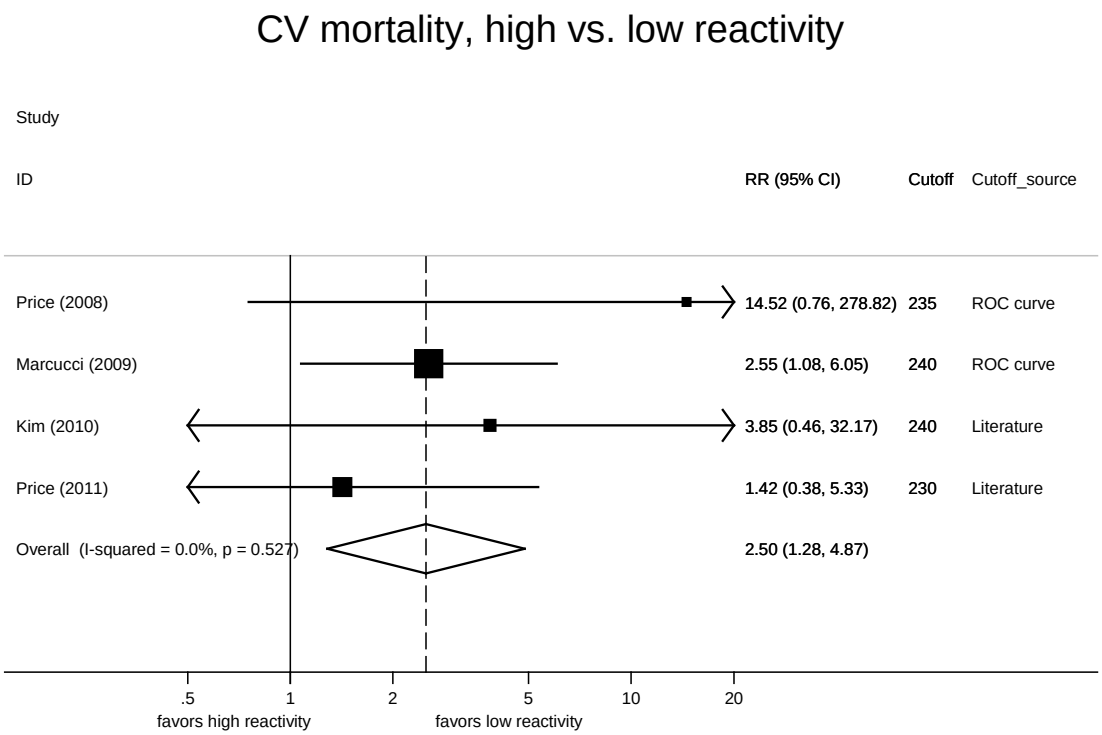
CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of all-cause mortality (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Cardiovascular Mortality

Six studies reported information on the ability of the VerifyNow P2Y12 assay to predict cardiovascular mortality for patients receiving clopidogrel-based treatment.[141,160,162,166,169,170] The assay uses ADP as the agonist to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four of the six studies and derived from the observed study data in two of the studies. Studies had moderate to large sample sizes, ranging from 110 to 1691 included patients.

Figure 20 presents a meta-analysis of the four studies that used cutoff values based on platelet reactivity units (meta-analysis was not performed for 2 other studies using percent platelet inhibition to define reactivity). The summary RR was 2.50 (95% CI 1.28, 4.87); P=0.007, indicating a significant association between high platelet reactivity and cardiovascular mortality. There was little evidence of between study heterogeneity (P_Q=0.527; I^2=0 percent). The two studies not included in the meta-analysis did not report a significant association between higher platelet reactivity and increased cardiovascular mortality.

Figure 20: Meta-analysis of All-Cause Mortality Comparing Patients with High vs. Low Reactivity Measured Using the VerifyNow P2Y12 Assay



CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of cardiovascular mortality (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Acute Coronary Syndromes

Fourteen studies reported information on the ability of the VerifyNow P2Y12 assay to predict myocardial infarction[156,161,163-165,173] or acute coronary syndromes over a longer followup[115,116,141,154,160,166,169,170] for patients receiving clopidogrel-based treatment. One study used a combination of ADP and AA (in different assays) to define a population of combined clopidogrel and aspirin responders.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in seven studies; derived from the observed study data in five studies; and it was not explicitly reported in two studies . Studies were generally large; sample sizes ranged from 110 to 1691. Because of heterogeneity in the study populations, and the metrics and thresholds used to define increased platelet reactivity, we did not perform meta-analyses of predictive ability for this group of studies. Of the six studies reporting information on periprocedural myocardial infarctions, four studies reported a statistically significant association between higher reactivity levels and periprocedural events and two did not report statistically significant results. Of the eight studies reporting information on acute coronary syndromes during followup, four reported a statistically significant association between increased reactivity and risk of acute coronary syndromes, three did not report statistically significant results, and one reported that patients with low response to both aspirin and clopidogrel had significantly higher risks compared to patients with adequate response.

Taken together, studies suggested an association between increased platelet reactivity by VerifyNow and both periprocedural and non-periprocedural acute coronary syndromes in patients with ischemic heart disease.

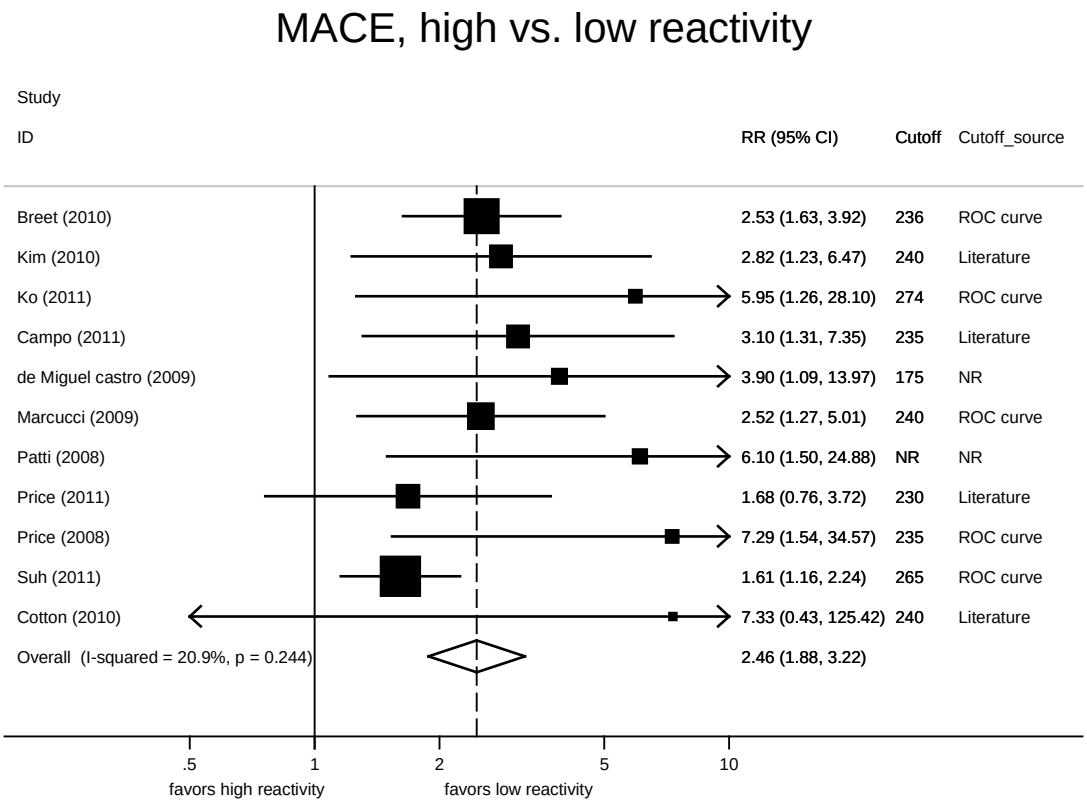
Stent Thrombosis

Nine studies reported information on the ability of the VerifyNow P2Y12 assay to predict stent thrombosis for patients receiving clopidogrel-based treatment.[77,115,116,141,154,160,162,169,170] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in seven of the nine studies and derived from the observed study data in two of the studies. Studies were generally large; sample sizes ranged from 110 to 1691. Because of heterogeneity in the metrics used to define platelet reactivity we did not perform meta-analyses of predictive ability for this group of studies. Of the nine studies reporting relevant information, six did not report statistically significant results and produced relatively wide CIs, indicating substantial uncertainty around estimates of the RR; three studies reported statistically significant associations between high reactivity with risk of stent thrombosis. Overall, there was weak evidence to support an association between increased platelet reactivity by VerifyNow and stent thrombosis in patients with ischemic heart disease; results were inconclusive due to heterogeneity that precluded quantitative synthesis.

MACE

Seventeen studies reported information regarding the ability of the VerifyNow P2Y12 assay to predict MACE for patients receiving clopidogrel-based treatment. One study used a combination of ADP and AA (different assays) to define a population of combined clopidogrel and aspirin responders[116]. Of the 16 remaining studies, 11 enrolled nonoverlapping patients populations and used cutoff values for platelet reactivity based on platelet reactivity units (the 5 studies not included in meta-analysis used other methods to define platelet reactivity or did not provide adequate data for inclusion). The summary RR was 2.46 (95% CI 1.88, 3.22); P<0.001 and there was little evidence of heterogeneity (P_Q=0.244; I^2=21 percent). Results from the meta-analysis are presented in **Figure 21.** Among the five studies not included in the meta-analysis, three used percentage of platelet inhibition to define platelet reactivity and two studies did not provide adequate data for inclusion. Among the three studies that used percentage of platelet inhibition to define platelet reactivity, two studies reported significantly higher rates of MACE at 6 months in those with a low response to clopidogrel; one study reported lower rates of MACE at 30 days in those with a low response to clopidogrel.

Figure 21: Meta-analysis of Major Adverse Cardiovascular Events Comparing Patients with High vs. Low Reactivity Measured Using the VerifyNow P2Y12 Assay



CI = confidence interval; MACE = major adverse cardiovascular events; NR = not reported; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of major adverse cardiovascular events (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Stroke

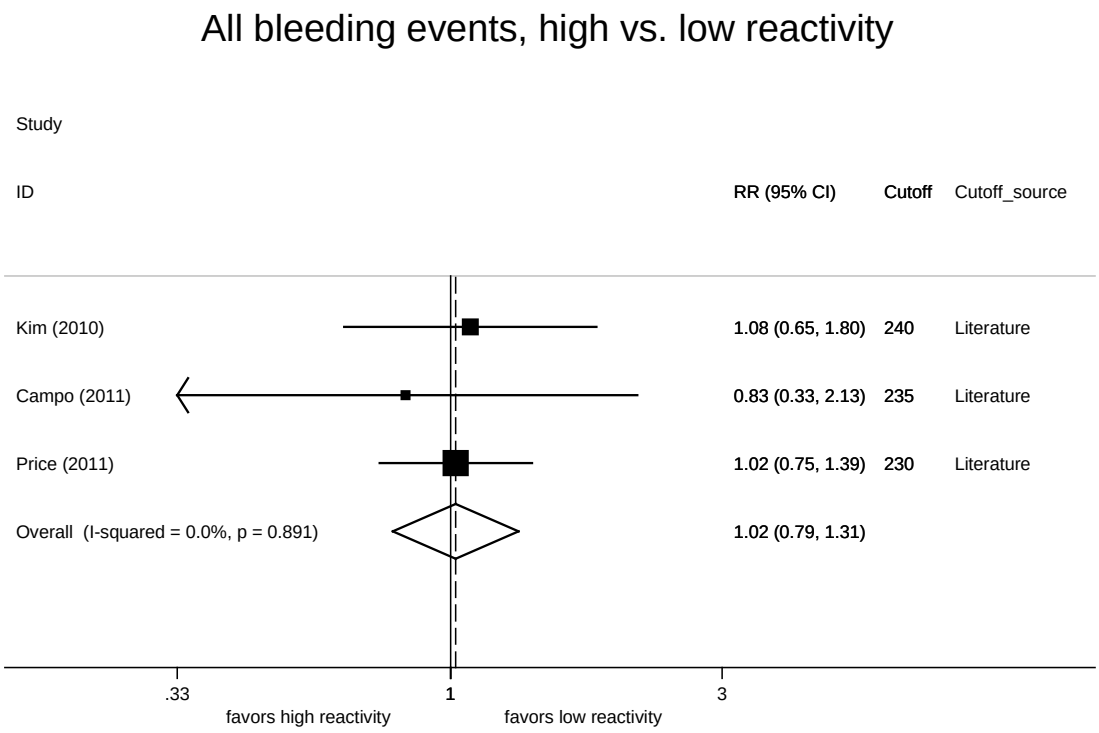
Five studies reported information on the ability of the VerifyNow P2Y12 assay to predict strokes for patients receiving clopidogrel-based treatment.[115,116,141,154,160] One study used a combination of ADP and AA (different assays) to define a population of combined clopidogrel and aspirin responders.[116] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four of the five studies; and derived from the observed study data in one study.[115] Studies were generally large; sample sizes ranged from 110 to 1069. Because of heterogeneity in the metrics used to define platelet reactivity we did not perform meta-analyses of predictive ability for this group of studies. Four studies using ADP as the agonist did not report statistically significant associations. The fifth study did not find a statistically significant difference in the risk of stroke between patients with low response to both aspirin and clopidogrel compared to patients with adequate response.

Bleeding Events

Eight studies reported information regarding the ability of the VerifyNow P2Y12 assay to predict bleeding events for patients receiving clopidogrel-based treatment. Of these, three studies reported results based on percent inhibition and used different cutoff values for reactivity; thus they were not considered adequately similar for meta-analysis. Of the remaining five studies one reported that no events were observed (regardless of reactivity status), leaving four nonoverlapping studies for meta-analysis (3 reported information on bleeding events regardless of severity and all 4 reported information on major bleeding events).

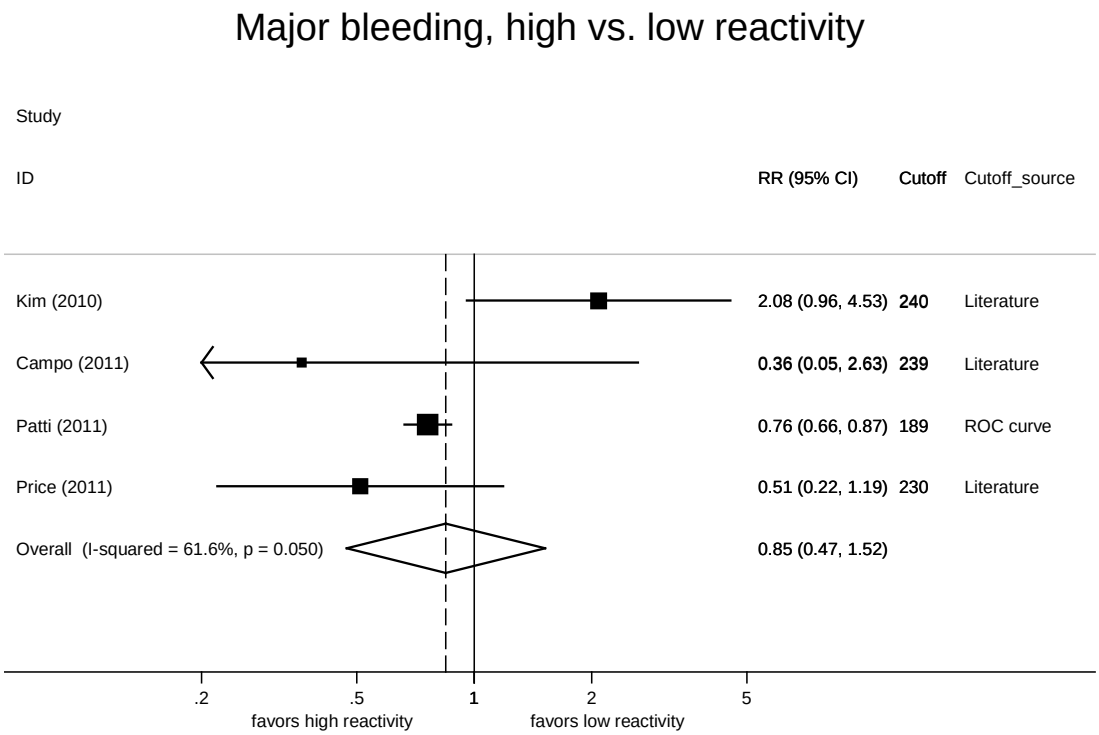
Figure 22 presents meta-analysis results for all bleeding events combined. The summary RR was 1.02 (95% CI 0.79, 1.31); P=0.881, with little evidence for heterogeneity (P_Q=0.891; I^2=0 percent). **Figure 23** presents meta-analysis results for major bleeding events. The summary RR was 0.85 (95% CI 0.47, 1.52); P=0.575, with evidence of substantial heterogeneity (P_Q=0.050; I^2=62 percent). Interestingly, heterogeneity appeared to be due to the inclusion of a single study that used a lower threshold to define platelet reactivity compared to others.

Figure 22: Meta-analysis of Bleeding Events Comparing Patients with High vs. Low Reactivity Measured Using the VerifyNow P2Y12 Assay



CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of bleeding events (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Figure 23: Meta-analysis of Major Bleeding Events Comparing Patients with High vs. Low Reactivity Measured Using the VerifyNow P2Y12 Assay



CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of major bleeding events (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Other Clinical Outcomes

Three studies reported information on the ability of the VerifyNow P2Y12 assay to predict other clinical outcomes.[160,166,168] Two studies reported information on target level revascularization,[160,166] and one study on a composite of PCI entry-site complications such as hematoma, pseudoaneurysm, arteriovenous fistula, and bleeding[168] Thresholds for defining "positive" results were derived from the observed study data in two of the studies and based on prior literature (or other sources external to the study) in two studies (one study used both approaches). The metrics used to define positive results were calculated differently across studies. Studies were generally large; sample sizes ranged from 110 to 683. Because of heterogeneity in the outcomes and thresholds used, we did not perform meta-analyses of predictive ability for this group of studies. Two studies did not report any statistically significant associations; one study reported a statistically significant association between increased platelet reactivity and risk of PCI entry-site complications.

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

One study[155] reported information on the predictive value of baseline platelet reactivity status (ascertained with the VerifyNow P2Y12) on platelet reactivity during followup measured as a continuous outcome. In this study, baseline reactivity was assessed using the VerifyNow assay. The study enrolled patients with acute coronary syndromes[155] and was conducted in the setting of interventional cardiac procedures—a mix of PCI and PCI with stenting.[155] The study reported that patients with poor response at baseline had significantly higher reactivity 1 month after PCI compared to those with adequate response.

Reactivity as a Categorical Outcome

Two studies[77,158] reported information on the value of baseline platelet reactivity status (ascertained using VerifyNow P2Y12) for predicting platelet reactivity during followup as a categorical outcome. In both studies, baseline reactivity was assessed using the VerifyNow assay. Cutoff values and metrics for defining reactivity were variable; one study used absolute reactivity as the metric with a cutoff value derived from literature.[77] The other study used the percent within-patient change to define reactivity.[158] Both studies enrolled patients with chronic ischemic heart disease and both were conducted in the setting of interventional cardiac procedures.

Results were reported for different followup periods (ranging from a 7 days to 1 month). Response status during followup in all studies was measured by the same assays that were used at baseline. One of the studies reported a 100 percent sensitivity of baseline response status in predicting status at 7 day status;[158] the other study reported a statistically significant difference in platelet reactivity during followup comparing nonresponders and responders at baseline.[77]

In summary, while suggested an association between platelet reactivity at baseline and measurement obtained during followup, results were inconclusive due to the small number of available studies.

Patients with Cerebrovascular Disease

Three studies[175-177] included patients with cerebrovascular disease and reported information on the predictive value of the VerifyNow assay on clinical outcomes. No studies reported information on the predictive value of the VerifyNow assay on platelet reactivity during followup. All three studies were conducted in the setting of interventional procedures (stent implantation and coil embolization).

Clinical Outcomes

Studies reported information on the ability of the VerifyNow P2Y12 assay to predict only clinical outcomes for clopidogrel-treated patients with cerebrovascular disease. No studies reported information on the ability of the VerifyNow P2Y12 assay to predict platelet reactivity as a continuous or categorical outcome for clopidogrel-treated patients with cerebrovascular disease.

Three studies[175-177] reported information on the ability of the VerifyNow P2Y12 assay to predict various clinical outcomes. One study reported outcomes such as intraprocedural thrombosis, thromboembolic event, good function score on modified Rankin Scale, good function Glasgow Outcome Score;[177] another reported procedure-related thromboembolism and procedure-related aneurysm perforation[176]; the third study reported thromboembolic

complications of the procedure.[175] Thresholds for defining "positive" results were not explicitly reported in any of the studies. The metrics used to define positive results were calculated differently across studies. Studies were small; sample sizes ranged from 52 to 186. Because of heterogeneity in the thresholds used, we did not perform meta-analyses of predictive ability for this group of studies. The results from two studies indicated that increased reactivity was statistically significantly associated with increased event rates of the outcomes being studied; another study reported 75 percent sensitivity for clopidogrel resistance to predict thromboembolic events.

VASP Assay with Flow Cytometry

Fourteen studies reported information on the predictive ability of the VASP assay.[113,124,178-189] All 14 studies included patients with ischemic heart disease; none involved patients with cerebrovascular or peripheral arterial disease. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E**.

Patients with Ischemic Heart Disease

Fourteen studies included patients with ischemic heart disease and reported information on the predictive value of the VASP assay. Of these, 10 assessed the value of the test for predicting clinical outcomes and five for predicting platelet reactivity during followup (1 study reported both clinical and platelet reactivity outcomes). Of the 14 studies, six enrolled patients with acute coronary syndromes, four enrolled patients with chronic stable coronary artery disease, and four enrolled mixed populations with chronic and acute presentations.

All-Cause Mortality

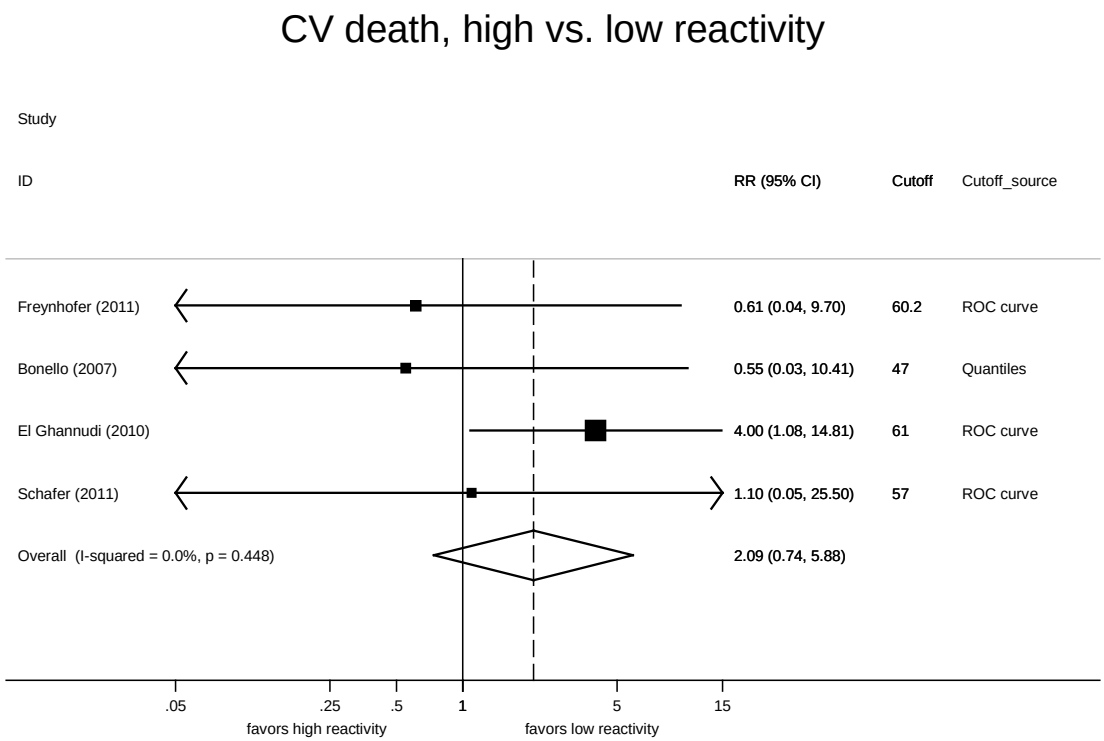
Three studies reported information on the ability of the VASP assay to predict all-cause mortality for patients receiving clopidogrel-based treatment.[181,182,185] The assay uses ADP as the agonist to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in two of the studies;[182,185] and derived from the observed study data in one study.[181] Studies were generally large; sample sizes ranged from 436 to 460. All of the three studies reported statistically significant association of higher mortality with high platelet reactivity or low response to clopidogrel (however, we considered that all studies enrolled overlapping patient populations on the basis of their population base and enrollment periods). In summary, data suggested an association between increased platelet reactivity by VASP and all-cause mortality in patients with ischemic heart disease; however, results were inconclusive due to limited effective information size.

Cardiovascular Mortality

Six studies reported information on the ability of the VASP assay to predict all-cause mortality for patients receiving clopidogrel-based treatment. Two of the studies were conducted in overplapping patient populations (on the basis of study base and enrollment periods). **Figure 24** presents a meta-analysis of the remaining four studies that used cutoff values based on platelet reactivity units (meta-analysis was not performed for the other two studies using percent platelet inhibition to define reactivity). The summary RR was 2.09 (95% CI 0.74, 5.88); P=0.164. There was little evidence of between study heterogeneity (P_Q=0.448; I^2=0 percent). Even though the test for heterogeneity was not statistically significant, studies produced point

estimates that ranged from substantial protective effects to large increases in risk. Results were imprecise and confidence intervals could not exclude a clinically significant predictive effect.

Figure 24: Meta-analysis of Cardiovascular Mortality Comparing Patients with High vs. Low Reactivity Measured Using the VASP Assay with Flow Cytometry

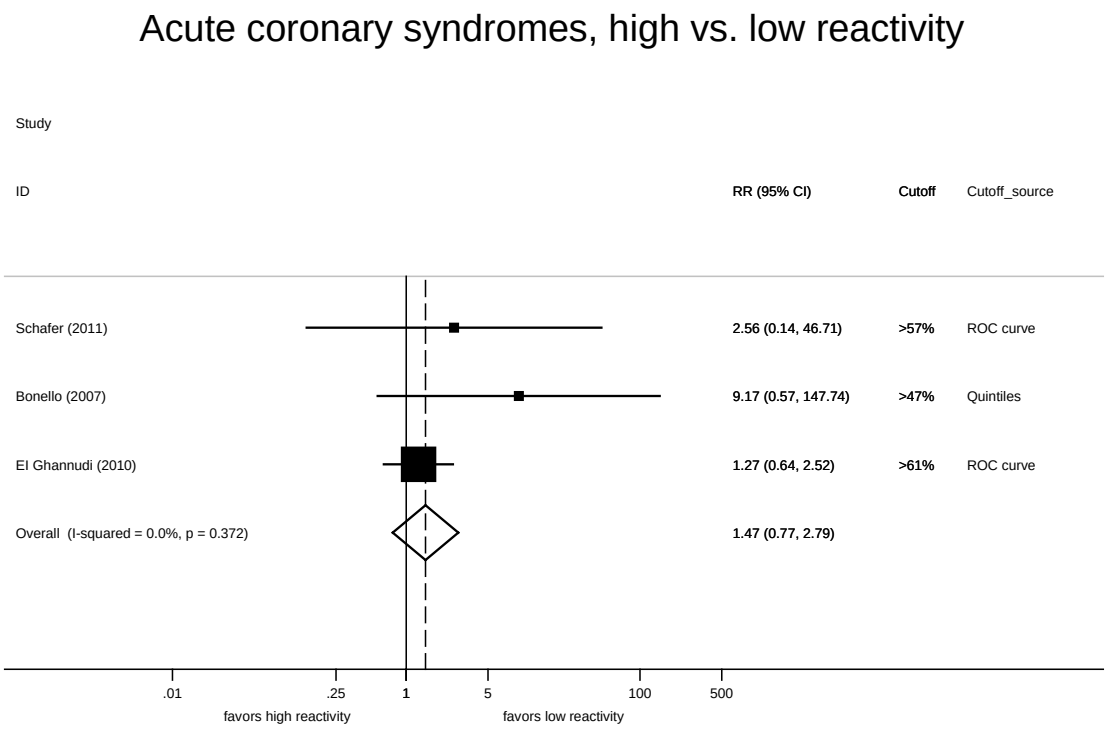


CI = confidence interval; CV = cardiovascular; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk; VASP = vasodilator-stimulated phosphoprotein.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of CV mortality (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Acute Coronary Syndromes

Six studies reported information on the ability of the VASP assay to predict acute coronary syndromes for patients receiving clopidogrel-based treatment. One study reported that no events occurred regardless of platelet reactivity status. Of the remaining five studies, three reported on overlapping populations (on the basis of study base and enrollment periods). **Figure 25** presents a meta-analysis of the three studies (one study contributed separate estimates by diabetes status). The summary RR was 1.47 (95% CI 0.77, 2.79); P=0.246, indicating that the association between high platelet reactivity and acute coronary syndromes was not statistically significant. There was little evidence of between-study heterogeneity (P_Q=0.372; I^2=0 percent).

Figure 25: Meta-analysis of Acute Coronary Syndromes Comparing Patients with High vs. Low Reactivity Measured Using the VASP Assay with Flow Cytometry

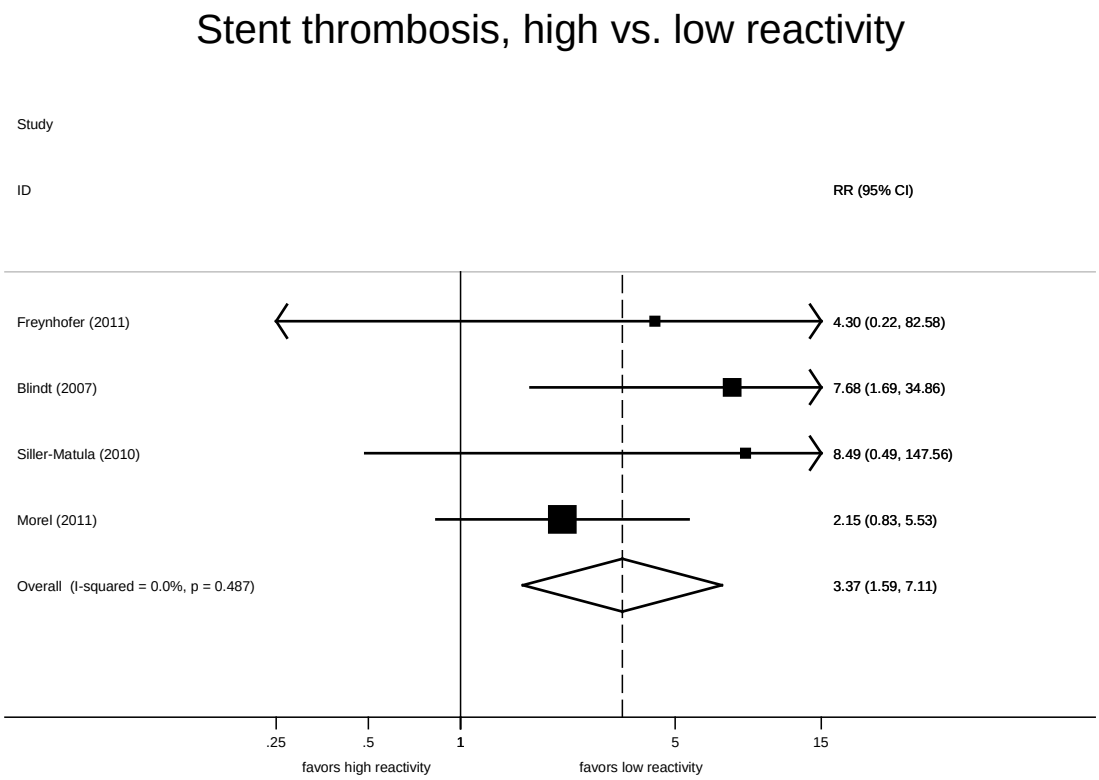


CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk, VASP = vasodilator-stimulated phosphoprotein.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of acute coronary syndromes (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Stent Thrombosis

Seven studies provided information on stent thrombosis (definite or probable, when study definitions were available). One study reported that no events were observed (regardless of platelet reactivity status). Of the remaining six studies, three enrolled overlapping populations (on the basis of study base and enrollment periods; we used data from the publication reporting the largest number of events). **Figure 26** presents a meta-analysis of the four nonoverlapping studies. The summary RR was 3.37 (95% CI 1.59, 7.11); P=0.015, indicating a significant association between high platelet reactivity and stent thrombosis. There was evidence of moderate between-study heterogeneity (P_Q=0.487; I^2=0 percent).

Figure 26: Meta-analysis of Stent Thrombosis Comparing Patients with High vs. Low Reactivity Measured Using the VASP Assay with Flow Cytometry

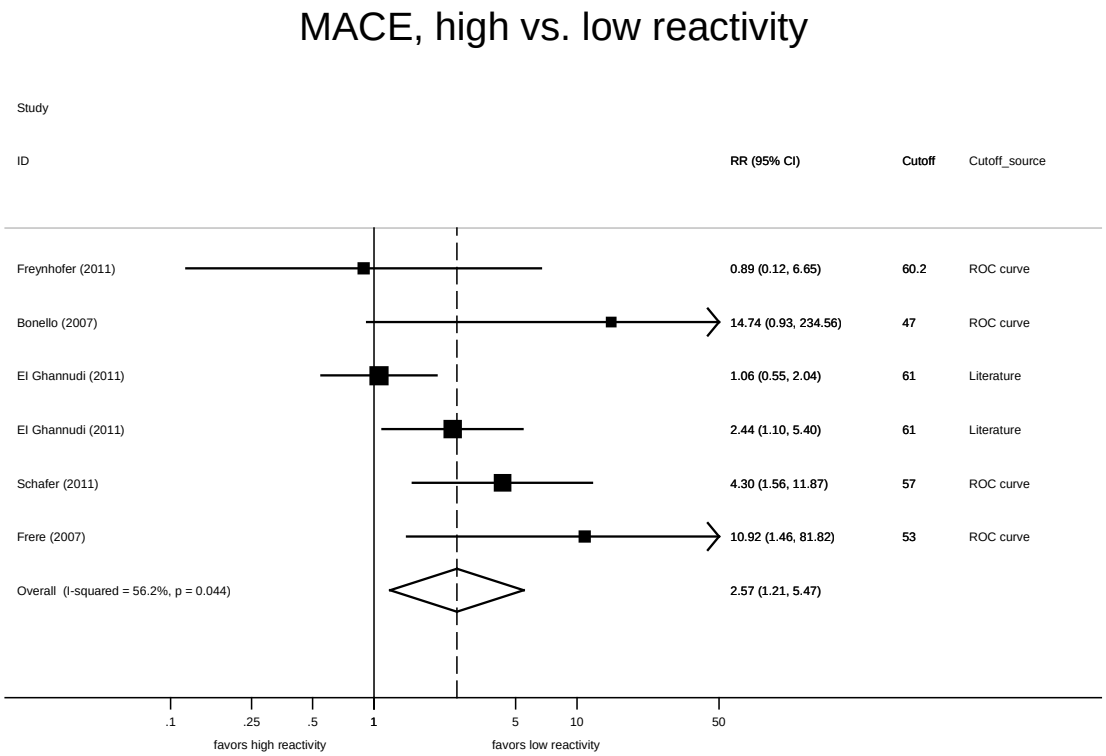


CI = confidence interval; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk, VASP = vasodilator-stimulated phosphoprotein.
The solid squares (and horizontal lines) indicate the RR of stent thrombosis (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

MACE

Seven studies reported information on the ability of the VASP assay to predict MACE for patients receiving clopidogrel-based treatment. Two studies reported on overlapping patient populations based on source population and enrollment periods (in meta-analyses we included the publication reporting on the largest total number of cardiovascular events). **Figure 27** presents a meta-analysis of the six nonoverlapping studies (one study contributed separate estimates by diabetes status). The summary RR was 2.57 (95% CI 1.21, 5.47); P=0.015, indicating a significant association between high platelet reactivity and major adverse cardiovascular events. There was evidence of moderate between-study heterogeneity (P_Q=0.044; I^2=56 percent).

Figure 27: Meta-analysis of MACE Comparing Patients with High vs. Low Reactivity Measured Using the VASP Assay with Flow Cytometry



CI = confidence interval; MACE = major adverse cardiovascular events; ROC curve = receiver-operating-characteristic curve analysis; RR = relative risk; VASP = vasodilator-stimulated phosphoprotein.
Cutoff values, expressed in platelet reactivity units, are shown for high (above the cutoff) and low (below the cutoff) reactivity; the source of the cutoff value is also shown. The solid squares (and horizontal lines) indicate the RR of major adverse cardiovascular events (and the corresponding 95% CI) for individual studies. The size of the squares is proportional to the weight of each study in the meta-analysis. The dashed vertical line represents the summary RR, with the open diamond showing the corresponding CI. The solid line indicates an RR of 1.

Stroke

One study reported information on the ability of the VASP assay to predict strokes for patients receiving clopidogrel-based treatment.[187] The study used ADP as an agonist to assess reactivity and the thresholds for defining "positive" results were derived from both the observed study data and from literature. The study enrolled 54 patients with acute ST elevation myocardial infarction admitted for a coronary intervention with a followup time of 12 months. This suggests an association between increased platelet reactivity by the VASP assay and stroke, but the result is inconclusive owing to insufficient information.

Other Clinical Outcomes

Four studies reported information on the ability of the VASP assay to predict other clinical outcomes for patients receiving clopidogrel-based treatment.[179,180,182,187] Target lesion revascularization was studied in three studies; other outcomes that were reported were the rates of followup interventions such as PCI and CABG surgery. All the studies used ADP as an agonist to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in two studies; they were not explicitly reported

in one study; and they were derived from the observed study data and literature in the one study. Study sample sizes ranged from 54 to 436; followup time ranged from 6 to 12 months. Because of clinical heterogeneity across studies we did not perform meta-analyses of predictive ability. Of the three studies reporting information on target-vessel revascularization, two did not report statistically significant results and one study reported that no events occurred (regardless of reactivity status). The studies reporting on followup interventions documented statistically significantly increased rates of the intervention with increased reactivity.

No studies reported information on bleeding events for this patient population.

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

One study[184] reported information on the value of the VASP assay for predicting platelet reactivity during followup as a continuous outcome. In this study, reactivity at baseline was assessed using LTA with different concentrations of ADP as an agonist. Platelet reactivity was also assessed using P-selectin expression, platelet/monocyte conjugates, and platelet/neutrophil conjugates after ADP stimulation. The study enrolled patients with acute coronary syndromes and was conducted in the setting of interventional cardiac procedures (PCI). The study showed that the patients with a poor response at baseline had significantly higher reactivity one month after PCI, compared to patients with adequate response at baseline, irrespective of the methods used to assess reactivity.

Reactivity as a Categorical Outcome

Five studies[178,180,184,186,188] reported information on the value of the VASP assay for predicting platelet reactivity during followup as a continuous outcome. In these studies reactivity during followup was assessed using the VASP assay in four studies and by LTA in one study. Three of the five studies enrolled patients with acute coronary syndromes,[178,180,186] whereas two studies[184,188] enrolled patients with chronic ischemic heart disease. All five studies were conducted in the setting of interventional cardiac procedures—three, in patients with PCI[184,186,188]; one, in patients with PCI with stenting[180]; and one, in patients with coronary angiography.[178]

Of the four studies with available data, three studies showed that the VASP assay was not a significant predictor of future platelet reactivity.[180,186,188] In one study, baseline reactivity measured with the VASP assay was significantly associated with response status at 1 month.[184] One study did not allow for conclusions because none of the patients at baseline could be categorized as nonresponders.[178]

Multiplate Analyzer

Twelve studies reported information on the predictive ability of the Multiplate analyzer assay. Of these, 11 included patients with ischemic heart disease and one included patients with cerebrovascular disease. No study enrolled patients with peripheral arterial disease. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E**.

Patients with Ischemic Heart Disease

Eleven studies included patients with ischemic heart disease and reported information on the predictive value of the Multiplate analyzer assay.[161,183,188-196] All 11 studies assessed the value of

the test for predicting clinical outcomes. No study assessed the value of the test for predicting platelet reactivity during followup. Eight of the 11 enrolled patients with chronic stable coronary artery disease; the remaining included mixed populations with chronic coronary disease and acute coronary syndromes.

Clinical Outcomes

All-Cause Mortality

Four studies reported information on the ability of the Multiplate analyzer assay to predict all-cause mortality for patients receiving clopidogrel-based treatment.[190,192,193,196] Three studies appear to have been based on the same patient populations, reporting results at 30 days,[193]6 months,[196] and 1 year of followup.[192] All the studies used ADP as an agonist to assess reactivity. One study also used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[190] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in the three studies of the same patient population[192,193,196]; they were derived from the observed study data and literature in the other study.[190] The three studies of the same patient population enrolled 1608 patients; the other study had a sample size of 219 patients. Followup durations were 30 days to 12 months. Because of the limited number of studies in nonoverlapping populations we did not perform meta-analyses of predictive ability. Of the three studies conducted in the same patients, the study with the longest followup did not find a statistically significant difference in the risk of all-cause mortality among patients with high platelet reactivity.[192] The other study, reporting on groups stratified by a combination of high reactivity to both clopidogrel (using ADP as the agonist) and aspirin (using AA as the agonist), also did not report statistically significantly higher event rates in patient groups having high reactivity to clopidogrel only or to both clopidogrel and aspirin, as compared to the other groups.[190] In summary, the reviewed studies do not suggest an association between increased platelet reactivity by the Multiplate analyzer assay and all-cause mortality in patients with ischemic heart disease; however, results were inconclusive due to the limited number of studies in nonoverlapping patient populations providing information.

Cardiovascular Mortality

Four studies reported information on the ability of the Multiplate analyzer assay to predict cardiovascular mortality for patients receiving clopidogrel-based treatment.[183,191,192,196] Two studies reported information from the same patient population, with one reporting outcomes at 6 months of followup[196] and the other at 1 year.[192] All studies used ADP as the agonist to assess reactivity. One study also used ADP in combination with AA to evaluate both clopidogrel and aspirin.[191] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three studies;[183,192,196] they were not explicitly reported in the other study.[191] Study sample sizes ranged from 182 to 1608; followup time ranged from 6 to 14 months. Because of limited number of studies reporting on nonoverlapping population and the diversity of metrics used to define high platelet reactivity we did not perform meta-analyses of predictive ability. Of the two studies reporting information on the same patient population, the study with the longest followup did not find a statistically significant effect of platelet reactivity on the odds of cardiovascular mortality.[192] One study reported a significantly higher number of deaths in patients with high platelet reactivity.[183] The study that assessed both clopidogrel and aspirin response status reported a lower risk of death among clopidogrel nonresponders than among responders to at least one of the drugs, but the difference was not statistically

significant.[191] In summary, reviewed studies do not provide sufficient evidence of an association between increased platelet reactivity by the Multiplate analyzer assay and cardiovascular mortality in patients with ischemic heart disease.

Acute Coronary Syndromes

Seven studies reported information on the ability of the Multiplate analyzer assay to predict myocardial infarction or other acute coronary syndromes for patients receiving clopidogrel-based treatment.[161,183,190-193,196] Three studies reported information from the same patient population over different followup durations.[192,193,196] All studies used ADP as the agonist to assess reactivity. Two studies used ADP in combination with AA to assess response to both clopidogrel and aspirin.[190,191] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four studies,[183,192,196] were not explicitly reported in one study,[191] and in one study used both external sources and the observed study data.[190] Sample sizes ranged from 182 to 1608; followup time ranged from 6 to 14 months. Because of the limited number of nonoverlapping studies using similar metrics and cutoff values to define high platelet reactivity, we did not perform meta-analyses of predictive ability. Of the three studies reporting information on acute coronary syndrome risk stratified by reactivity status (using ADP as the agonist), two did not report statistically significant results and one reported that the risk myocardial infarction was higher in the group with low reactivity. Additionally, both studies that evaluated combined response to clopidogrel and aspirin reported that the association between dual nonresponsiveness and myocardial infarction risk was not statistically significant.[190,191]

Stent Thrombosis

Seven studies reported information on the ability of the Multiplate analyzer assay to predict stent thrombosis for patients receiving clopidogrel-based treatment.[189,190,192-196] Two sets of studies appear to each have been based on one population–with three studies reporting results for the same population at different time points[192,193,196] and wo other studies reporting on two different classifications of reactivity in another population.[194,195]

The results of the studies with the longest followup were considered. All the studies used ADP as an agonist to assess reactivity. One study used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[190] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four studies[189,192,196] and were derived from the observed study data in two studies[194,195]; one study used both approaches.[190] Study sample sizes were variable, ranging from 30 to 2533 patients. Followup time was 1 day to 14 months. Because of the diversity of the metrics and cutoffs used to define high platelet reactivity, and substantial variability in followup durations, we did not perform meta-analyses of predictive ability.

In three of the four studies, high on-clopidogrel platelet reactivity was significantly associated with a higher risk of stent thrombosis. In one study that evaluated combined clopidogrel and aspirin response status, higher number of deaths were observed in the group with dual clopidogrel and aspirin nonresponse but the difference was not statistically significant.[190] Overall, the studies provided weak evidence of an association between increased platelet reactivity (as measured by the Multiplate analyzer) and acute coronary syndromes in patients with ischemic heart disease; however, given the substantial clinical heterogeneity, the results are inconclusive.

MACE

Nine studies reported information on the ability of the Multiplate analyzer assay to predict MACE for patients receiving clopidogrel-based treatment.[161,183,190-196] Two sets of studies reported on overlapping patient populations: one set comprised three studies reporting results on the same population over different followup durations[192,193,196]; the second set comprised two studies employing two different classifications of reactivity on the same population.[194,195] In both cases, we based our conclusions on the studies with the longest followup duration. The definitions of composite outcomes were variable across studies. All studies used ADP as the agonist to assess reactivity. Two studies used ADP in combination with AA to assess response to both clopidogrel and aspirin.[190,191] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in four studies and not explicitly reported in two studies; they were derived from the observed study data and literature in four studies. Study sample sizes were variable, ranging from 182 to 2533 patients; followup durations ranged from 1 month to 14 months. Because of the diversity of the metrics and cutoff values used to define high platelet reactivity, and the substantial variability in followup durations, we did not perform meta-analyses of predictive ability for this group of studies.

All three studies reporting information on the predictive value of on-clopidogrel platelet reactivity (using ADP as the agonist) did not report statistically significant results. Among the two studies that evaluated a combination of clopidogrel and aspirin responders, both reported significantly higher rates of composite outcome events in patient groups with dual clopidogrel and aspirin nonresponse.[190,191] In summary, there was insufficient evidence to support an association between increased platelet reactivity measured by the Multiplate analyzer assay and composite clinical outcomes in patients with ischemic heart disease; however, given the heterogeneity in the studies, the results were inconclusive.

Stroke

Three studies reported information on the ability of the Multiplate analyzer assay to predict strokes for patients receiving clopidogrel-based treatment.[192,193,196] All three studies were based on the same patient population (1608 patients) and reported results over different followup durations. ADP was the agonist used to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in all studies. Followup duration ranged from 30 days to 12 months. The study reporting followup results at one year documented a statistically significant association between high platelet reactivity and ischemic stroke; the association was not statistically significant for hemorrhagic or overall stroke.[192]

Bleeding Events

Five studies reported information on the ability of the Multiplate analyzer assay to predict bleeding events for patients receiving clopidogrel-based treatment.[192-196] Two sets of studies reported on overlapping patient populations: one set comprised three studies reporting results on the same population over different followup durations[192,193,196]; the second set comprised two studies employing two different classifications of reactivity on the same population.[194,195] In both cases we based our conclusions on the studies with the longest followup duration. ADP was used as the agonist to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three studies; they were derived from the observed study data and literature in two studies. Study sample sizes were 1608 and 2533 for each of the two sets of studies. Followup duration ranged from 6 months to 1 year. Because of

the limited number of studies on nonoverlapping patient populations (n=2), we did not perform meta-analyses of predictive ability. One study did not find a statistically significant association between platelet reactivity measured by the Multiplate analyzer and risk of bleeding; one study reported that major and minor bleeding risk was significantly higher in the group of patient with high on-clopidogrel reactivity.

Other Clinical Outcomes

Four studies reported information on the ability of the Multiplate analyzer assay to predict target lesion revascularization for patients receiving clopidogrel-based treatment[191-193,196] Three of the four studies reported results based on the same patient population at different followup durations.[192,193,196] We based our conclusions on the study with the longest followup duration. All studies used ADP as an agonist to assess reactivity. One study also used ADP in combination with AA to assess the response to both clopidogrel and aspirin.[191] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three studies; they were not explicitly reported in one study. Study sample sizes ranged from 182 to 1608; followup time ranged from 6 to 14 months. Because of limited number of studies with nonoverlapping patient populations, we did not perform meta-analyses of predictive ability. One study did not find a statistically significant association between increased platelet reactivity and target lesion revascularization (using ADP as the agonist).[192] The study that assessed the impact of combined response to clopidogrel and aspirin on target lesion revascularization found a higher risk among patients exhibiting nonresponse (to both drugs).[191]

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

No studies reported information on the ability of the Multiplate analyzer assay to predict platelet reactivity as a continuous outcome for clopidogrel-treated patients with ischemic heart disease.

Reactivity as a Categorical Outcome

One study[188] reported on the effect of tests using the Multiplate analyzer assay on platelet reactivity as a categorical outcome. In this study, reactivity during followup was assessed using LTA with ADP as an agonist. The study enrolled patients with chronic ischemic heart disease and was conducted in the setting of interventional cardiac procedures, specifically PCI. The study reported inadequate quantitative data to assess the predictive value of the Multiplate analyzer assay on platelet reactivity as a categorical outcome.

Patients with Cerebrovascular Disease

One study reported information on the predictive value of the Multiplate analyzer assay for composite clinical outcomes among clopidogrel-treated patients with cerebrovascular disease.[197] No studies reported on the test's ability to predict platelet reactivity.

MACE

The study reported information on the ability of the Multiplate analyzer assay to predict composite MACE (transient intra-interventional thrombosis, transient ischemic attack, or cerebral infarction for clopidogrel-treated patients with cerebrovascular disease).[197] ADP was used as the agonist to assess reactivity. The rationale for selecting the threshold for defining a

"positive" result was not explicitly reported in the study. This small study (n=50) reported that increased reactivity was statistically significantly associated with increased risk of the composite outcome.

Thromboelastography

Five studies reported information on the predictive ability of thromboelastography as an assay for measuring platelet reactivity. All five studies included patients with ischemic heart disease. No studies reporting information on this assay were conducted in other patient populations. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E**.

Patients with Ischemic Heart Disease

Five studies included patients with ischemic heart disease and reported information on the predictive value of thromboelastography.[112,134,136,155,198] All of the five studies assessed the value of the test for predicting clinical outcomes (all-cause mortality, acute coronary syndromes, composite cardiovascular outcomes, and bleeding events). No studies assessed the value of the test for predicting other clinical outcomes or platelet reactivity during followup. Of the five studies, four enrolled patients with chronic stable coronary artery disease; the remaining study included patients with acute coronary syndromes.

Clinical Outcomes

All-Cause Mortality

One study reported on the predictive value of thromboelastography to predict all-cause mortality for patients receiving clopidogrel-based treatment.[112] In this study, reactivity was assessed using ADP as agonist. The study enrolled patients with chronic stable coronary artery disease and was conducted in the setting of interventional cardiac procedures (PCI). The study reported that no deaths occurred (regardless of reactivity status).

Acute Coronary Syndromes

One study reported on using thromboelastography to predict myocardial infarction for patients receiving clopidogrel-based therapy.[112] In this study, reactivity was assessed by thromboelastography using ADP as agonist. The study enrolled patients with chronic stable coronary artery disease and was conducted in the setting of interventional cardiac procedures (PCI). Over a course of one year, the study reported a higher risk of myocardial infarction among patients with high platelet reactivity; however, the difference was not statistically significant.

MACE

Four studies reported information on the ability of thromboelastography to predict MACE in patients receiving clopidogrel-based treatment.[112,134,136,155] Two of the studies reported information on the same patient population, with the one study reporting information after 6 months of followup[134] and the other after 36 months of followup.[136] We based our conclusions on the study with the longest followup. Definitions of composite clinical outcomes were different across studies. The studies conducted on the same population used thrombin and ADP as agonists[134,136]; the other two used ADP. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in two studies and were derived from the

observed study data in two studies. Study sample sizes ranged from 49 to 225 patients and followup time ranged from 6 months to 36 months. Because of the limited number of studies reporting on nonoverlapping patient population and the diversity of the cutoff values used to define high platelet reactivity, we did not perform meta-analyses of predictive ability. Two of the three studies reported that the risk of composite cardiovascular events was statistically significantly higher in groups identified as having high platelet reactivity when both ADP and thrombin were used as agonists.[112,136] The third study also reported significantly higher event rates in groups having high reactivity (using ADP as the agonist).[155] In summary, reviewed studies suggested an association between increased platelet reactivity by thromboelastography and composite adverse cardiovascular events in patients with ischemic heart disease; however, the evidence was limited by clinical heterogeneity across the few available studies.

Bleeding Events

Two studies reported on using thromboelastography to predict bleeding events for patients receiving clopidogrel-based treatment.[112,198] In both studies, reactivity was assessed by thromboelastography using collagen and ADP as agonists. Both studies enrolled patients with chronic stable coronary artery disease and were conducted in the setting of interventional cardiac procedures: PCI in one[112] and CABG surgery in the other.[198] One study assessed major and minor bleeding events;[112] one study assessed post-operative transfusion requirement as an indicator for loss from bleeding.[198] The postoperative transfusion requirement was significantly higher with increased platelet reactivity[198]; the bleeding events did not differ statistically significantly between groups in the other study.[112] Overall, there was insufficient information to evaluate the predictive value of platelet reactivity as measured by thromboelastography and bleeding events in patients with ischemic heart disease.

PFA-100

Eight studies reported information on the predictive ability of PFA-100 assay. All of the studies included patients with ischemic heart disease. No relevant studies of this assay enrolled patients with cerebrovascular or peripheral arterial disease. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E**.

Patients with Ischemic Heart Disease

Eight studies included patients with ischemic heart disease and reported information on the predictive value of the PFA-100 assay.[115,129,188,199-203] One also assessed a variation of the PFA-100 assay called the INNOVANCE PFA P2Y assay.[115] Seven studies assessed the value of the test for predicting clinical outcomes and one for predicting platelet reactivity during followup. Of the eight studies, four enrolled patients with acute coronary syndromes[199-202]; the remaining four enrolled patients with chronic stable coronary artery disease.[115,129,188,203]

Clinical Outcomes

All-Cause Mortality

Two studies reported information on the ability of the PFA-100 and the INNOVANCE PFA P2Y assay to predict all-cause mortality for patients receiving clopidogrel-based treatment.[115,200] One study evaluated both assays;[115] the other study used only PFA-100.[200] All assays used

collagen and ADP as agonists to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in one study; they were derived from the observed study data in the other. Study sample sizes were 612 and 1069 patients. Because of the limited number of available studies, we did not perform meta-analyses of predictive ability for this group of studies. Both studies reporting information on reactivity as measured by PFA-100 did not report statistically significant associations between reactivity status and all cause mortality;[115,200] High on-treatment platelet reactivity as measured by INNOVANCE PFA P2Y assay was statistically significantly associated with risk of death.[115] The reviewed studies did not suggest an association between increased platelet reactivity by PFA-100 assay and all-cause mortality; however, data were very limited. Similarly, the association between increased platelet reactivity measured by the INNOVANCE PFA P2Y assay and all-cause mortality was supported by only a single study.

Cardiovascular Mortality

Two studies reported information on the ability of the PFA-100 assay to predict cardiac mortality for patients receiving clopidogrel-based treatment.[200,202] One study used two agonist combinations: collagen and epinephrine, and collagen and ADP to assess both aspirin and clopidogrel nonresponsiveness, respectively.[202] The other study used collagen and ADP as agonists.[200] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in both studies. Study sample sizes were 125 and 612. Because of the limited number of available studies, we did not perform meta-analyses of predictive ability for this group of studies. One study reported that the risk of cardiac death was significantly higher among patients with high platelet reactivity while assessing clopidogrel responsiveness.[200] The other study, which assessed combined aspirin and clopidogrel responsiveness, reported that the risk of cardiac death was statistically significantly higher among patients with dual nonresponsiveness.[202] In summary, reviewed studies suggested an association between increased platelet reactivity by PFA-100 assay and cardiac mortality in patients with ischemic heart disease; however, results were inconclusive due to the limited number of publications and heterogeneity in the methods for reactivity assessment.

Acute Coronary Syndromes

Five studies reported information on the ability of the PFA-100 assay to predict acute coronary syndromes for patients receiving clopidogrel-based treatment.[115,199,200,202,203] One study used a combination of collagen and epinephrine, and collagen and ADP, to assess both aspirin and clopidogrel nonresponsiveness, respectively.[202] The other studies used collagen and ADP as agonists. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three studies; they were derived from the observed study data in one study; and not explicitly reported in one study. Study sample sizes ranged from 91 to 1069 patients. Because of the heterogeneity in the cutoff values used, we did not perform meta-analyses of predictive ability for this group of studies. Three studies did not report a statistically significant association between high reactivity as measured by PFA-100 and risk of myocardial infarction; the remaining two studies reported a statistically significantly higher risk of myocardial infarction among patients with high platelet reactivity. One study assessing high on-treatment platelet reactivity as measured by INNOVANCE PFA P2Y assay did find a statistically significant difference in the risk of myocardial infarction between groups.[115] In summary, studies did not suggest an association between increased platelet reactivity as measured by PFA-100 assay and acute coronary syndromes outcomes in patients with ischemic

heart disease; however, results were inconclusive due to heterogeneity in the methods used to assess reactivity that precluded quantitative synthesis.

Stent Thrombosis

Two studies reported information on the ability of the PFA-100 and the INNOVANCE PFA P2Y assays to predict stent thrombosis for patients receiving clopidogrel-based treatment.[115,129] One study used measurements of platelet reactivity from both assays (used separately)[115]; the other study used a combination of collagen and epinephrine, and collagen and ADP, to assess aspirin and clopidogrel nonresponsiveness, respectively.[129] Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in one study; they were derived from the observed study data in the other. Study sample sizes were 746 and 1069 patients. Because of the limited number of available studies, we did not perform meta-analyses of predictive ability. One study reported 100 percent sensitivity of PFA-100 assay to predict stent thrombosis.[129] The other study did not report a statistically significant association between platelet reactivity and stent thrombosis.[115]The association between high on-treatment platelet reactivity as measured by the INNOVANCE PFA P2Y assay with stent thrombosis was not statistically significant.[115] In summary, reviewed studies did not provide adequate evidence to support the association between increased platelet reactivity as measured by PFA-100 assay and stent thrombosis in patients with ischemic heart disease.

MACE

Seven studies reported information on the ability of the PFA-100 and the INNOVANCE PFA P2Y assays to predict MACE in patients receiving clopidogrel-based treatment.[115,129,199-203] Only one study used measurements of platelet reactivity from both assays (used separately)[115]; the other studies used combinations of collagen and epinephrine and collagen and ADP to assess platelet reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in three studies and were derived from the observed study data in two studies; one study used both approaches; and one did not explicitly report the rationale for selecting a cutoff value. Study sample sizes ranged between 71 and 1069 patients. Because of the limited number of available studies and heterogeneity in the methods used to define high platelet reactivity, we did not perform meta-analyses of predictive ability.

Five of the seven studies using the PFA-100 assay reported statistically significant associations between increased platelet reactivity and increased risk of composite outcome events; the remaining two studies did not report statistically significant associations but produced effect sizes in the same direction (i.e., indicating an association between increased reactivity and higher risk of events). The association between high on-treatment platelet reactivity as measured by the INNOVANCE PFA P2Y assay with stent thrombosis was statistically significant indicating increased risk among patients with high on-clopidogrel reactivity.[115] In summary, the reviewed studies supported an association between increased platelet reactivity as measured by the PFA-100 assay and composite outcome events among patients with ischemic heart disease; however, the strength of these findings was limited by heterogeneity in definitions of increased reactivity that precluded quantitative synthesis.

Stroke

One study reported information on the ability of the PFA-100 assay to predict strokes for patients receiving clopidogrel-based treatment.[115] The study used collagen and ADP as an agonist. Thresholds for defining "positive" results were derived from the observed study data.

The study enrolled 1069 patients who were undergoing PCI with stent implantation. This study did not report a statistically significant association between high reactivity as measured by PFA-100 and stroke at one year of followup. The result did not suggest an association between increased platelet reactivity by PFA-100 assay and stroke; however, results were inconclusive due to paucity of relevant information.

Bleeding Events

One study reported on the ability of the PFA-100 assay to predict bleeding events for patients receiving clopidogrel-based treatment.[199] In this study, reactivity was assessed using collagen and ADP as agonists. The study enrolled patients with acute coronary syndromes and was conducted in the setting of interventional cardiac procedures (PCI). The study reported that no bleeding events occurred (regardless of reactivity status).

Other Clinical Outcomes

Two studies reported information on the ability of the PFA-100 assay to predict miscellaneous clinical outcomes for patients receiving clopidogrel-based treatment.[199,202] The outcomes included: recurrent angina, arrhythmia, pulmonary edema, cardiogenic shock,[199] and rehospitalization for congestive heart failure.[202] One study used ADP and collagen as the agonist to assess reactivity[199]; the other used a combination of collagen and epinephrine, and collagen and ADP to assess both aspirin and clopidogrel nonresponsiveness.[202] The studies generally showed that higher reactivity was associated with higher risk of outcome events; however, the strength of evidence was limited by the small number of available studies and the heterogeneity in reactivity measurement methods .

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

No studies reported information on the ability of the PFA-100 assay to predict platelet reactivity as a continuous outcome among clopidogrel-treated patients with ischemic heart disease.

Reactivity as a Categorical Outcome

One study[188] reported on ability of the PFA-100 assay to predict platelet reactivity as a categorical outcome. In this study, reactivity at baseline was assessed using collagen and ADP as agonists. The study enrolled patients with chronic ischemic heart disease and was conducted in the setting of interventional cardiac procedures (PCI). Reactivity status, as measured by the PFA-100 assay at baseline, was not statistically significantly associated with response status during followup.

Other Tests for Measuring Platelet Reactivity

Seven studies evaluated the predictive value of platelet reactivity by a diverse group of tests: one study used iron as an agonist[201]; two studies used the Plateletworks assay to measure reactivity[204,205]; one study used two separate assays (the Ichor Plateletworks and the Impact-R assay with ADP)[115]; one study used flow cytometry to assess ADP-stimulated P-selectin expression[132]; one study used a the results of two assays assessed jointly (LTA and the VerifyNow assay) to define platelet response[141]; and one study used a combination of three assays (conventional LTA, thromboelastography and Plateletworks assay).[206] All seven studies

included patients with ischemic heart disease. None were conducted on other patient populations. Detailed information on the selection criteria, settings, and treatment strategies (including adjunctive treatments) is presented in **Appendix E.**

Patients with Ischemic Heart Disease

All seven studies using diverse tests for reactivity included patients with ischemic heart disease. Five of the seven studies assessed the value of the test for predicting clinical outcomes, and two for predicting platelet reactivity during followup. Three enrolled patients with acute coronary syndromes[201,204,205]; three included patients with chronic stable coronary artery disease[115,132,206]; and one enrolled mixed populations with chronic and acute presentations.[141]

Clinical Outcomes

All-Cause Mortality

Two studies reported information on all-cause mortality. One study used measurements of platelet reactivity from two assays—the Ichor Plateletworks and the Impact-R assay;[115]the other study used measure of reactivity by only the Plateletworks assay.[204] In all cases ADP was the agonist used to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in one study; they were derived from the observed study data in the other. Study sample sizes were 31 and 1069 patients. Because of limited number of available studies we did not perform meta-analyses of predictive ability. Of the two studies reporting relevant information on reactivity as measured by Plateletworks, one did not report a statistically significant association between platelet reactivity status and risk of death.[115] The second study reported that no deaths occurred, regardless of reactivity.[204] Platelet reactivity as measured by the Impact R assay was also not statistically significantly associated with risk of death.[115]

Acute Coronary Syndromes

Two studies reported information on acute coronary syndromes among patients receiving clopidogrel-based treatment. One study used the measure of platelet reactivity from two assays—the Ichor Plateletworks and the Impact-R assay[115]; the other study used measure of reactivity by only the Plateletworks assay.[204] In all cases ADP was the agonist used to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in one study; they were derived from the observed study data in one study. Study sample sizes were 31 and 1069 patients. Because of the limited number of available studies we did not perform meta-analyses. Of the two studies reporting information on reactivity as measured by the Plateletworks assay, one reported that not myocardial infarctions occurred, regardless of reactivity.[204] The other study reported that the risk of myocardial infarction was significantly higher among patients with high on-treatment platelet reactivity.[115] Platelet reactivity as measured by Impact R ADP was not statistically significantly associated with myocardial infarction at 1 year.[115]

Stent Thrombosis

One study including 1069 patients reported information on stent thrombosis. The study used measurements of platelet reactivity from two assays—the Ichor Plateletworks and the Impact-R assay.[115] Both the assays used ADP as the agonist to assess reactivity. The threshold for defining

"positive" results was derived from the observed study data. The study reported that the high platelet reactivity as measured by either Plateletworks or Impact R was not statistically significantly associated with stent thrombosis.

MACE

Four studies reported information on composite MACE (of varying definitions). One study used iron as an agonist in LTA and also used the Plateletworks assay[201]; one measured platelet reactivity using two assays—the Ichor Plateletworks and the Impact-R[115]; one study used a the joint results of two assays, conventional LTA and VerifyNow, to define platelet response; and one study used a combination of three assays—conventional LTA, thromboelastography, and Plateletworks—to categorize response to clopidogrel. Apart from the study using iron as an agonist, the other three studies used ADP as the agonist to assess reactivity. Thresholds for defining "positive" results were based on prior literature (or other sources external to the study) in one study, were derived from the observed study data in one study, and were not explicitly reported in two studies. Study sample sizes ranged from 50 to 1069 patients and followup durations ranged from 1 month to a year. Because of limited number of studies evaluating each test, we did not perform meta-analyses of predictive ability for this group of studies. Of the two studies reporting information on the predictive value of reactivity as measured by Plateletworks, one did not report a statistically significant difference between reactivity groups[201] and the other reported significantly higher risk the composite cardiovascular event among patients with high on-treatment platelet reactivity.[115] Platelet reactivity as measured by the Impact R assay was not statistically significant associated with outcome events.[115] Increased reactivity based on combined assay results (LTA and VerifyNow or LTA, thromboelastography, and PlateletWork) was not significantly associate with increased event rates.

Stroke

One study reported information on the ability of Plateletworks and the Impact-R assay to predict strokes for patients receiving clopidogrel-based treatment. All the assays used ADP as the agonist to assess reactivity. Thresholds for defining "positive" results were derived from the observed study data in one study. The study enrolled 1069 patients undergoing PCI with stent implantation. This study did not find a statistically significant association between high reactivity as measured by either the Impact R or Plateletworks assay with stroke at 1 year of followup.

Bleeding Events

Two studies reported information on bleeding events for patients receiving clopidogrel-based treatment. One study used measurements of reactivity by the Plateletworks assay[204] and the other used two assays jointly – conventional LTA and VerifyNow assay – to assess platelet reactivity.[141] In all cases ADP was used as the agonist to assess reactivity. One study reported information on hematoma development as an outcome[204] while the other reported bleeding events. [141] Neither platelet reactivity as measured by the Plateletworks assay nor combined assessment with LTA and VerifyNow were statistically significantly associated with hematomas or bleeding events, respectively.

Other Clinical Outcomes

Two studies reported information on the ability of the different platelet function tests to predict bleeding events for patients receiving clopidogrel-based treatment. One study used

measurements of reactivity by the Plateletworks assay[204] and one used a combination of three assays—conventional LTA, thromboelastography, and Plateletworks assay—to categorize response to clopidogrel.[206] In all cases ADP was used as the agonist to assess reactivity. One study reported angina recurrence as an outcome[204]; the other study reported rates of followup intervention, both PCI and CABG surgery.[206] Platelet reactivity measured with the Plateletworks assay and combined assessment with LTA, thromboelastography, and Plateletworks assays were not statistically significantly associated with the outcomes assessed.

Intermediate Outcome: Platelet Reactivity During Followup

Reactivity as a Continuous Outcome

No studies reported information on the ability of other tests of platelet reactivity to predict platelet reactivity as a continuous outcome for clopidogrel-treated patients with ischemic heart disease.

Reactivity as a Categorical Outcome

Three studies evaluated the predictive value of baseline platelet reactivity on platelet reactivity as a continuous outcome.[132,204,205] Two studies used the Plateletworks assay[204,205] and one study used flow cytometry to assess P-selectin expression.[132] Two of the three studies enrolled patients with acute coronary syndromes[204,205] while one study[132] enrolled patients with chronic ischemic heart disease. All three studies were conducted in the setting of interventional cardiac procedures—one PCI[204], one PCI with stent implantation,[132] and one CABG surgery.[205]

Results were reported with different followup periods (ranging from a one day to 30 days). Because of differences in the tests used, we did not perform meta-analyses for this group of studies. Baseline reactivity measured with Plateletworks and ADP-stimulated P-selectin expression was not statistically significantly associated with reactivity status during followup.

Studies Reporting Comparative Information on Test Performance

Sixteen studies reported information on the predictive value of two or more tests applied to the same population (we refer to these studies as "comparative studies"). Although some of these studies performed informal comparisons between the predictive value of tests (e.g., by comparing effect sizes of metrics for predicting clinical outcomes), no study was specifically designed and analyzed in a way to allow formal comparisons between tests. All comparative studies have been considered with regards to the individual tests they evaluated in the preceding sections of this chapter. Their risk of bias is discussed in the next section; overall, they did not differ substantially (in terms of risk of bias) from studies assessing only a single test.

Eleven of the comparative studies reported extractable information on clinical outcomes for at least one of the assessed tests; sometimes information on an outcome was reported for one of the tests but not for others, leaving a total of nine comparative studies for our analyses. Complete information for the populations included, assays used, and study results are presented in **Appendix E**. Here, we focus on outcomes that were addressed by at least two comparative studies: major adverse cardiovascular events (composite outcome, seven studies); acute coronary syndromes (two studies); and stent thrombosis (three studies). All studies were conducted in ischemic heart disease populations.

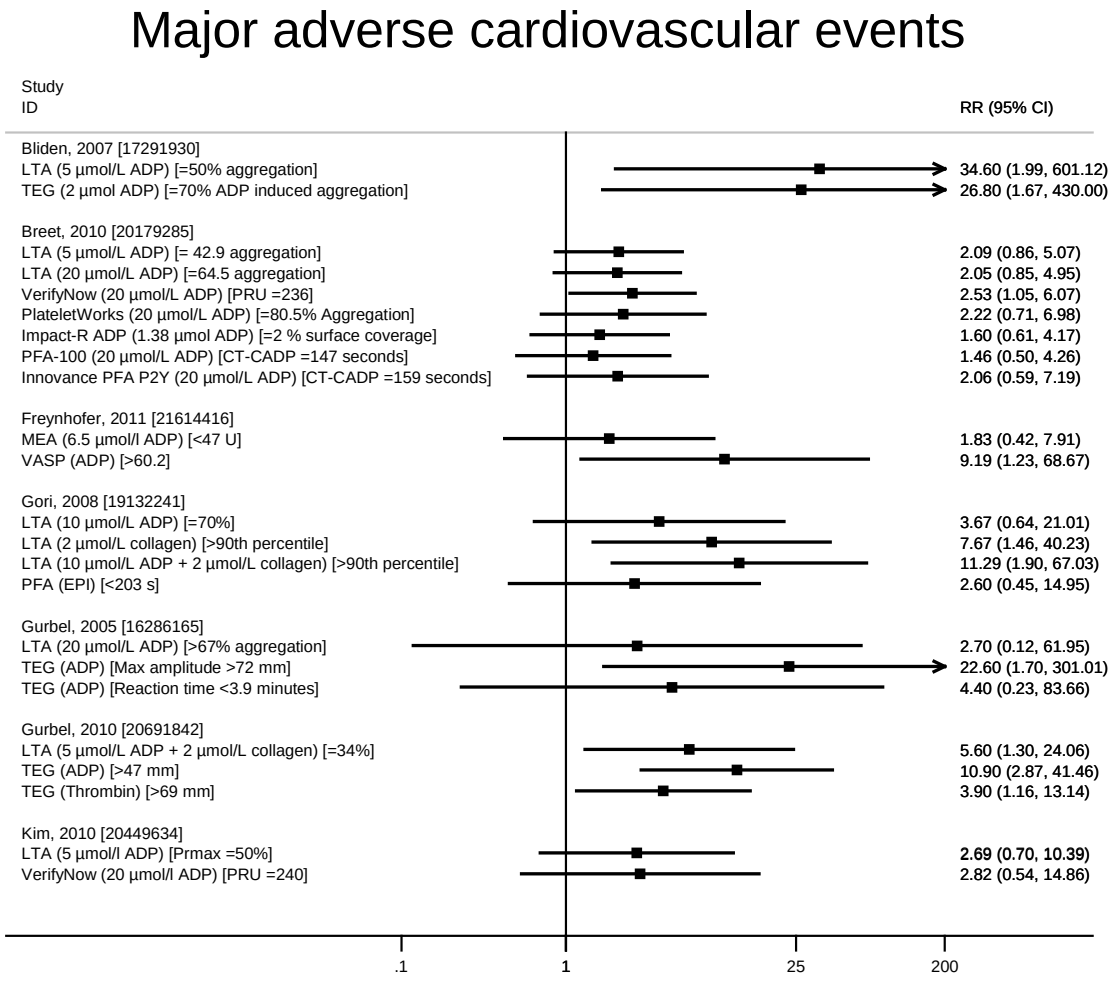
For each of these outcomes, we plotted the predictive effect sizes reported for each of the tests evaluated (grouped by study), along with information on the assay and threshold used

(results have been organized by study to facilitate direct within-study, cross-assay comparisons). Unfortunately the data cannot be quantitatively synthesized, because when multiple assays are applied to the same patient population results for are likely to be correlated (because the population is shared and assays applied to the same sample produce correlated—if not identical—results) and because the within-study correlation of results cannot be accounted for using aggregate data extracted from the available published studies. Similar problems would be encountered for other measures of test performance, again due to the within-study correlation of reported effect sizes. In the Discussion section of the report we provide suggestions on how future research studies could provide additional information regarding comparative test performance.

MACE

Seven studies reported comparative information regarding the ability of assays for measuring platelet reactivity to predict major adverse cardiovascular events. The most commonly used test was LTA, which was compared to various comparator tests (thromboelastography and VerifyNow P2Y12 were the most commonly used comparators, in three and two studies, respectively). Results have been summarized in **Figure 28**. Overall, point estimates were similar between alternative test methods (within each study) and CIs were overlapping, suggesting that the predictive ability of the compared tests is fairly similar.

Figure 28: Comparative Studies Assessing the Predictive Ability of Platelet-Reactivity Assays for Major Adverse Cardiovascular Events

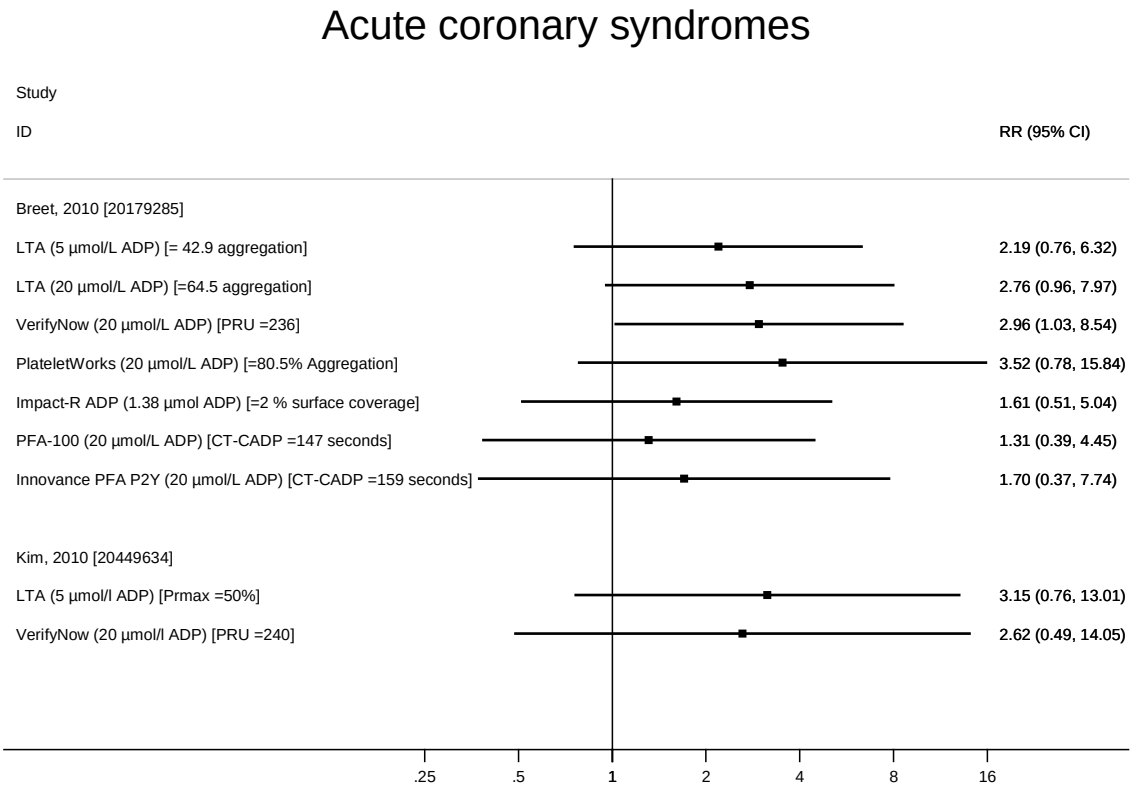


ADP = adenosine diphosphate; CI = confidence interval; CT = closure time; EPI = epinephrine; LTA = light-transmission aggregometry; max = maximum; MEA = Multiplate analyzer; PFA = Platelet Function Analyzer; PRU = platelet reactivity units; RR = relative risk; TEG = thromboelastography; U = units; VASP = vasodilator-stimulated phosphoprotein.
RRs compare event rates among patients with high versus low platelet reactivity (as defined by each study, for each test employed). Solid squares (and horizontal lines) indicate the RR of acute coronary syndromes (and the corresponding 95% CI) for individual studies. The vertical line indicates an RR of 1.

Acute Coronary Syndromes

Two studies reported comparative information regarding the ability of assays for measuring platelet reactivity to predict acute coronary syndromes. Both studies compared LTA and VerifyNow P2Y12; one study assessed four additional assay types (**Figure 29**). Overall, point estimates were similar between alternative test methods (within each study) and CIs were fairly wide and overlapping. These findings suggest that the predictive ability of the compared assays may be similar; however, there is substantial uncertainty regarding their relative test performance.

Figure 29: Comparative Studies Assessing the Predictive Ability of Platelet-Reactivity Assays for Acute Coronary Syndromes

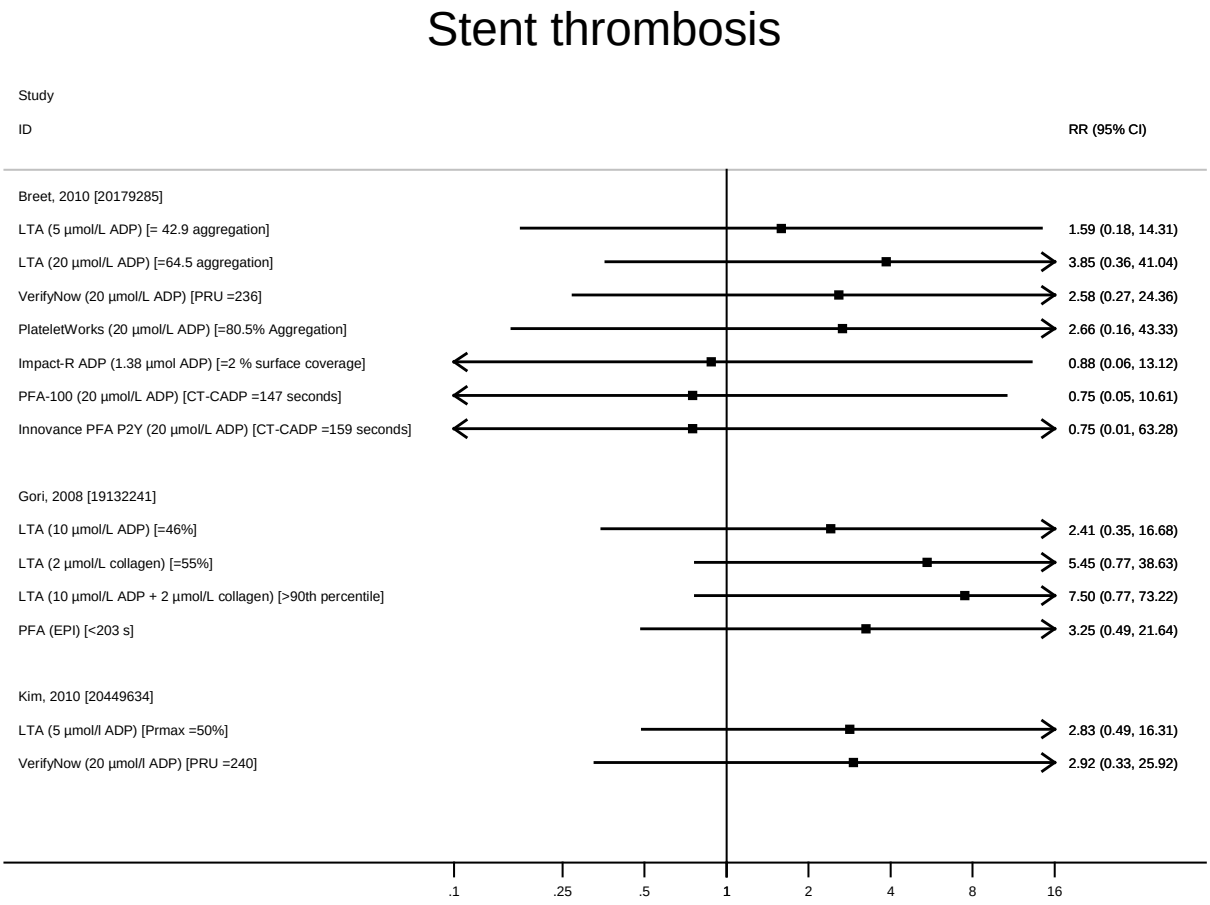


ADP = adenosine diphosphate; CI = confidence interval; CT = closure time; EPI = epinephrine; LTA = light-transmission aggregometry; max = maximum; MEA = Multiplate analyzer; PFA = Platelet Function Analyzer; PRU = platelet reactivity units; RR = relative risk; TEG = thromboelastography; U = units; VASP = vasodilator-stimulated phosphoprotein.
RRs compare event rates among patients with high versus low platelet reactivity (as defined by each study, for each test employed). The solid squares (and horizontal lines) indicate the RR of acute coronary syndromes (and the corresponding 95% CI) for individual studies. The vertical line indicates an RR of 1.

Stent Thrombosis

Three studies reported comparative information regarding the ability of assays for measuring platelet reactivity to predict stent thrombosis for patients undergoing PCI with stent implantation (**Figure 30**). All studies compared LTA against other reactivity assays (VerifyNow P2Y12 was the comparator in two studies). Overall, point estimates for the predictive ability of alternative test methods were variable (within each study); however, CIs were extremely wide (and overlapping), suggesting that there is substantial uncertainty regarding the relative predictive ability of the compared tests for stent thrombosis and that there is insufficient evidence on comparative test performance for this outcome.

Figure 30: Comparative Studies Assessing the Predictive Ability of Platelet-Reactivity Assays for Stent Thrombosis



ADP = adenosine diphosphate; CI = confidence interval; CT = closure time; EPI = epinephrine; LTA = light-transmission aggregometry; max = maximum; PFA = Platelet Function Analyzer; PRU = platelet reactivity units; RR = relative risk; U = units. RRs compare event rates among patients with high versus low platelet reactivity (as defined by each study, for each test employed). The solid squares (and horizontal lines) indicate the RR of stent thrombosis (and the corresponding 95% CI) for individual studies. The vertical line indicates an RR of 1.

Risk of Bias for Studies Reviewed for Key Question 2b

The detailed assessment of 11 risk-of-bias items for studies evaluating the predictive ability of phenotypic testing regarding clinical outcomes or platelet reactivity (during followup) is presented in **Appendix E**. Because of the large number of eligible studies, we did not generate a risk of bias graph showing individual items results for each study. Instead, we provide averages across studies for each item assessed (**Appendix Figure E2**). Briefly, all studies used a longitudinal design (not case–control; per our inclusion criteria) and no studies had substantial loss-to-followup. Inappropriate exclusions were uncommon however information on blinding was often not reported (particularly for the index test) or not used. Using the arbitrary cutoffs based on the number of adequately addressed risk of bias items 21 studies were rated as quality "A," 67 studies were rated as quality "B," and 12 were rated as quality "C."

Assessing Small-Study Effects

For the two assays (VerifyNow P2Y12 and VASP) and for outcomes for which we performed meta-analyses of predictive value we assessed differences between more precise (larger) and less precise (smaller) studies using the Egger regression-based test for small study effects. **Table 9** summarizes our key findings.

Table 9: Small Study Effects in Meta-Analyses of the Value of Platelet Reactivity for Predicting Clinical Outcomes among Clopidogrel-Treated Patients with Ischemic Heart Disease

Assay	Outcome	P Value for Small-Study Effects
VerifyNow P2Y12	All-cause mortality	0.016
	Cardiovascular mortality	0.383
	MACE	0.001
	Bleeding events (all severity levels)	0.596
	Bleeding events (major)	0.865
VASP assay	MACE	0.208
	Cardiovascular mortality	0.048
	Stent thrombosis	0.316
	Acute coronary syndromes	0.311

MACE = major adverse cardiovascular events, VASP = vasodilator-stimulated phosphoprotein.
Significant P values are in bold type.

Statistically significant "small study effects" were identified among studies of the VerifyNow P2Y12 assay included in meta-analyses of all-cause mortality and major adverse cardiovascular events, as well as among studies of the VASP assay included in the meta-analysis of cardiovascular mortality. Again, we caution that these significant effects should not be overinterpreted as proof for the presence of publication bias given both the statistical properties of the Egger test and the fact that alternative causes (true heterogeneity between smaller and larger studies, chance, or other biases) can also produce significant results. Nonetheless, these results suggest that selective publication of more extreme results in smaller studies may have affected the available data and limits somewhat the strength of the conclusions that can be drawn.

Key Question 2c: What is the Impact of Modifying Factors on the Association Between Phenotypic Test Results and Clinical Outcomes?

We reviewed studies to identify any evidence that patient- or system-level factors or test characteristics could modify the predictive ability of phenotypic testing for platelet reactivity. Evidence of effect modification by person-level characteristics (e.g., age, sex, disease severity) is best obtained from comparisons within a given study (i.e., among subgroups of the patient population that were selected using the same criteria and managed in a similar way). Additional evidence on effect modification can be derived from exploratory comparisons across studies, by assessing study-level characteristics (e.g., the setting of care or study design characteristics) or characteristics of the overall patient population (e.g., populations selected on the basis of ancestry).[j] In the following sections we discuss each of these sources of information regarding effect modification separately.

Effect Modification Within Studies

In total, five studies reported information on effect modification of the predictive effect of platelet reactivity. All studies reported information on clinical outcomes. Relevant results have been summarized in **Table 10**; however, we caution that because only a small subset of the eligible studies provided adequate information to statistically assess effect modification, selective reporting is highly likely.

Studies assessed the following factors as potential modifiers: IIb/IIIa inhibitor as an adjunct treatment for PCI (two studies), diabetes mellitus (two studies), and chronic kidney disease (one study). Two studies used the VASP assay to assess platelet reactivity, two used the VerifyNow P2Y12 assay (one of which also used VerifyNow ASA[k]), and one used LTA (with ADP as the agonist). Statistically significant interaction effects were reported only in the study by Morel et al. 2011[185] that assessed whether the predictive value of the VASP assay is different among patients with coronary artery disease who have coexisting chronic kidney disease versus those who do not. The study found statistically significant interaction effects for several clinical outcomes (all-cause mortality, cardiac death, and a composite outcome of all-cause mortality, myocardial infarction, or target-lesion revascularization), in all cases suggesting that the predictive effect of high on-clopidogrel platelet reactivity was stronger among patients with chronic kidney disease.

[j] We refer to the information obtained from cross-study comparisons as "exploratory" because comparisons for a given characteristics across studies may be confounded (by other study features). Our ability to statistically "control" for such differences is typically limited by the relatively small number of available studies available for meta-analysis.

[k] This assay uses arachidonic acid as the agonist, to measure "aspirin resistance."

Table 10: Studies Reporting Information on Effect Modification

Author Year Country PMID Study name (when available)	Patient Population	Antiplatelet Regimen	Platelet Reactivity Measurement Method (agonist) [assay and manufacturer] Threshold Definition	Effect Modifier	Summary of Findings[a]
Bliden 2007 US 17291930 NR	Patients on clopidogrel-based therapy for ≥1 mo undergoing scheduled PCI	Aspirin ≥81 mg/d for ≥7 d before PCI; clopidogrel 75 mg/d for ≥1 mo	LTA (ADP 5 μmol/L) [Chronolog Lumi-Aggregometer; Model 490-4D; Chronolog, Havertown, PA] ≥50% on-clopidogrel reactivity [based on prior literature]	IIb/IIIa inhibitor treatment (used vs. not)	Predictive effect of on-treatment reactivity stratified by IIb/IIIa inhibitor treatment for the composite outcome of death, MI, TVR, stroke, non-TVR, or rehospitalization for ischemia [calculated values] *IIb/IIIa inhibitor used:* high reactivity vs. normal reactivity OR=29.33 (95% CI 2.40, 357.85) *IIb/IIIa inhibitor not used:* high reactivity vs. normal reactivity OR=32.67 (95% CI 7.12, 149.79) P for interaction=0.94 [chi-square test for heterogeneity]
Campo 2010 Multinational 20951320 3T/2R ancillary study	Patients undergoing PCI were screened for aspirin and clopidogrel responsiveness (poor-responders were randomized to tirofiban or placebo[b]); the current study reported on long term outcomes regardless of baseline response status	Aspirin 100 mg/d indefinitely; clopidogrel 75 mg/d for ≥1 mo for patients with stable CAD receiving BMS, or for ≥1 yr for patients with UA or receiving DES	VerifyNow Aspirin (AA agonist) for aspirin responsiveness; VerifyNow P2Y12 (ADP agonist) for clopidogrel responsiveness [Accumetrics, San Diego, CA] For VerifyNow Aspirin ARU ≤550 and VerifyNow P2Y12 % platelet inhibition ≥40% were considered indicative of full response to aspirin and clopidogrel, respectively [cutoff values were those used in the 3T/2R study]	IIb/IIIa inhibitor treatment (used vs. not); in poor responders at baseline, use of IIb/IIIa inhibitor was randomized; the choice was "operator's choice" in responders	Predictive effect of "nonresponder status" stratified by IIb/IIIa inhibitor treatment [calculated values] Periprocedural ischemic events (up to 3 d post-PCI) *IIb/IIIa inhibitor used:* high reactivity vs. normal reactivity OR=1.63 (95% CI 0.54, 4.92) *IIb/IIIa inhibitor not used:* high reactivity vs. normal reactivity OR=2.76 (95% CI 1.42, 5.36) P for interaction=0.42 [chi-square test for heterogeneity] Periprocedural MI (up to 3 d post-PCI) *IIb/IIIa inhibitor used:* high reactivity vs. normal reactivity OR=4.20 (95% CI 1.77, 9.93) *IIb/IIIa inhibitor not used:* high reactivity vs. normal reactivity OR=7.72 (95% CI 4.60, 13.04) P for interaction=0.22 [chi-square test for heterogeneity] Death, MI, or stroke (3 d–1 yr post-PCI) *IIb/IIIa inhibitor used:* high reactivity vs. normal reactivity OR=2.97 (95% CI 0.95, 9.28)

[a] The description "calculated values" indicates that the results are based on statistical procedures we conducted in cases where studies provided sufficient statistics for the assessment of effect modification but did not assess its statistical significance.

[b] Information from the randomized comparison has been reviewed in the section of this report relevant to Key Question 3.

Author Year Country PMID Study name (when available)	Patient Population	Antiplatelet Regimen	Platelet Reactivity Measurement Method (agonist) [assay and manufacturer] Threshold Definition	Effect Modifier	Summary of Findings[a]
					IIb/IIIa inhibitor not used: high reactivity vs. normal reactivity OR=3.59 (95% CI 1.74, 7.39) P for interaction=0.78 [chi-square test for heterogeneity]
El Ghannudi 2011 France 21524751 NR	Patients undergoing PCI with stent placement for ACS or stable CAD	Clopidogrel (various loading doses); all patients were on aspirin treatment	VASP assay (ADP ± PGE1) [Diagnostica Stago (Biocytex), Asnieres, France] using flow cytometry Poor response was defined as VASP PRI>61% [based on prior literature]	Diabetes mellitus (yes vs. no)	Results from univariate analyses (platelet reactivity was not a significant predictor of outcomes in nondiabetic patients and adjusted results were NR) *Diabetic patients* HR for PRI >61% vs. ≤61% for CV death=5.798 (95% CI, 1.25, 26.86) HR for PRI >61% vs. ≤61% for all-cause mortality=3.84 (95% CI, 1.04, 14.23) HR for PRI >61% vs. ≤61% for MACE=2.01 (95% CI, 0.96, 4.23) *Non-diabetic patients* HR for PRI >61% vs. ≤61% for CV death=1.74 (95% CI, 0.43, 7.00) HR for PRI >61% vs. ≤61% for all-cause mortality=1.53 (95% CI, 0.47, 5.04) HR for PRI >61% vs. ≤61% for MACE=1.06 (95% CI, 0.55, 2.03) Interaction P value [diabetic vs. nondiabetic] for CV death=0.26; for all-cause mortality=0.31; for MACE=0.20 [calculated values, chi-square test for heterogeneity]
Mangiacapra 2010 Multinational 20723634 NR	Patients undergoing elective PCI with stent implantation for stable angina or NSTE ACS	Pre-PCI: clopidogrel loading dose 600 mg or on-clopidogrel chronic treatment (75 mg/d for ≥5 d); post-PCI: clopidogrel maintenance (75 mg/d for ≥1 mo after BMS implantation or 12 mo after DES implantation or patients with ACS) All patients received aspirin (100 mg/d) pre-PCI and continued with the same maintenance dose indefinitely	VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA] High platelet reactivity was defined as VerifyNow PRU > 240 [based on prior literature]	Diabetes mellitus (yes vs. no)	Effect of high vs. low platelet reactivity, stratified by diabetes status (calculated values) on periprocedural MI *Diabetic patients:* OR=5.89 (95% CI 1.46, 23.81) *Nondiabetic patients,* OR=1.82 (95% CI 0.44, 7.65) Interaction P value [diabetic vs. nondiabetic]=0.25 [chi-square test for heterogeneity]
Morel 2011 France 21251579 NR	Patients undergoing PCI for stable CAD or ACS	Clopidogrel loading dose (300 or 600 mg); all patients received aspirin	VASP assay (ADP ± PGE1) [Biocytex, Diagnostica Stago, Asnieres, France] using flow cytometry Poor response was defined as VASP PRI>61% [based on prior work from the same	CKD (present vs. not)	Effect of nonresponse to clopidogrel (vs. response), stratified by CKD status (calculated values) *Patients with CKD* All-cause mortality, OR=12.145 (95% CI 2.604, 56.647) Cardiac death, OR=10.923 (95% CI 2.325, 51.314) STE MI, OR=1.449 (95% CI 0.197, 10.637) NSTE MI, OR=2.219 (95% CI 0.357, 13.780)

Author Year Country PMID Study name (when available)	Patient Population	Antiplatelet Regimen	Platelet Reactivity Measurement Method (agonist) [assay and manufacturer] Threshold Definition	Effect Modifier	Summary of Findings[a]
			authors]		TVR, OR=1.157 (95% CI 0.295, 4.538) Definite stent thrombosis, OR=1.440 (95% CI 0.088, 23.567) Probable stent thrombosis, OR=6.128 (95% CI 0.664, 56.528) Definite/probable stent thrombosis, OR=3.859 (95% CI 0.718, 20.730) Possible stent thrombosis, OR=17.387 (95% CI 0.939, 321.819) All types of stent thrombosis, OR=8.659 (95% CI 1.808, 41.457) MACE, OR=3.556 (95% CI 1.433, 8.822) *Patients without CKD* All-cause mortality, OR=0.648 (95% CI 0.164, 2.557) Cardiac death, OR=0.762 (95% CI 0.138, 4.228) STE MI, OR=0.507 (95% CI 0.052, 4.930) NSTE MI, OR=1.242 (95% CI 0.476, 3.241) TVR, OR=1.146 (95% CI 0.552, 2.382) Definite stent thrombosis, OR=2.079 (95% CI 0.457, 9.456) Probable stent thrombosis, OR=0.764 (95% CI 0.069, 8.523) Definite/probable stent thrombosis, OR=1.556 (95% CI 0.441, 5.490) Possible stent thrombosis, OR=0.507 (95% CI 0.021, 12.559) All types of stent thrombosis, OR=1.289 (95% CI 0.385, 4.321) MACE, OR=1.026 (95% CI 0.538, 1.956) Interaction P values [chi-square tests for heterogeneity], by outcome (CKD vs. no CKD) All-cause mortality, P=0.005; cardiac death, P=0.023; STE MI, P=0.495; NSTE MI, P=0.581; TVR, P=0.990; definite stent thrombosis, P=0.821; probable stent thrombosis, P=0.213; definite/probable stent thrombosis, P=0.396; possible stent thrombosis, P=0.107; all types of stent thrombosis, P=0.057; MACE, P=0.029

3T/2R = Tailoring Treatment with Tirofibam in patients showing Resistance to aspirin and/or Resistance to clopidogrel; AA = arachidonic acid; ACS = acute coronary syndromes; ADP = adenosine diphosphate; ARU = aspirin reactivity units; BMS = bare metal stent; CAD = coronary artery disease; CI = confidence interval, CKD = chronic kidney disease; CV = cardiovascular, d = days, DES = drug eluting stent; HR = hazard ratio, LTA = light-transmission aggregometry, MACE = major adverse cardiovascular events; MI = myocardial infarction; mo=months, NR = not reported; NSTE = non–ST-elevation; OR = odds ratio; PCI = percutaneous coronary intervention; PGE1 = prostaglandin E1, PRI = platelet reactivity index, PRU = platelet reactivity units, STE = ST-elevation; TVR = target-vessel revascularization; UA = unstable angina, VASP = vasodilator-stimulated phosphoprotein; yr = years.

Effect Modification Across Studies

On the basis of our protocol and the availability of data from studies considered eligible for Key Question 2b, we assessed the following characteristics as potential modifiers of the predictive effect of platelet reactivity by performing comparisons across studies using subgroup analysis: disease subtype (acute coronary syndromes vs. mixed coronary artery disease populations), setting of care (PCI vs. other), duration of followup (≤30 days vs. >30 days); and year when enrollment was started (continuous variable). For setting of care (PCI versus not) there was no variability across studies (all were conducted in the setting of interventional procedures); as such no meta-analysis was performed. Meta-regressions were performed only for one outcome (major adverse cardiovascular events) reported in more than 10 studies and for high versus low reactivity measured with the VerifyNow assay (all other assays had less than 10 studies considered sufficiently similar for meta-analysis for all outcomes assessed). **Table 11** summarizes the findings of our meta-regression analyses.

Table 11: Meta-regression Results for Study-Level Effect Modifiers of the Predictive Value of Phenotypic Testing for Platelet Reactivity Using the VerifyNow P2Y12 Assays in Patients with Ischemic Heart Disease: Major Adverse Cardiovascular Events

Effect Modifier	Groups Compared	No. of Studies	RR (95% CI) Within Subgroup	rRR (95% CI); P Value
All studies	NA	11	2.46 (1.88, 3.22)	NA
ACS vs. ischemic heart disease	Other ischemic heart disease or mixed	7	2.32 (1.68, 3.21)	1.47 (0.74, 2.91); 0.273
	ACS	4	3.24 (1.88, 5.59)	
Duration of followup	>30 d	9	2.25 (1.76, 2.87)	2.62 (0.87, 7.91); 0.088
	≤30 d	2	6.03 (2.13, 17.10)	
Enrollment start year	(continuous variable)	10	NA	1.01 (0.80, 1.28); 0.905

ACS = acute coronary syndromes; CI = confidence interval; d = days; NA = not applicable; RR = relative risk; rRR = relative RR (between groups).
Results across all studies are provided for comparison. One study did not report the year of start of enrollment.

Taken together, analyses of effect modification within-studies and across studies did not provide sufficient evidence that any factor substantially modifies the predictive effect of platelet reactivity.

Key Question 3a and 3b Regarding Alternative Testing Strategies

The overarching question of the impact of testing (genetic or phenotypic) on intermediate or patient-relevant clinical outcomes can be answered by comparative studies of alternative test-and treat strategies (potentially including a strategy of no testing). Direct comparisons of testing strategies, particularly those in which assignment to a specific test-based strategy is random, are generally very rare. However, other study designs can provide information on the clinical utility of the tests of interest to this review. We grouped the studies we identified for this Key Question into three categories:

4. *Randomized trials of test-and-treat strategies*: These studies randomize patients to alternative management strategies, at least one of which is based on a test of interest. Patients are then followed up for intermediate or clinical outcomes. Random treatment assignment provides an unconfounded estimate of the effect of test-based management compared to alternative therapeutic strategies on outcomes.
5. *Randomized treatment trials that evaluate treatment-effect modification:* These are randomized studies in which patients in all groups undergo the test of interest at baseline. Treatment assignment is based on randomization and thus is independent of test results. Because these studies include both test-positive and test-negative patients in each treatment arm, they can be used to assess test result × treatment interactions. The presence of an interaction effect indicates that the relative treatment benefits (or harms) differ by test status; the results can also be used to infer whether improved outcomes should be expected if treatment choice were guided by test results.
6. *Randomized trials with test-based selection:* These studies select patients on the basis of baseline test results and then randomize them into non–test-based treatment groups. When properly randomized and conducted, these studies can provide unconfounded estimates of the treatment effect conditional on a particular test result but do not provide information on whether the treatment effect is modified by the test results.

Key Question 3 focused on efficacy outcomes (platelet reactivity as an intermediate marker of response and patient-relevant outcomes such as cardiovascular death or other MACE). We also considered intermediate and clinical outcomes separately for each design. Similar information on harms (e.g., bleeding events) is summarized later in the Results section, under Key Question 4, subsection on test-directed treatment.

In the following sections, we discuss studies belonging to each of three designs—studies of test-and-treat strategies, studies of treatment-effect modification, and studies with test-based selection—separately for genetic testing (for CYP2C19 variants) and for phenotypic testing (of platelet reactivity). **Table 12** summarizes the number of studies and outcomes assessed for each of the designs we reviewed for Key Question 3.

Table 12: Number of Studies Relevant to Key Question 3a or 3b and Outcomes Assessed, by Study Design and Test Used

Study Design	Genetic Testing for CYP2C19 Variants	Phenotypic Testing for Platelet Reactivity
RCTs of test-and-treat strategies (testing vs. no testing or comparisons between alternative test-and-treat strategies)	0	5 (4 for intermediate outcomes; 5 for clinical outcomes)
RCTs of treatment-effect modification by test results (difference in treatment effects across levels of the biomarkers)	10 (5 for intermediate outcomes; 4 for clinical outcomes; 1 for both)	1 (intermediate outcomes only)
RCTs with test-based selection (comparison of treatments among patients selected on the basis of biomarker level)	0	5 (2 for phenotypic outcomes only; 2 for clinical outcomes only; 1 for both)

RCT = randomized controlled trial.

Key Question 3a: What Is the Comparative Effectiveness of Genetic Testing for CYP2C19 Variants and Phenotypic Testing for Platelet Reactivity for Therapeutic Decisionmaking?

Genetic Testing for CYP2C19 Variants

Studies of Test-and-Treat Strategies

No studies compared testing for CYP2C19 variants with alternative testing strategies (including a no testing strategy) to guide treatment decisionmaking.

Randomized Trials Reporting Information on Treatment-Effect Modification by CYP2C19 Genotype Status

We identified 11 publications (reporting on 10 study populations) describing randomized controlled trials that provide information on effect modification by CYP2C19 variants. Five studies (reported in six publications)[a] provided information on clinical outcomes, five on intermediate outcomes (platelet reactivity during followup), and one on both types of outcome.

Information on the genetic assays used, the specific variants genotyped, and the grouping of genotypes for analysis is presented **Table 13** for all studies of treatment-effect modification by CYP2C19 variants. In most cases, authors claimed to have based the classification of genotypes on prior published literature, however studies genotyped different variants, resulting in heterogeneous exposure groupings.

Table 13: Test Information for Studies of Treatment-Effect Modification by CYP2C19 Genotype Status

Author Year Country PMID Study Name (if available)	Assay for Genetic Testing (Manufacturer)	Test Time Point Interval Between Sampling and Genotyping Results Interval Between Collection and DNA Extraction	N Tested Test Success Rate (%)	Alleles Genotyped	Genotype Grouping	Rationale for Grouping Alleles
Pare[91] 2010 Multinational 20979470 ACTIVE A	TaqMan assays [no additional information provided]	NR NR NR	Not estimable (data presented only on the successfully genotyped patients)	*2 [rs4244285]; *3 [rs4986893]; *4 [rs12248560]	Poor metabolizers=*2/*2 or *2/*3 or *3/*3 Intermediate metabolizers=*2/*1 or *3/*1 Extensive metabolizers=*1/*1 Ultra metabolizers=*17/*1 or *17/*17 Unknown metabolizers=*2/*17 or *17/*17	Based on prior literature; however, results from an alternative classification were presented as Appendix material

[a] Three publications reported information on a single population each and one publication reported information on two independent populations.

Pare[91] 2010 Multinational 20979470 CURE	TaqMan assays [no additional information provided]	NR NR NR	Not estimable (data presented only on the successfully genotyped patients)	*2 rs4244285]; *3 rs4986893]; *4 [rs12248560]	Poor metabolizers=*2/*2 or *2/*3 or *3/*3 Intermediate metabolizers=*2/*1 or *3/*1 Extensive metabolizers=*1/*1 Ultra metabolizers=*17/*1 or *17/*17 Unknown=*2/*17 or *17/*17	Based on prior literature; however, results from an alternative classification were presented as Appendix material
Mega[85,207] 2009 Multinational 19106084 19414633 TRITON-TIMI 38	98% of the genotyping procedures were performed with the Targeted Human DMET 1.0 Assay [Affymetrix, no additional details provided]; for the *17 allele and for no-call samples on the DMET chip (2% of samples) genotyping was performed with bi-directional sequencing or exon-specific PCR	NR NR NR	Not estimable (data presented only on the successfully genotyped patients)	*1A, *2A, *3, *4, *5A, *6, *7, *8, *9, *10, *12, *13, *14	Noncarriers=*1A/*1A Carriers=*1/*2A, *1A/*3, *1A/*4, *1A/*8, *2A/*2A, *2A/*3, *2A/*4, *2A/*5A, *2A/*8 Unknown=*1A/*9, *1A/*10, *2A/*17, *6/*17	Based on prior literature
Mega[208] 2010 Multinational 20801494 TRITON-TIMI 38	Detailed information not provided in the paper however the study by Mega et al. 2009[85] (see above row) was cited for the genotyping methods and grouping of genotypes.	NR NR NR	Not estimable (data presented only on the successfully genotyped patients)	For CYP2C19: as above In addition, genotyping was performed for ABCB1 C3435T (rs1045642) and results by combining both tests were reported	For CYP2C19: as above For ABCB1 C3435T: TT carriers vs. CC or CT carriers Combined results were presented by grouping patients with CYP2C19 reduced-function alleles and ABCB1 TT carriers vs. CYP2C19 loss-of-function allele noncarriers and ABCB1 CC/CT carriers	Based on prior literature
Kim[209] 2011 S. Korea 21511217 ACCELAMI2 C19	Single base primer extension assay (SNaPshot kit, Applied Biosystems, Foster City, CA) on the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA)	NR NR NR	140 MI patients were identified; CYP2C19 genotype could be determined in 126 (88.6%)	*2 [rs4244285]; *3 [rs4986893]	Carriers=*2/*1 or *3/*1 or *3/*3 Noncarriers=*1/*1	Not explicitly reported
Wallentin[90] 2010 Multinational 20801498 PLATO	TaqMan assays [Applied Biosystems, Life Technologies, Pleasanton CA]	Blood samples were obtained "as close to [time of] randomization as possible" NR NR	10,285 98.8–99.5%	CYP2C19*1–*8, *17 In addition, genotyping was performed for ABCB1 C3435T (rs1045642) and results by combining both tests were reported	For CYP2C19: Any LOF allele (*2–*8) No LOF allele (*1 or *17) For major bleeding only: Any LOF allele (*2–*8) but no GOF allele (not *17) No LOF or GOF allele (*1) Any GOF allele (*17) For ABCB1 C3435T: High expression (C/C) Intermediate expression (C/T) Low expression (T/T) Combined results were presented by grouping patients with any CYP2C19 loss-of-function allele and ABCB1 TT carriers vs. all other patients	Based on prior literature

Varenhorst[36] 2009 Sweden 19429918 TABR	Targeted Human DMET 1.0 Assay (Affymetrix, Santa Clara, CA) for all alleles except *17; *17 was genotyped using PCR-RFLP methods	NR NR NR	For CYP2C19 variants: 98 (100% success rate)	*1A, *2A, *3, *4, *5A, *6, *7, *8, *9, *10, *12, *13, *14, *17	Extensive metabolizers=*17/*17, *1A/*17, *1A/*1A Reduced metabolizers=*1A/*2A, *1A/*8, *2A/*2A Uncertain functional status=*2A/*17	Based on prior literature
Tantry[210] 2010 USA and UK 21079055 ONSET/OFFSET and RESPOND Genotype Studies	TaqMan Assay (Life Technologies, Pleasanton, CA)	NR NR NR	NR (results reported only on successfully genotyped individuals)	*2 [rs4244285]; *3 [rs4986893]; *4 [rs28399504]; *5 [rs56337013]; *6 [rs72558184]; *7 [rs72558186]; *8 [rs41291556]; *17 [rs12248560]	3 genotype grouping schemes were used: *Grouping 1* Ultra metabolizers=*17/*17 or *17/*1 Extensive metabolizers=*1/*1 Intermediate metabolizers=*2-*8/*1 or *2-*8/*17 Poor metabolizers=*2-*8/*2-*8 *Grouping 2* Loss-of-function carriers=*2-*8/*1 or *2-*8/*17 or *2-*8/*2-*8 Loss-of-function noncarriers=*1/*1 or *17/*17 or *17/*1 *Grouping 3* Gain-of-function carriers=*17/*17 or *17/*1 Extensive metabolizers=*1/*1 Loss-of-function carriers=*2-*8/*1 or *2-*8/*17 or *2-*8/*2-*8 Results were also presented separately for the observed diplotypes.	Not explicitly reported
Hwang[211] 2010 S. Korea 20823393 ACCEL-RESISTANCE, DM, and COMPLEX trials	Single base primer extension assay (SNaPshot kit, Applied Biosystems, Foster City, CA) on the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA)	NR NR NR	140 patients undergoing PCI in the 3 RCTs were identified; CYP2C19 genotype could be determined in 114 (76%)	*2 [rs4244285]; *3 [rs4986893]	Carriers=*2/*1 or *3/*1 or *3/*3 Noncarriers=*1/*1	Not explicitly reported (however the authors cite a previous study from the same center)
Gladding[158] 2008 New Zealand 19463375 PRINC	TaqMan assay (Applied Biosystems, Foster City, CA) on ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA)	NR NR NR	60 patients participating in the PRINC study; analyses relevant to this KQ only included 43 patients (the genotyping success rate was not estimable)	*2 [rs4244285]; *4 [rs28399504]; *17 [rs12248560]; results on the *17 variant allele were not reported in analyses relevant to this KQ	Carriers=*2/*2 or *2/*4 or *4/*4 or *2/*1 or *4/*1 Noncarriers=*1/*1	Not explicitly reported (results relevant to this KQ were not reported for all genotyped alleles)
Park[212] 2011 S. Korea 21345843 CILON-T	TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA) for *2 and *3 SNaPshot multiplex kit (ABI PRISM) for *17	NR NR NR	483 (successful in 474/483=98.1%) [in 5 patients, the DNA amount was not enough to carry out the genotyping of all 3 SNPs, and genotyping of *2 failed in 3 patients and of *3 in 1 patient]	CYP2C19*2 [rs4244285]; *3 [rs4986893]; *17 [rs12248560]	Carriers of CYP2C19 LOF allele (*2 or *3 with any other allele) Noncarriers (*1/*1, *17/*17, or *1/*17)	

CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events; DM = diabetes mellitus; DMET = Drug Metabolizing Enzyme and Transporter; KQ = Key Question; NR = not reported; ONSET/OFFSET and RESPOND; PCI = percutaneous coronary intervention; PCR = polymerase chain reaction; PLATO = Platelet Inhibition and Patient Outcomes; PRINC = Plavix Response in Coronary Intervention; RCT = randomized controlled trial; RFLP = restriction fragment length polymorphism; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel – Thrombolysis in Myocardial Infarction 38; UK = United Kingdom.

Clinical Outcomes

Of the five studies (reported in six publications) providing clinical outcome information, four[85,85,90,91,207,207] were large (>1000 participants), multicenter, randomized trials of clopidogrel-based treatment versus alternative treatments (two compared aspirin plus clopidogrel vs. aspirin monotherapy, one compared aspirin plus clopidogrel vs. aspirin plus prasugrel, and one compared aspirin plus clopidogrel vs. aspirin plus ticagrelor); each of these had at least one outcome event. The fifth study[209] was a single-center study of 126 patients followed for 30 days, comparing dual-antiplatelet therapy (aspirin plus clopidogrel) versus triple antiplatelet therapy (aspirin, clopidogrel, and cilostazol) for patients with myocardial infarction treated with "uneventful PCI." Because of the short followup period, no deaths, nonfatal myocardial infarctions, or urgent target-vessel revascularizations were reported.

Of the four larger studies, one included patients with non–ST-elevation acute coronary syndromes (the CURE [Clopidogrel in Unstable Angina to Prevent Recurrent Events] trial), one involved patients with ST-elevation or non–ST-elevation acute coronary syndromes (the PLATO [Platelet inhibition and patient Outcomes] trial), one included those with moderate-to-high-risk acute coronary syndromes who were undergoing PCI (TRITON-TIMI 38 [Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction 38]), and one enrolled patients with atrial fibrillation who were not candidates for vitamin K antagonist therapy (ACTIVE A). CURE and ACTIVE A compared aspirin plus clopidogrel (at standard doses) with aspirin monotherapy, TRITON-TIMI 38 compared aspirin plus clopidogrel versus aspirin plus prasugrel, and the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. All trials were designed and powered to detect the main effect of antiplatelet therapies but were not specifically powered to detect heterogeneity of treatment effects and included only a subsample of the overall study population in the genetic substudy.

The genetic substudy of the CURE trial (of patients with non–ST-elevation acute coronary syndromes) reported treatment-effect estimates (for dual-antiplatelet therapy vs. aspirin monotherapy) among poor, intermediate, extensive, and ultra-metabolizers, as well as patients with genotypes of unknown functional status. (See **Table 13** for definitions of genotype groups as implemented in the study.) Detailed results from time-to-event analyses for the two primary composite outcomes assessed in the study (cardiovascular death, nonfatal myocardial infarction, or stroke; and cardiovascular death, nonfatal myocardial infarction, stroke, recurrent ischemia, or hospitalization for unstable angina) are presented in **Table 14**. Overall, there was no statistically significant heterogeneity of treatment effects across genotype groups (P=0.12 and 0.29 for the first and second composite outcomes, respectively).

The genetic substudy of TRITON-TIMI 38enrolled patients with moderate-to-high-risk acute coronary syndromes who underwent PCI. The substudy reported treatment-effect estimates (for clopidogrel plus aspirin vs. prasugrel plus aspirin) among carriers and noncarriers of reduced-function CY2C19 alleles (see **Table 13** for definitions of genotype groups as implemented in the study). Detailed results on the study's primary efficacy endpoint (a composite of cardiovascular death, myocardial infarction, or stroke) at 15 months of followup are presented in **Table 14**. The study reported that there was statistically significant treatment-effect heterogeneity among genotype groups (P=0.046); using the events reported in the study, we calculated that the odds ratio for the primary efficacy outcome among carriers of reduced-function alleles was 1.45 (95% CI 0.91, 2.31), compared with 0.81 (95% CI, 0.60, 1.10) among noncarriers (P=0.04 for the difference between estimates). These results indicate that there was statistically significant

treatment-effect heterogeneity between carriers and noncarriers, the results also suggest a qualitative interaction, highlighting their clinical relevance for therapeutic decisionmaking. The combined grouping of CYP2C19 and ABCB1[b] genotypes, was a statistically significant predictor of outcomes among patients receiving clopidogrel-based therapy (P=0.0018) but not among patients receiving prasugrel-based therapy (P=0.485). The study did not report results of a test of interaction between treatment and genotype groups defined jointly by CYP2C19 and ABCB1status.

The genetic substudy of the PLATO trial enrolled patients with acute coronary syndromes. The substudy reported treatment-effect estimates (for clopidogrel plus aspirin vs. ticagrelor plus aspirin) among carriers and noncarriers of loss-of-function CY2C19 alleles (see **Table 13** for definitions of genotype groups as implemented in the study). Detailed results on the study's primary efficacy endpoint (a composite of cardiovascular death, myocardial infarction, or stroke) at 12 months of followup are presented in **Table 14**; there was no statistically significant treatment-effect heterogeneity between carriers of any loss-of-function allele and those without a loss-of-function allele (P=0.46). Neither were the results statistically significant for subcomponents of the primary outcome (cardiovascular death or myocardial infarction; interaction P=0.30). For definite stent thrombosis, the study did not provide an interaction P value (because the number of events was too low); however, the treatment effect was similar in both genotype groups (same direction and magnitude; assuming normality for the log–hazard ratio of each group defined by genotype, we calculated a z-score P value of 0.76 for the relative hazard ratio). In addition, the combined grouping of CYP2C19 and ABCB1 genotypes did not yield a statistically significant interaction for the primary outcome between treatment group and genotype group (P=0.13).

Finally, the genetic substudy of the ACTIVE A trial enrolled patients with atrial fibrillation and at least one additional risk factor for stroke who were not considered appropriate candidates for vitamin K–antagonist therapy. Patients were randomized to aspirin monotherapy or dual-antiplatelet therapy with aspirin and clopidogrel. Results from time-to-event analyses were reported for the treatment effect among poor, intermediate, extensive, and ultra-metabolizers, as well as patients with genotypes of unknown functional status (see **Table 13** for definitions of genotype groups as implemented in the study). Detailed results regarding the composite outcome of any major vascular event (stroke, systemic embolism outside the central nervous system, myocardial infarction, or death from vascular causes) are presented in **Table 14**. Overall, there was no significant heterogeneity of treatment effects across genotype groups (P=0.32).

[b] ABCB1 is a gene that encodes a protein involved in the intestinal absorption of clopidogrel.[213]

Table 14: Clinical Outcomes in Studies of Treatment-Effect Modification by CYP2C19 Genotype Status

Author Year Country PMID Study name (if available)	Outcome Definition (timing)	Subgroup defined by genotype	Treatment group	Number with outcome/total number within phenotype-treatment group	Comparison between treatments, within genotype groups ES (95% CI); P-value [statistical test]	Comparison of treatment effects across genotype groups
Pare[91]c 2010 Multinational 20979470 CURE	First primary composite outcome=CV death, nonfatal MI, or stroke	Poor metabolizers	Dual antiplatelet therapy	4/61	HR=0.44 (0.12 – 1.61) [Cox proportional hazards model; adjusted for age, sex, ancestry]	P=0.12
			Aspirin monotherapy	6/55		
		Intermediate metabolizers	Dual antiplatelet therapy	37/437	HR=0.72 (0.48 – 1.10) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	54/442		
		Extensive metabolizers	Dual antiplatelet therapy	112/1033	HR=0.92 (0.71 – 1.19) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	121/987		
		Ultra metabolizers	Dual antiplatelet therapy	66/847	HR=0.53 (0.39 – 0.72) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	112/826		
		Unknown metabolizers	Dual antiplatelet therapy	11/152	HR=0.69 (0.33 – 1.47) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	18/176		
	Second primary composite outcome=CV death, nonfatal MI, or stroke, recurrent ischemia, or hospitalization for UA	Poor metabolizers	Dual antiplatelet therapy	13/61	HR=0.93 (0.41 – 2.11) [Cox proportional hazards model; adjusted for age, sex, ancestry]	P=0.29
			Aspirin monotherapy	11/55		
		Intermediate metabolizers	Dual antiplatelet therapy	70/437	HR=0.87 (0.63 – 1.19) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	84/442		
		Extensive metabolizers	Dual antiplatelet therapy	193/1033	HR=0.90 (0.74 – 1.10) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	206/987		
		Ultra metabolizers	Dual antiplatelet therapy	123/847	HR=0.68 (0.53 – 0.85) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	167/826		

[c] The authors reported extensive subgroup and sensitivity analyses across different genotype grouping and after including or excluding subgroups of patients by ethnicity. The results relevant to treatment effect heterogeneity from these analyses are presented in the text of this section.

		Unknown metabolizers	Dual antiplatelet therapy	19/152	HR=0.63 (0.36 – 1.11) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	34/176		
Pare[91] 2010 Multinational 20979470 ACTIVE A	Primary efficacy outcome=any major vascular event (stroke, systemic embolism outside the CNS, MI, death from vascular causes)	Poor metabolizers	Dual antiplatelet therapy	1/9	HR=0.74 (0.05 – 12.04) [Cox proportional hazards model; adjusted for age, sex]	P=0.32
			Aspirin monotherapy	1/12		
		Intermediate metabolizers	Dual antiplatelet therapy	21/93	HR=0.85 (0.47 – 1.54) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	24/92		
		Extensive metabolizers	Dual antiplatelet therapy	34/199	HR=0.55 (0.36 – 0.83) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	68/235		
		Ultra metabolizers	Dual antiplatelet therapy	49/222	HR=0.97 (0.65 – 1.45) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	50/200		
		Unknown metabolizers	Dual antiplatelet therapy	7/37	HR=0.55 (0.21 – 1.47) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	10/35		
Mega[85,207][d] 2009 Multinational 19106084 19414633 TRITON-TIMI 38	Primary efficacy outcome=CV death, MI, or stroke; analysis for this outcome was performed base on intention-to-treat (as assigned)	Carriers of a reduced function allele	Clopidogrel + aspirin	46/395	OR=1.45 (0.91 – 2.31) [calculated values]	P=0.046 based on a test-for-interaction; P=0.04 [calculated from z-test between odds ratios]
			Prasugrel + aspirin	34/407		
		Noncarriers	Clopidogrel + aspirin	83/1064	OR=0.81 (0.60 – 1.10) [calculated values]	
			Prasugrel + aspirin	99/1048		
Mega[85,207] 2010 Multinational 20801494 TRITON-TIMI 38	Primary efficacy outcome=CV death, MI, or stroke; analysis for this outcome was performed base on intention-to-treat (as assigned) at 15 mo	CYP2C19 reduced-function alleles and ABCB1 TT carriers	Clopidogrel + aspirin	17/125	OR for the treatment effect among CYP2C19 reduced-function allele noncarriers and ABCB1 CC/CT carriers=NR [not possible to calculate] OR for the treatment effect among CYP2C19 reduced-function allele carriers or ABCB1 TT carriers=NR [not possible to calculate]	P-value for the genotype effect across genotypes=0.0018 (clopidogrel-treated); 0.485 (prasugrel-treated)
			Prasugrel + aspirin	NR/110		

[d] Patients with genotypes of "unclear" functional significance were excluded.

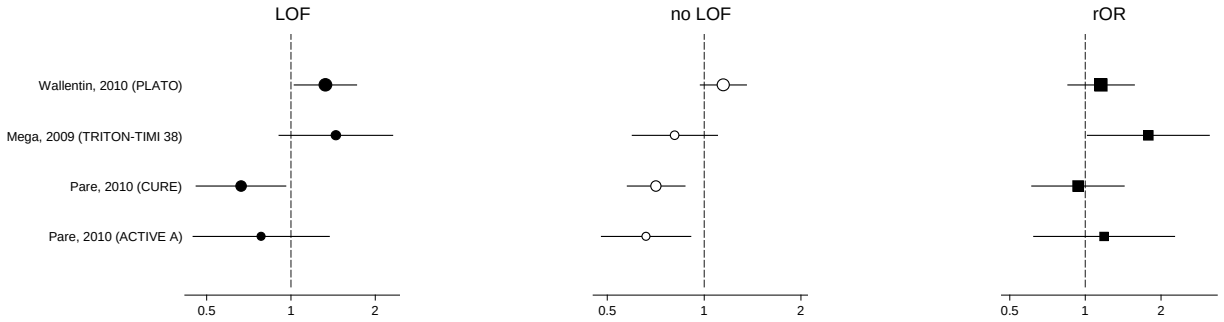
		CYP2C19 reduced-function allele noncarriers and ABCB1 TT carriers	Clopidogrel + aspirin	35/288		
			Prasugrel + aspirin	NR/278		
		CYP2C19 reduced-function alleles and ABCB1 CC/CT carriers	Clopidogrel + aspirin	29/268		
			Prasugrel + aspirin	NR/296		
		CYP2C19 reduced-function allele noncarriers and ABCB1 CC/CT carriers	Clopidogrel + aspirin	48/773		
			Prasugrel + aspirin	NR/767		
Kim[209] 2011 S. Korea 21511217 ACCELAMI2C19	Major cardiovascular events=death, nonfatal MI, or urgent target vessel revascularization	Carriers	Triple therapy (adjunctive cilostazol)	0/39	OR not estimable	NA
			High-maintenance dose clopidogrel	0/38		
		Noncarriers	Triple therapy (adjunctive cilostazol)	0/25		
			High-maintenance dose clopidogrel	0/24		
Wallentin[90] 2010 Multinational 20801498 PLATO	Primary composite outcome: CV death, MI, or stroke: 30 d	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	NR/1384	HR=0.73 (0.52–1.03) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.20
			Clopidogrel+ aspirin	NR/1388		
		No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	NR/3554	HR=0.96 (0.76–1.22) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	NR/3516		
	Primary composite outcome: CV death, MI, or stroke: 12 mo	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	115/1384	HR=0.77 (0.60–0.99) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.46
			Clopidogrel+ aspirin	149/1388		
		No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	296/3554	HR=0.86 (0.74–1.01) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	332/3515		
		Any CYP2C19 LOF allele (*2–*8) + ABCB1 C/C	Ticagrelor + aspirin	NR/2253	HR=0.75 (0.62–0.91) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.13

		Clopidogrel+ aspirin	NR/2248		
	No CYP2C19 LOF allele (*1 or *17) + ABCB1 C/T or T/T	Ticagrelor + aspirin	NR/2710	HR=0.92 (0.77–1.11) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
		Clopidogrel+ aspirin	NR/2698		
CV death or MI	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	102/1384	HR=0.73 (0.57–0.95) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.30
		Clopidogrel+ aspirin	138/1388		
	No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	273/3554	HR=0.86 (0.74–1.01) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
		Clopidogrel+ aspirin	306/3516		
Definite stent thrombosis (in patients who underwent stenting)	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	15/943	HR=0.71 (0.36–1.37) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	NR (no. of events too low to calculate)
		Clopidogrel+ aspirin	21/934		
	No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	22/2341	HR=0.62 (0.36–1.05) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
		Clopidogrel+ aspirin	35/2300		

ACTIVE A Trial = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A trial; CABG = coronary artery bypass grafting; CI = confidence interval; CNS = central nervous system; CURE Trial = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; CV = cardiovascular; d = day; ES = effect size; GOF = gain of function; HR = hazard ratio; LOF = loss of function; MI = myocardial infarction; mo = month; NA = not applicable; NR = not reported; OR = odds ratio; PLATO trial = PLATelet inhibition and patient Outcomes trial; PPI = proton-pump inhibitor; TRITON TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis In Myocardial Infarction 38.

Because of the large differences in included populations, treatments compared, and exposure and outcome definitions among studies reporting on treatment-effect modification by CYP2C19 variants on clinical outcomes, we did not perform a meta-analysis. Given that the drugs compared (clopidogrel, prasugrel, and ticagrelor) have different mechanisms of action, it is plausible that interaction effects could have different magnitudes or directions across studies. For purposes of illustration, we used the counts reported in the studies to compare the treatment effect among carriers of CYP2C19 loss-of-function alleles versus noncarriers (i.e., those with normal or gain-of-function alleles). **Figure 31** presents the results for the four studies (in three publications) with at least one composite cardiovascular outcome occurred (see **Table 14** for definitions of outcomes) and with the results stratified by genotype group. The figure also demonstrates the relative treatment effect (i.e., the relative odds ratio) across the genotype groups—the equivalent of the genotype × treatment interaction. The relative treatment effect was statistically significant in only one study.

Figure 31: Results from Large Randomized Trials Assessing Effect Modification by CYP2C19 Variants on MACE



Forest plots of treatment effects (odds ratios) on major adverse cardiovascular events (MACE) among carriers of at least one loss-of-function (LOF) allele (left panel); treatment effects (odds ratios) among noncarriers of LOF alleles (middle panel); and relative effects (relative odds ratios [rOR]) comparing the treatment effect among LOF carriers and LOF noncarriers (right panel). The CURE and ACTIVE A trials compared aspirin plus clopidogrel versus aspirin monotherapy; the TRITON-TIMI 38 trial compared aspirin plus clopidogrel versus aspirin plus prasugrel; the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. The CURE and ACTIVE A trials compared aspirin plus clopidogrel versus aspirin monotherapy; the TRITON-TIMI 38 trial compared aspirin plus clopidogrel versus aspirin plus prasugrel; the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. Point estimates for treatment effects are shown as black circles (carriers) or white circles (noncarriers); point estimates for relative treatment effects are shown as black squares. Horizontal lines denote 95 percent confidence intervals for all estimates. Vertical dashed lines denote no effect. Please see **Tables 13** and **14** for full definitions of the genotype categories and outcomes reported by each study.
ACTIVE A = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; PLATO = Platelet inhibition and patient Outcomes trial; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction.

Intermediate Outcome: Platelet Reactivity During Followup

Six studies assessing treatment-effect modification by CYP2C19 variants provided information on platelet reactivity during followup as an intermediate outcome (**Table 13** and **15**).[36,158,209-212] All six were based on randomized trials comparing clopidogrel-based treatment to alternative therapies, had small to moderate sample sizes (range, 60 to 474 participants), and enrolled heterogeneous populations: two included patients with stable coronary artery disease, one included patients with myocardial infarction undergoing PCI, one included patients undergoing elective PCI, one enrolled patients with coronary artery disease receiving drug-eluting stents, and one included "high-risk" patients undergoing PCI. All had short followup periods(<7 days to 6 weeks).

Table 13 describes the assays used, alleles genotyped, and genotype groupings used in the six studies. Four of the six did not provide a rationale for the genotype grouping. The studies differed in the alleles genotyped and the genotype groupings used, leading to heterogeneity in the exposure definition. Platelet reactivity during followup was assessed by the VerifyNow P2Y12 assay in all six studies, as well as by LTA in three studies and the VASP assay (based on flow cytometry) in two studies. Key findings from these studies are summarized in **Table 16**. The overall results were variable and incomplete reporting often precluded the quantitative assessment of test × treatment interactions. Because of the differences in designs, populations, treatments compared, and followup durations among the included studies, we did not perform a meta-analysis.

Table 15: Study Characteristics in Studies of Treatment-Effect Modification by CYP2C19 Genotype Status with Intermediate Outcomes

Author Year Country PMID Study Name (if available)	Selection Criteria	Study Design	N Enrolled Male (%) Age[e] Dyslipidemia (%) Smokers (%) HTN (%) Diabetes (%) Race/Ethnicity (%)	Treatments Compared	Followup Duration
Varenhorst[36f] 2009 Sweden 19429918 TABR	Patients with stable coronary artery disease; patients were participants in a 2-center, double-blind, double-dummy RCT	Pre-specified genetic substudy of an RCT	98 89 (91%) 63.8 (6.0) NR 12 (12%) [unclear definition] NR 19 (19%) White=98 (100%)	All study subjects received aspirin 75 mg/d for 5–21 days prior to randomization and continued throughout the study. Following the open-label aspirin run-in period, subjects continuing to meet enrolment criteria were assigned to two groups: *Aspirin + prasugrel:* prasugrel loading dose 60 mg, followed by prasugrel 10 mg/d maintenance dose *Aspirin + clopidogrel:* clopidogrel 600 mg loading dose; clopidogrel 75 mg/d maintenance dose	30 d
Tantry[g] Tantry, 2010 39 /id} 2010 USA and UK 21079055 ONSET/OFFSET and RESPOND Genotype Studies	Patients with documented stable coronary artery disease; patients were participants in the double-blind, double-blind, parallel arm multicenter ONSET-OFFSET study or the double-blind, double-blind, crossover multicenter RESPOND study	Genetic substudies based on 2 RCTs [data from both were combined]	174 129 (74%) 63.9 (8.6) 164 (94%) 15 (9%) [current smokers] 137 (79%) 39 (22%) White=153 (88%); Black=15 (7%); other NR	In the ONSET/OFSET trial: Ticagrelor group: ticagrelor (180 mg) loading dose; ticagrelor maintenance (90 mg) in the evening with a 12-hour interval between dosing, followed by ticagrelor maintenance 90 mg BID for 6 w Clopidogrel group: clopidogrel (600 mg) loading dose; clopidogrel maintenance (90 mg) in the evening with a 12-hour interval between dosing, followed by clopidogrel maintenance 75 mg/d for 6 w Placebo group: placebo (no treatment) loading dose; followed by placebo administration for 6 w In the RESPOND trial, previously identified responsive or nonresponsive to clopidogrel 300 mg loading dose (cut-off ≤10% absolute change of reactivity to LTA) patients were randomized into two groups for the first trial period: *Clopidogrel group:* 600 mg clopidogrel loading dose, followed by 75 mg/d maintenance dose for 2 w *Ticagrelor group:* 80 mg ticagrelor loading dose, followed by 90 mg BID maintenance dose Period 2 data for the RESPOND trial were not used in the pharmacodyamic investigation.	6 w for ONSET/OFFSET participants; 2 w for RESPOND participants
Kim[209] 2011 S. Korea 21511217 ACCELAMI2C19	Consecutive MI patients admitted to a single university hospital treated with "uneventful PCI"; patients were participants in the genetic expanded study of ACCEL-AMI	Genetic substudy of RCT	126 [with available genotyping information] 92 (73%) 61.7 (12.3) 35 (28%) 75 (60%) [current smokers] 59 (47%) 35 (28%) east Asian=126 (100%)	All patients received clopidogrel loading dose of 600 mg upon admission; followed by 75 mg/d maintenance dose. *High-maintenance dose clopidogrel:* clopidogrel 150 mg/d maintenance *Triple therapy (adjunctive cilostazol):* cilostazol 100 mg BID + clopidogrel 75/d maintenance All patients received aspirin 300 mg loading dose, followed by 200 mg/d maintenance dose for the duration of the study.	30 d

[e] Mean (standard deviation), unless otherwise stated.
[f] Some information extracted from Wallentin et al. 2008.[214]
[g] Some information extracted from Gurbel et al. 2009[215] and Gurbel et al. 2010.[137]

Hwang[211] 2010 S. Korea 20823393 ACCEL-RESISTANCE, DM, and COMPLEX trials (ACEL-POLYMORPHISM)	High-risk patients undergoing PCI; patients were participants in the ACELL trials (patients in the RESISTANCE trial had HPPR; in the DM trial had diabetes mellitus; and in the COMPLEX trial had complex lesions for stent placement)[h]	Prespecified genetic substudy of 3 RCTs	114 [with available genotyping information] 88 (77%) 63.2 (9.5) 33 (29%) 51 (45%) [current smokers] 76 (67%) 37 (32%) East Asian=114 (100%)	All patients received clopidogrel loading dose of 300 mg upon ≥12 h before PCI or where on chronic clopidogrel treatment (75 mg/d for ≥7 d); followed by 75 mg/d maintenance dose. *High-maintenance dose clopidogrel:* clopidogrel 150 mg/d maintenance *Triple therapy (adjunctive cilostazol):* cilostazol 100 mg BID + clopidogrel 75/d maintenance All patients received aspirin 300 mg loading dose, followed by 200 mg/d maintenance dose for the duration of the study.	30 d
Gladding[158i] 2008 New Zealand 19463375 PRINC	Patients undergoing elective PCI who were on aspirin treatment; patients were participants In the randomized PRINC trial	Genetic substudy of RCT	60 50 (83%) 68 (10) NR 6 (10%) [current smokers] 34 (57%) 11 (18%) White=57 (95%); others NR	The PRINC study design had 2 phases: a 2×2 factorial, randomized, placebo-controlled, double-blind study over the first 24 h, followed by a 1 w randomized, placebo-controlled, double-blind study. Patients were first randomized in a 2 × 2 manner to receive either 5 mg verapamil or placebo at baseline and either placebo or 600 mg clopidogrel, 2 h from baseline. All patients received 600 mg clopidogrel at the start of the PCI procedure, 10 min after administration of verapamil or placebo. The next day, patients were separately randomized to receive clopidogrel 75 or 150 mg once daily for 1 week, followed by 75 mg once daily thereafter.	7 d
Park[212] 2011 S. Korea 21345843 CILON-T	Patients undergoing implantation of drug-eluting stents; patients were participants in the CILON-T randomized trial	Genetic substudy of RCT	474 330 (70%) 63.3 (8.7) 220 (46%) 98 (21%) 322 (68%) 159 (34%) 474 (100%) Korean	Patients who had not taken aspirin or clopidogrel before PCI were given a loading dose of aspirin (300 mg) and clopidogrel (300–600 mg) and then were randomized to: *Dual-antiplatelet therapy:* Aspirin (100 mg daily) and clopidogrel (75 mg daily) *Triple-antiplatelet therapy:* Aspirin (100 mg daily), clopidogrel (75 mg daily), and cliostazol (200 mg loading dose + 100 mg twice daily)	NR ("at discharge ")

d=day; NR=not reported; PCI=percutaneous coronary intervention; PRINC=Plavix Response in Coronary Intervention; RCT=randomized clinical trial.

Table 16: Effect Modification on the Outcome of Platelet Reactivity in Randomized Trials Stratifying Patients by CYP2C19 Genotype Status

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
Varenhorst[36] 2009 Sweden 19429918 TABR	*Aspirin + prasugrel*	Extensive metabolizers Reduced metabolizers Uncertain functional status	35 15 1	VASP assay (ADP ±PGE1) [Byocytex, Marseille, France] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	By PRI VASP: at 24h, 14d, and 29d the difference between extensive and reduced metabolizers was P<0.05 in clopidogrel treated patients but P=NS for prasugrel treated patients. By PRU VerifyNow: at 24h, 14d, and 29d the difference between extensive and reduced metabolizers was P<0.05 in clopidogrel treated patients but P=NS for prasugrel treated patients. Exact p-values were not reported; additional information provided only in graphical form (Figure 3 of the paper).

[h] Although the HPPR study required presence of resistance to antiplatelet therapy based on reactivity testing we included ht study by Hwang et al. in this section (and not in the section on studies randomizing patients after selection with reactivity testing) because the paper did not report separate results for patients in the ACCEL-RESISTANCE study, and such criteria were not used in the ACCEL-DM or ACCEL-COMPLEX trials.

[i] Some information extracted from Gladding et al. 2008.[216]

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
	Aspirin + clopidogrel	Extensive metabolizers Reduced metabolizers Uncertain functional status	37 9 1		
Tantry[j] Tantry, 2010 39 /id} 2010 USA and UK 21079055 ONSET/OFFSET and RESPOND Genotype Studies	*Ticagrelor group*	Ultra metabolizers Extensive metabolizers Intermediate metabolizers Poor metabolizers	27 28 35 2	LTA (ADP 5 or 20 µmo/L) [Chronolog Optical Aggregometer, model 490-4D, no additional information provided] VASP assay (ADP ±PGE1) [Byocytex, Marseille, France] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	LTA (ADP 5 µM); P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Ultra metabolizers, P=0.0016 Extensive metabolizers, P=0.0004 Intermediate metabolizers, P<0.0001 Poor metabolizers, P =0.149 LTA (ADP 20 µM); P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Ultra metabolizers, P=0.001 Extensive metabolizers, P=0.0001 Intermediate metabolizers, P<0.0001 Poor metabolizers, P=0.139 VerifyNow; P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Ultra metabolizers, P<0.0001 Extensive metabolizers, P<0.0001 Intermediate metabolizers, P<0.0001 Poor metabolizers, P=0.138 VASP assay; P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Ultra metabolizers, P<0.0001 Extensive metabolizers, P<0.0001 Intermediate metabolizers, P<0.0001 Poor metabolizers, P =0.149 P-values from Wilcoxon rank sum test. In all groups reactivity was lower in the ticagrelor group. Additional information provided only in graphical form (Figures 3-5)
	Clopidogrel group	Ultra metabolizers Extensive metabolizers Intermediate metabolizers Poor metabolizers	28 31 20 3		

[j] Results were extracted for the longest followup reported (2–6 weeks); results for each diplotype not presented.

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
	Ticagrelor group	LOF carriers LOF noncarriers GOF carriers	27 28 37	LTA (ADP 5 and 20 µmo/L) [Chronolog Optical Aggregometer, model 490-4D, no additional information provided] VASP assay (ADP ±PGE1) [Byocytex, Marseille, France] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	LTA (ADP 5 µM); P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Loss-of-function carriers, P<0.0001 Loss-of-function noncarriers, P<0.0001 Gain-of-function carriers, P =0.0016 LTA (ADP 20 µM); P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Loss-of-function carriers, P<0.0001 Loss-of-function noncarriers, P=0.0001 Gain-of-function carriers, P=0.001 VerifyNow; P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Loss-of-function carriers, P<0.0001 Loss-of-function noncarriers, P<0.0001 Gain-of-function carriers, P<0.0001 VASP assay; P for the treatment effect within genotype group (ticagrelor vs. clopidogrel) Loss-of-function carriers, P<0.0001 Loss-of-function noncarriers, P<0.0001 Gain-of-function carriers, P<0.0001 P-values from Wilcoxon rank sum test. In all groups reactivity was lower in the ticagrelor group. Additional information provided only in graphical form (Figures 3-5)
	Clopidogrel group	LOF carriers LOF noncarriers GOF carriers	28 31 23		
Kim[k209] 2011 S. Korea 21511217 ACCELAMI2 C19	*High-maintenance dose clopidogrel*	Carriers Noncarriers	38 24	LTA (ADP 5 and 20 µmol/L) [AggRAM aggregometer, Helena Laboratories Corp., Beaumont, TX] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	*Maximal platelet aggregation by LTA 5 µmol/L at 30 d* *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=37.6 (SD=16.0) Mean reactivity in triple therapy group=24.9 (SD=14.3) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=30.1 (SD=10.1) Mean reactivity in triple therapy group=28.2 (SD=11.5) P for the treatment effect among noncarriers=0.547 *Maximal platelet aggregation by LTA 20 µmol/L at 30 d (mean reactivity)* *Carriers:* High-maintenance dose clopidogrel group, 52.3 (SD=17.5) triple therapy group=35.0 (SD=19.3) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=38.6 (SD=12.0) Mean reactivity in triple therapy group=37.5 (SD=13.2) P for the treatment effect among noncarriers=0.752 *Late platelet aggregation by LTA 5 µmol/L at 30 d* *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=26.7 (SD=16.5) Mean reactivity in triple therapy group=15.2 (SD=12.4) P for the treatment effect among carriers=0.001

[k] Results were extracted for the longest followup reported (30 d).

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
					Noncarriers Mean reactivity in high-maintenance dose clopidogrel group=16.4 (SD=9.2) Mean reactivity in triple therapy group=13.6 (SD=11.7) P for the treatment effect among noncarriers=0.347
					Late platelet aggregation by LTA 20 µmol/L at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=40.6 (SD=22.2) Mean reactivity in triple therapy group=23.5 (SD=19.2) P for the treatment effect among carriers=0.0001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=21.8 (SD=14.8) Mean reactivity in triple therapy group=19.7 (SD=16.8) P for the treatment effect among noncarriers=0.649
					VerifyNow PRU at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=184.2 (SD=80.6) Mean reactivity in triple therapy group=171.9 (SD=86.3) P for the treatment effect among carriers=0.518 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=152.2 (SD=70.4) Mean reactivity in triple therapy group=136.6 (SD=70.0) P for the treatment effect among noncarriers=0.441
					VerifyNow %inhibition at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=40.3 (SD=23.5) Mean reactivity in triple therapy group=48.0 (SD=24.0) P for the treatment effect among carriers=0.157 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=54.7 (SD=22.3) Mean reactivity in triple therapy group=56.4 (SD=20.7) P for the treatment effect among noncarriers=0.783
					HPR (cut-off >59% using LTA with ADP 20 µmol/L) at 30 d *Carriers* N (%) among high-maintenance dose clopidogrel group=17 (44.7%) N (%) among triple therapy group=6 (15.4%) P for the treatment effect among carriers=0.005 *Noncarriers* N (%) among high-maintenance dose clopidogrel group=2 (8.3%) N (%) among triple therapy group=0 (0%) P for the treatment effect among noncarriers=0.235
	Triple therapy (adjunctive cilostazol)	Carriers Noncarriers	25 39		
Gladding[158] 2008 New Zealand 19463375 PRINC	*1200 mg clopidogrel loading*	Carriers Noncarriers	11 15	VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	% inhibition (Mann-Whitney U test) among carriers at 4 h (600 mg vs. 1200 mg): median=14% vs. 37%; P=0.002 % inhibition (Mann-Whitney U test) among noncarriers at 4 h (600 mg vs. 1200 mg): median=35% vs. 43%; P=0.3 % inhibition (Mann-Whitney U test) among carriers at 7 h (600 mg vs. 1200 mg): median=22% vs. 42%; P=0.09 % inhibition (Mann-Whitney U test) among noncarriers at 7 h (600 mg vs. 1200 mg): median=29% vs. 63%; P=0.05

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
					% inhibition (Mann-Whitney U test) among carriers at 7 d (150 mg vs. 75 mg): median=14% vs. 51%; P=0.042 % inhibition (Mann-Whitney U test) among noncarriers at 7 h (150 mg vs. 75 mg): median=32% vs. 51%; P=0.2
	600 mg clopidogrel loading	Carriers Noncarriers	8 9		
	150 mg clopidogrel maintenance	Carriers Noncarriers	5 12		
	75 mg clopidogrel maintenance	Carriers Noncarriers	9 6		
Hwang[l211] 2010 S. Korea 20823393 ACCEL-RESISTANCE, DM, and COMPLEX trials	*High-maintenance dose clopidogrel*	Carriers Noncarriers	43 22	LTA (ADP 5 and 20 µmol/L) [AggRAM aggregometer, Helena Laboratories Corp., Beaumont, TX] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	*Maximal platelet aggregation by LTA 5 µmol/L at 30 d* *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=42.9 (SD=18.1) Mean reactivity in triple therapy group=28.4 (SD=13.9) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=31.1 (SD=12.9) Mean reactivity in triple therapy group=26.7 (SD=15.2) P for the treatment effect among noncarriers=0.304
					Maximal platelet aggregation by LTA 20 µmol/L at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=55.4 (SD=15.9) Mean reactivity in triple therapy group=40.5 (SD=16.7) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=41.9 (SD=16.4) Mean reactivity in triple therapy group=36.0 (SD=19.2) P for the treatment effect among noncarriers=0.276
					Late platelet aggregation by LTA 5 µmol/L at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=30.7 (SD=22.2) Mean reactivity in triple therapy group=16.7 (SD=11.5) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=19.0 (SD=13.4) Mean reactivity in triple therapy group=14.9 (SD=13.4) P for the treatment effect among noncarriers=0.318
					Late platelet aggregation by LTA 20 µmol/L at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=43.2 (SD=22.0) Mean reactivity in triple therapy group=26.4 (SD=17.4) P for the treatment effect among carriers<0.001 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=26.9 (SD=18.8) Mean reactivity in triple therapy group=21.2 (SD=19.0) P for the treatment effect among noncarriers=0.321
					VerifyNow PRU at 30 d

[l] Results were extracted for the longest followup reported (30 d).

Author Year Country UID Study name (if available)	Treatment group	Subgroups defined by genotype	N	Assay (agonist) [manufacturer] for the assessment of platelet reactivity	Summary of results
					Carriers Mean reactivity in high-maintenance dose clopidogrel group=214.1 (SD=68.5) Mean reactivity in triple therapy group=191.6 (SD=78.4) P for the treatment effect among carriers=0.153 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=149.7 (SD=65.4) Mean reactivity in triple therapy group=129.4 (SD=76.4) P for the treatment effect among noncarriers=0.348
					VerifyNow %inhibition at 30 d *Carriers* Mean reactivity in high-maintenance dose clopidogrel group=33.5 (SD=19.2) Mean reactivity in triple therapy group=45.8 (SD=21.2) P for the treatment effect among carriers=0.005 *Noncarriers* Mean reactivity in high-maintenance dose clopidogrel group=65.2 (SD=12.8) Mean reactivity in triple therapy group=70.5 (SD=7.6) P for the treatment effect among noncarriers=0.317
					HPR (cut-off >50% using LTA with ADP 5 µmol/L) at 30 d *Carriers* N (%) among high-maintenance dose clopidogrel group=16 (37.2%) N (%) among triple therapy group=3 (6.4%) P for the treatment effect among carriers<0.001 *Noncarriers* N (%) among high-maintenance dose clopidogrel group=3 (13.6%) N (%) among triple therapy group=1 (4.5%) P for the treatment effect among noncarriers=0.607
	Triple therapy (adjunctive cilostazol)	Carriers Noncarriers	47 22		
Park[212] 2011 S. Korea 21345843 CILON-T	*Dual-antiplatelet therapy:*	LOF carriers Noncarriers	132 104	VerifyNow P2Y12 (ADP) [Accumetics, San Diego, CA]	*Mean on-treatment platelet reactivity (in PRU)* Dual therapy, carrier (254±7) vs. noncarrier (208±7), P<0.001 Triple therapy, carrier (215±7) vs. noncarrier (199±9), P=0.167 Noncarrier, dual therapy (211±8) vs triple therapy (196±9), P=0.242 Carrier, dual therapy (256±7) vs triple therapy (213±6), P<0.001 *High on-treatment platelet reactivity (>240 PRU):* Dual therapy, carrier (60.8%) vs. noncarrier (36.5%), P<0.001 Triple therapy, carrier (43.7%) vs. noncarrier (33.3%), P=0.115 Noncarriers, triple therapy (33.3%) vs. dual therapy (36.5%), P=0.241 Carriers, triple therapy (43.7%) vs. dual therapy (60.8%), P<0.001 P=0.241 for proportion of high on-treatment platelet reactivity among triple therapy noncarrier, triple therapy carrier, and dual therapy noncarrier
	Triple-antiplatelet therapy (adjunctive cilostazol)	LOF carriers Noncarriers	151 87		Multivariate analysis for independent predictors of high on-treatment platelet reactivity (adjusted for decade of age, sex, smoking, diabetes, CKD, and treatment group): *OR (95% CI) [P value] vs. dual therapy, noncarrier:* Dual therapy, carrier 2.93 (1.64–5.21) [<0.001] Triple therapy, noncarrier 0.75 (0.39–1.44) [0.388] Triple therapy, carrier 1.19 (0.68–2.05) [0.545]

ADP = adenosine diphosphate; CI = confidence interval; CKD = chronic kidney disease; d = day(s); GOF = gain-of-function; LOF = loss-of-function; LTA = light-transmission aggregometry; PRINC = Plavix Response in Coronary Intervention; PRU = platelet reactivity units; VASP = vasodilator-stimulated phosphoprotein.

Assessment of Risk of Bias of Individual Studies

A detailed assessment of 17 risk-of-bias items for studies providing information on effect modification by CYP2C19 variants is presented in **Appendix Table F1** (the table includes both studies reporting on clinical and those reporting on intermediate outcomes) and is summarized in **Appendix Figure F1**. Overall, the risk of bias of individual studies was variable. Generally, studies reporting on clinical outcomes (which were generally larger and had longer followup) rather than platelet reactivity as an outcome used robust methods for randomization and allocation concealment. However, these studies included only a small proportion of the patients included in the corresponding parent trials (15 to 40 percent in trials reporting clinical followup of more than a month). In contrast, in studies reporting information on laboratory outcomes (which tended to be smaller with short followup periods), 79 to 100 percent of the patients enrolled in the parent randomized trials were also included in the genetic substudies. These smaller studies provided adequate descriptions of the methods used for generating the randomization sequence but did not provide sufficient information to assess methods of allocation concealment.

Studies with Genetic Test–Based Selection of Patients

No studies fulfilling our inclusion criteria selected patients on the basis of CYP2C19 genotype to compare alternative treatment strategies.

Phenotypic Testing for Platelet Reactivity

Studies of Test-and-Treat Strategies

We identified five studies directly comparing alternative test-and-treat strategies.[217-221] All studies had a randomized design; no nonrandomized comparative studies of test-and-treat strategies were identified. Four compared VASP assay–guided therapy and non–test-guided therapy; the fifth compared VerifyNow P2Y12–guided therapy versus non–test-guided therapy. The four evaluating the use of the VASP assay were of moderate size (the smallest enrolled 153 patients; the largest, 429 patients); three were multicenter studies and 1 was a single-center investigation. The single study assessing VerifyNow was smaller (had 60 patients) and had been conducted in a single research center.

All five studies directly comparing alternative test-and-treat strategies assessed patients undergoing PCI; 4 enrolled patients with stable coronary artery disease or acute coronary syndromes and one enrolled exclusively patients undergoing elective stenting. In four studies, the average patient age was older than 65 years; the average age was 64.9 years in the fifth study. Patients had a relatively high burden of risk factors for cardiovascular disease. Four studies selected patients on the basis of a baseline assessment of platelet reactivity (enrolling only those with reactivity above a predetermined threshold); the fifth study included all patients, regardless of baseline reactivity.

The experimental groups in four studies (three using the VASP assay and one using the VerifyNow assay) employed repeat reactivity monitoring at multiple time points with modification of the administered clopidogrel dose on the basis of test results (**Table 17** summarizes the treatment strategies). The fifth study performed only a single assessment of platelet reactivity, after 15 days of treatment, with subsequent treatment modification in patients

found to have reactivity values above a predefined threshold. Control groups were given clopidogrel-based therapy at standard doses.

Table 17 summarizes the selection criteria, population characteristics, and treatments compared in the five test-and-treat studies involving phenotypic testing.

The five studies are relatively recent (with enrollment started between 2005 and 2009), and had relatively short followup durations (three had maximum followup of 30 days; one, 6 months; and one, a year). Only two studies, conducted by the same investigator group but with nonoverlapping enrollment periods, reported a prospective power calculation (and both had attained the enrollment goal). **Table 18** summarizes the study design of included studies. Information on the assays used, test timing, and the number of patients screened for inclusion is presented in **Table 19**.

Table 17: Descriptive Characteristics of Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Selected Population	Total N Enrolled Male (%) Age Dyslipidemia (%) Current smokers (%) HTN (%) Diabetes (%)	Initial and Compared Treatment Strategies
Wang[217] 2011 China 21538380	Patients with VASP PRI >50% 1 mo after undergoing PCI for refractory angina pectoris, silent ischemia on thallium scintigraphy, or NSTE ACS	N=306 214 (70%) 67 (11) 156 (51%) 118 (39%) 188 (61%) 132 (43%)	All patients underwent angiography after a loading dose of aspirin (100 mg) and clopidogrel (300 mg); maintenance doses were 100 mg/d for aspirin and 75–375 mg/d for clopidogrel. One mo after PCI patients were randomized to 2 groups: *VASP-guided treatment:* VASP PRI was ascertained at 3, 6, 9, and 12 mo after the first analysis (1 mo post-PCI), when patients with PRI >50% received clopidogrel at a dose of 150 mg/d. At each subsequent monitoring visit the dose was increased by 75 mg/d if PRI remained > 50% (for a maximum dose of 375 mg/d at 1 yr). If PRI was <25% at the 12-mp visit, the dose was decreased to 75mg/d. For PRI between 25% and 50%, the dose was not changed. *Control treatment:* A maintenance regimen of clopidogrel 75 mg/d was used.
Bonello[218] 2009 France 19101221	Patients with VASP PRI >50% undergoing nonemergent PCI for refractory angina pectoris under "optimal medical therapy," silent ischemia on thallium scintigraphy or NSTE ACS	N=429 345 (80%) 66 (11) 255 (59%) 238 (55%) 264 (62%) 155 (36%)	All patients received a clopidogrel loading dose of 600 mg. Patients with PRI >50% afterward were randomized to 2 groups: *VASP-guided treatment:* Clopidogrel pretreatment was adjusted individually (pre-PCI) to obtain a VASP PRI <50% by prescribing up to 3 additional boluses of clopidogrel 600 mg 24 h after the previous dose; PRI was assessed 12 h after each administration until the value was <50%. If PRI remained >50% after the 3 additional loading doses, PCI was performed. *Control treatment*: PCI was carried out without an additional clopidogrel loading dose.
Bonello[219] 2008 France 18387444	Patients with VASP PRI >50% undergoing nonemergent PCI for refractory angina pectoris under "optimal medical therapy" or having silent ischemia on thallium scintigraphy or NSTE ACS	N=162 126 (78%) 66 (11) 86 (53%) 62 (38%) 98 (60%) 67 (41%)	All patients received a clopidogrel loading dose of 600 mg. Patients with PRI >50% afterward were randomized to 2 groups: *VASP-guided treatment:* Clopidogrel pretreatment was adjusted individually (pre-PCI) to obtain a VASP PRI <50% by prescribing up to 3 additional boluses of clopidogrel 600 mg 24 h after the previous dose; PRI was assessed 12 h after each administration until the value was <50%. If PRI remained >50% after the 3 additional loading doses, PCI was performed. *Control treatment*: PCI was carried out without an additional clopidogrel loading dose.
Tousek[220] 2011 Czech Republic 21663983	Patients with VerifyNow PRU >240 undergoing PCI for stable angina, STE or NSTE MI, or UA	N=60 45 (75%) 66 (12) NR 22 (37%) 40 (67%) 18 (30%)	All patients received a clopidogrel loading dose of 600 mg. Patients with VerifyNow PRU >240 afterward were randomized to 2 groups: *VerifyNow-guided treatment:* clopidogrel up-titration to 150 mg/d with further dose increases according to VerifyNow results every 7 days; the target PRU was <240 by 30 d. *Control treatment:* Standard clopidogrel maintenance dose of 75 mg/d.

Aleil[221] 2008 France 19463377 VASP-02	Patients undergoing elective coronary stenting	N=153 64.9 (9.7) 126 (82%) 100 (65%) 24 (16%) 97 (63%) 37 (24%)	The day before PCI patients received clopidogrel (300 to 600 mg) loading dose and were then randomized into 2 groups (3:2 ratio): *VASP-guided treatment:* Clopidogrel maintenance dose of 75 mg/d for 2 wk. At 2 wk, VASP analysis was performed; patients with PRI ≥69% were administered a maintenance dose of 150 mg/d, and those with PRI <69% were kept on 75 mg/d, for an additional 2 wk. *Control treatment:* Clopidogrel maintenance dose of 150 mg/d for 4 wk.

Age is given as mean years (standard deviation), unless otherwise stated.
ACS = acute coronary syndrome; d = day(s); h = hours; HTN = hypertension; MI = myocardial infarction; mo = month; NR = not reported; NSTE = non-STEMI; PCI = percutaneous coronary intervention; PRI = platelet reactivity index; PRU = platelet reactivity units (arbitrary units); STE = ST-elevation; UA = unstable angina; VASP = vasodilator-stimulated phosphoprotein; wk = weeks.

Table 18: Study Design Characteristics of Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Enrollment period	Randomization Procedure	Blinding	Number of Participating Centers	Followup Duration	Setting	Target Enrollment from a priori Power Analysis	Procedure for Multiple Comparisons	Funding
Wang[217] 2011 China 21538380	August 2008–October 2009	NR	Endpoints were recorded by an investigator who was not aware of treatment assignment or patient characteristics	Single center	1 yr	Cardiology department of university hospital	NR	Not used	Nonindustry only
Bonello[218] 2009 France 19101221	August 2007–March 2008	NR	Endpoints were recorded by an investigator who was not aware of treatment assignment or patient characteristics	Multicenter	30 d	4 cardiology centers	400 (429 actually enrolled)	Not used	NR
Bonello[219] 2008 France 18387444	March 2007–July 2007	NR	Endpoints were recorded by an investigator who was not aware of treatment assignment or patient characteristics	Multicenter	30 d	4 cardiology centers	160 (162 actually enrolled)	Not used	Nonindustry only
Tousek[220] 2011 Czech Republic 21663983	May 2009–July 2010	NR	NR	Single center	6 mo	Cardiology department of university hospital	NR	Not used	Nonindustry only
Aleil[221] 2008 France 19463377 VASP-02	April 2005–December 2007	Centralized randomization; no additional details reported	NR	Multicenter	30 d[m]	Multiple cardiology departments	NR	Not used	Partly industry; also assays provided by the manufacturer

d = days; mo = months; NR = not reported; yr = years.

Table 19: Test Information in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country	Platelet Reactivity Assay (Manufacturer)	Timing of Test	N Tested; N Included	Rationale for the Platelet Reactivity Threshold

[m] We extracted information at 30 d because this is the only time point when outcomes could have been affected by testing (in the VASP-guided group).

PMID Study Name (if available)		Interval Between Sample Collection and Analysis Interval Between Sampling and Assay Results	Test Success Rate	Chosen
Wang[217] 2011 China 21538380	VASP assay (VASP kit; Becton Dickinson, Franklin Lakes, NJ) using flow cytometry (Coulter EPICS XL cytometer, FACSCalibur, Becton Dickinson)	1 mo post-PCI for all patients; at 3, 6, 9, and 12 mo for patients in the VASP-guided treatment group NR NR	538 patients screened for inclusion; 306 had PRI >50% and fulfilled all other inclusion criteria NR	Not explicitly provided
Bonello[218] 2009 France 19101221	VASP assay (VASP kit; Diagnostica Stago, Asnieres, France) using flow cytometry (Coulter EPICS XL cytometer, Beckman Coulter Inc., Fullerton, CA)	≥6 h and within 24 h after the initial clopidogrel bolus; in the VASP-guided group, 12 h after each additional clopidogrel bolus NR NR	1122 patients screened for inclusion; 429 had PRI ≥50% and fulfilled all other inclusion criteria	Statement (in the introduction section) that measurements above the cut-off threshold are associated with higher risk of adverse cardiovascular outcomes; also, a previous randomized study from the same team of investigators.
Bonello[219] 2008 France 18387444	VASP assay (VASP kit; Diagnostica Stago, Asnieres, France) using flow cytometry (Coulter EPICS XL cytometer, Beckman Coulter Inc., Fullerton, CA)	24 h after the initial clopidogrel bolus; in the VASP-guided group, 12 h after each additional clopidogrel bolus NR NR	406 patients screened for inclusion; 162 had PRI ≥50% and fulfilled all other inclusion criteria	A previous study by the same team of investigators where a threshold of 50% could predict adverse cardiovascular outcomes with a sensitivity of 100%
Tousek[220] 2011 Czech Republic 21663983	VerifyNow P2Y12 assay (Accumetrics, San Diego, CA)	24-48 h after PCI (post–clopidogrel loading); in the VerifyNow-guided group, every 7 days thereafter for up to 30 d NR NR	378 patients screened for inclusion; 134 had PRU ≥240 and fulfilled all other inclusion criteria; and 60 patients were randomized	Not explicitly provided
Aleil[221] 2008 France 19463377 VASP-02	VASP assay (VASP kit; Diagnostica Stago/Biocytex, Asnieres, France) using flow cytometry (FACS Calibur, Becton Dickinson, Plymouth, UK)	Before clopidogrel administration, 10-12 h post clopidogrel dosing, at 2 wk (to identify responders and switch nonresponders in the group originally assigned to 75 mg/d maintenance dose), and at 4 wk (to assess outcomes)	Inclusion was not based on baseline testing; 153 patients were included	Previous studies by the same team of investigators

h = hour(s); mo = month(s); NR = not reported; PCI = percutaneous coronary intervention; VASP = vasodilator-stimulated phosphoprotein; wk = week(s).

Clinical Outcomes

All five studies comparing alternative test-and-treat strategies reported information on cardiovascular mortality and composite MACE; four also reported on recurrent acute coronary syndromes, two on all-cause mortality, three on stent thrombosis, and two on repeat revascularization or myocardial infarction. Overall, the studies had short followup durations and included moderate numbers of participants; thus, the outcome rates were low and relative effect estimates (when possible to calculate) were often extreme (e.g., odds ratios <0.5) and had substantial uncertainty (wide CIs). Studies generally indicated that the groups with test-based monitoring had better outcomes (lower event rates) than the groups without test-based monitoring; however, the differences were often not statistically significant. Detailed outcome information is summarized in **Tables 20–25**. To facilitate qualitative synthesis, we present forest plots of study estimates for the outcomes assessed by all five studies: cardiovascular mortality

(**Figure 32**) and MACE (**Figure 33**). Meta-analyses were not performed owing to the differences in the populations included, interventions compared, and durations of followup used.

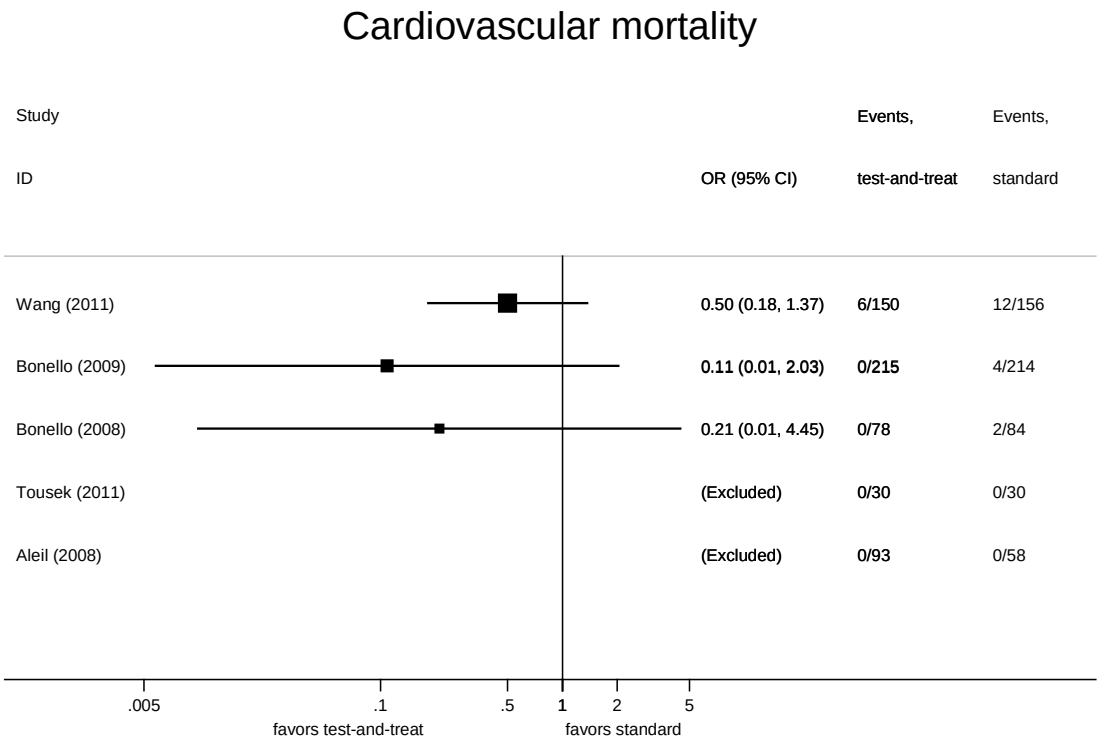
Table 20: Cardiovascular Mortality in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Time Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	1 yr	6 (4.0%)	OR=0.50 (calculated)	0.18 to 1.37 (calculated)	P=0.003[n] [unclear]
	Control N=156		12 (7.7%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	30 d	0 (0%)	OR=0.11 (calculated)	0.01 to 2.03 (calculated)	P=0.06 [Fisher exact test]
	Control N=214		4 (1.8%)			
Bonello[219] 2008 France 18387444	VASP-guided treatment N=78	30 d	0 (0%)	OR=0.21 (calculated)	0.01 to 4.45 (calculated)	P=0.498 [Fisher exact test; calculated]
	Control N=84		2 (2.4%)			
Tousek[220] 2011 Czech Republic 21663983	VerifyNow-guided treatment N=30	6 mo	0 (0%)	Not defined	Not defined	NA
	Control N=30		0 (%)			
Aleil[221] 2008 France 19463377 VASP-02	VASP-guided treatment N=95 (93 included in analyses)	30 d	0 (0%)	Not defined	Not defined	NA
	Control N=58		0 (0%)			

CI = confidence interval; d = days; mo = months; NA = not applicable; NR = not reported; OR = odds ratio; VASP = vasodilator-stimulated phosphoprotein; yr = year.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

[n] Although P=0.003 was the reported P value, recalculation using a chi-square test gives a P value of 0.170 and recalculation using the Fisher exact test gives a P value of 0.225. It is unclear how the reported P value was calculated.

Figure 32: Cardiovascular Mortality in Studies of Test-and-Treat Strategies using Phenotypic Testing for Platelet Reactivity



CI = confidence interval; OR = odds ratio. See **Table 17** for details on the strategies compared and the definitions of outcomes employed in each study.

Table 21: All-Cause Mortality in Studies Assessing Test-and-Treat Strategies

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Time Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	1 yr	NR	NR	NR	P<0.01 [log-rank test
	Control N=156		NR			
Tousek[220] 2011 Czech Republic 21663983	VerifyNow-guided treatment N=30	6 mo	0 (0%)	Not defined	Not defined	NA
	Control N=30		0 (0%)			

CI = confidence interval; mo = months; NA = not applicable; NR = not reported; VASP = vasodilator-stimulated phosphoprotein; yr = year.

Table 22: Stent Thrombosis in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Outcome Definition	Timie Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	Angiographically confirmed late stent thrombosis	1 yr	3 (2.0%)	OR=0.51 (calculated)	0.13 to 2.08 (calculated)	P=0.03[o] [unclear]
	Control N=156			6 (3.8%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	Early definite stent thrombosis=total occlusion originating in or within 5 mm of the stent or visible thrombus within the stent or within 5 mm of the stent associated with ≥1 of the following signs present within 48 h: new onset of ischemic symptoms at rest; recent changes suggestive of acute ischemia on ECG at rest; typical increase and decrease in cardiac biomarkers according to Academic Research Consortium criteria	30 d	1 (0.5%) [subacute thrombosis]	OR=0.10 (calculated)	0.01 to 0.75 (calculated)	P<0.01 for early definite stent thrombosis (P=0.25 for acute thrombosis; P=0.02 for subacute thrombosis) [Fisher exact test] P<0.01 [log-rank test]
	Control N=214			10 (4.7%) [8 subacute; 2 acute]			
Bonello[219] 2008 France 18387444	VASP-guided treatment N=78	Early definite stent thrombosis=Angiographic confirmation of stent thrombosis associated with at least 1 of the following signs present within 48 h: new onset of ischemic symptoms at rest; recent changes suggestive of acute ischemia on ECG at rest; typical increase and decrease in cardiac biomarkers according to Academic Research Consortium criteria	30 d	0 (0%)	OR=0.11 (calculated)	0.01 to 2.15 (calculated)	P=0.121 [Fisher exact test; calculated]
	Control N=84			4 (4.8%) [3 subacute; 1 acute]			

CI = confidence interval; d = days; ECG = electrocardiogram; h = hour(s); OR = odds ratio; VASP = vasodilator-stimulated phosphoprotein; yr = year.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

Table 23: Major Adverse Cardiovascular Events (Composite) in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Outcome Definition	Time Point	No. with Outcome (%)	Comparative metric	95% CI	P-Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	Cardiovascular death, angiographically confirmed stent thrombosis, recurrent ACS, or recurrent revascularization	1 yr	14 (9.3%)	OR=0.43 (calculated)	0.22 to 0.85 (calculated)	P=0.008[p] [unclear]

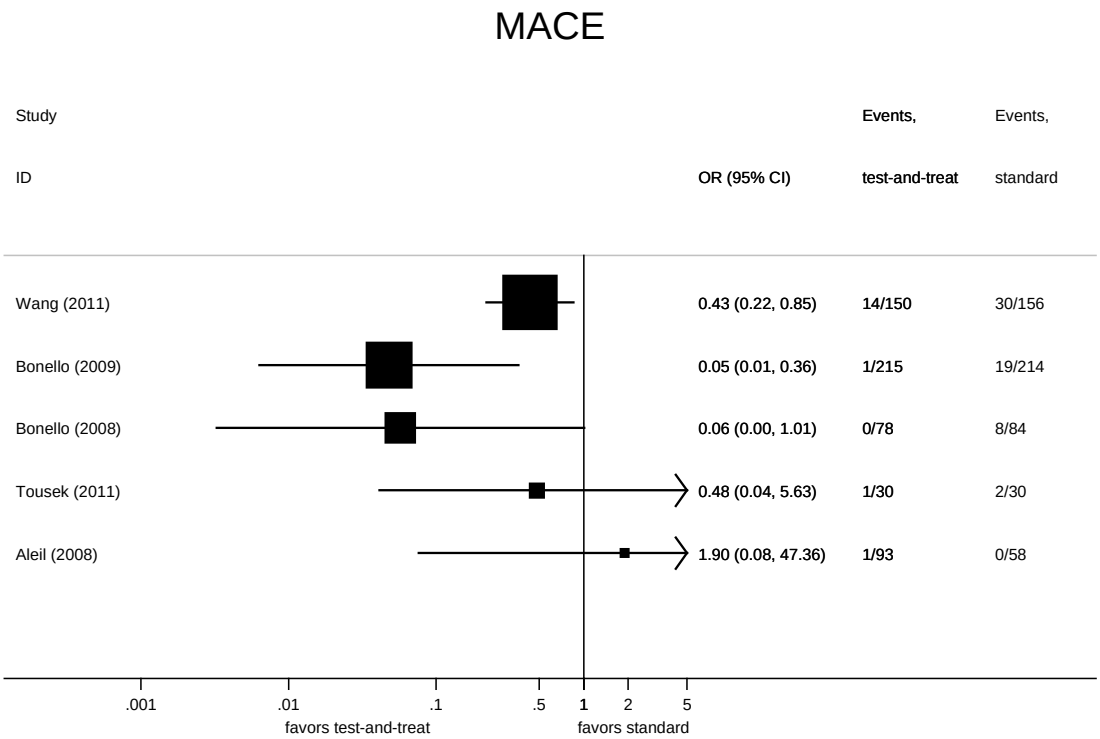
[o] Although P=0.03 was the reported P value, recalculation using a chi-square test gives a P value of 0.339 and recalculation using the Fisher exact test gives a P value of 0.502. It is unclear how the reported P value was calculated.

	Control N=156			30 (19.2%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	Cardiovascular death, MI, or urgent repeat revascularization	30 d	1 (0.5%)	OR=0.05 (calculated)	0.01 to 0.36 (calculated)	P<0.001 [Fisher exact test]
	Control N=214			19 (8.9%)			
Bonello[219] 2008 France 18387444	VASP-guided treatment N=78	Cardiovascular death, angiographically confirmed stent thrombosis, recurrent ACS, or repeat revascularization	30 d	0 (0%)	OR=0.06 (calculated)	<0.01 to 1.01 (calculated)	P=0.007 [Fisher exact test] P<0.005 [log-rank P value; log-rank statistic=7.75]
	Control N=84			8 (9.5%)			
Tousek[220] 2011 Czech Republic 21663983	VerifyNow-guided treatment N=30	Death, MI, or stroke	6 mo	1 (3.3%)	OR=0.48	0.04 to 5.6	P=0.55 [chi-square test]
	Control N=30			2 (6.7%)			
Aleil[221] 2008 France 19463377 VASP-02	VASP-guided treatment N=95 (93 included in analyses)	CV death, stroke, MI, documented ischemia requiring target-vessel revascularization, or "improvement" of medical treatment	30 d	1 (1%) [patient was a nonresponder]	OR=1.90 (calculated)	0.08 to 47.36 (calculated)	P>0.99 [Fisher exact test; calculated]
	Control N=58			0 (0%)			

ACS = acute coronary syndrome; CI = confidence interval; CV cardiovascular; d = days; mo = months; MI = myocardial infarction; OR = odds ratio; VASP = vasodilator-stimulated phosphoprotein; yr=year.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

[p] Although P=0.008 was the reported P value, recalculation using a chi-square test gives a P value of 0.014 and recalculation using the Fisher exact test gives a P value of 0.015. It is unclear how the reported P value was calculated.

Figure 33: Major Adverse Cardiovascular Events in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity



CI = confidence interval; MACE = major adverse cardiovascular events; OR = odds ratio. See **Table 17** for details on the strategies compared.

Table 24: Repeat Revascularization in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Outcome Definition	Time Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	Recurrent revascularization by coronary angioplasty or CABG	1 yr	3 (2.0%)	OR=0.33 (calculated)	0.09 to 1.26 (calculated)	P=0.027[q] [unclear]
	Control N=156			9 (5.8%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	Recurrent revascularization by urgent coronary angioplasty or CABG	30 d	0 (0%)	OR=0.09 (calculated)	<0.01 to 1.61 (calculated)	P=0.06 [unclear]
	Control N=214			5 (2.3%)			

[q] Although P=0.027 was the reported P value, recalculation using a chi-square test gives a P value of 0.089 and recalculation using the Fisher exact test gives a P value of 0.139. It is unclear how the reported P value was calculated.

CABG = coronary artery bypass grafting; CI = confidence interval; d = days; OR = odds ratio; VASP = vasodilator-stimulated phosphoprotein; yr = year.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

Table 25: Recurrent ACS in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Outcome Definition	Time Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	Recurrent ACS	1 yr	2 (1.3%)	OR=0.69 (calculated)	0.11 to 4.18 (calculated)	P=0.1[r] [unclear]
	Control N=156			3 (2.1%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	MI	30 d	1 (0.5%)	OR=0.10 (calculated)	0.01 to 0.75 (calculated)	P=0.01 [unclear]
	Control N=214			10 (4.8%)			
Bonello[219] 2008 France 18387444	VASP-guided treatment N=78	Recurrent ACS	30 d	0 (0%)	OR=0.21 (calculated)	0.01 to 4.45 (calculated)	P=0.498 [Fisher exact test; calculated]
	Control N=84			2 (2.4%)			
Tousek[220] 2011 Czech Republic 21663983	VerifyNow-guided treatment N=30	MI	6 mo	1 (3.3%)	OR=0.48	0.04 to 5.6	P=0.55 [unclear]
	Control N=30			2 (6.7%)			

ACS = acute coronary syndrome; CI = confidence interval; d = days; mo = months; MI = myocardial infarction; OR = odds ratio; VASP = vasodilator-stimulated phosphoprotein; yr = year.
"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

Intermediate Outcome: Platelet Reactivity During Followup

Four of the five studies directly comparing alternative test-and-treat strategies reported information on platelet reactivity as an intermediate outcome (**Appendix Tables F2** and **F3**). Although results generally indicated that platelet reactivity at the last followup assessment was lower in the groups that received test-based treatment than in those that received standard treatment, reporting was often incomplete and precluded statistical comparisons between groups. Furthermore, studies had short followup periods and it was unclear whether the observed differences in reactivity affected clinical outcomes.

[r] Although P=0.1 was the reported P value, recalculation using a chi-square test gives a P value of 0.684 and recalculation using the Fisher exact test gives a P value of 1.00. It is unclear how the reported P value was calculated.

Assessment of Risk of Bias of Individual Studies

A detailed assessment of 17 risk-of-bias items for studies comparing alternative test-and-treat strategies based on phenotypic testing for platelet reactivity is presented in **Appendix Table F2** and is summarized in **Appendix Figure F2**. Generally, studies had low-to-moderate risk of bias. Because studies were prospectively conducted and because phenotypic testing is performed immediately after sample collection, the index test was assessed without knowledge of clinical or intermediate outcomes in all cases. However, information to judge whether outcomes were assessed without knowledge of the index-test result was often not reported. Subjects and personnel were not blinded and reporting was incomplete regarding the methods of generating the randomization sequence and concealing allocation. In one study[217] we observed substantial discrepancies between the statistical results provided by the authors and results from analyses that we performed using the reported event counts (despite using the same statistical procedures described in the paper's methods section).

One concern about test-and-treat studies pertains to delaying PCI until a particular level of reactivity is reached (particularly, in studies that perform repeat testing and modify treatment accordingly over the course of several weeks): the time to PCI tends to be longer in the experimental group than in the control group, although only one of the included studies provided relevant data (indicating that the time-to-PCI was indeed longer in patients assigned to test-based monitoring). This delay is potentially of concern because several studies have shown that delayed PCI may adversely impact clinical outcomes.

Studies of Treatment-Effect Modification by Baseline Platelet Reactivity

We identified a single study reporting information on effect modification by baseline platelet reactivity in patients randomized to alternative antiplatelet therapies.[222] Four academic centers in France enrolled a total of 56 patients with unstable angina or non–ST-elevation myocardial infarction who received a loading dose of 900 mg of clopidogrel. Platelet reactivity was then assessed with four assays (LTA with 5 µM ADP, LTA with 20 µM ADP, VerifyNow P2Y12, and the VASP assay using flow cytometry). The study used a randomized cross-over design comparing aspirin plus prasugrel and aspirin plus clopidogrel (14 days of exposure to each treatment). Detailed information about the patient selection and patient characteristics is summarized in **Table 26**. Information about the assays used, test timing, and thresholds for platelet reactivity is presented in **Table 27**.

Table 26: Descriptive Characteristics of the Study of Treatment-Effect Modification using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Selection Criteria	Study Design	N Enrolled Male (%) Age[s] Dyslipidemia (%) Smokers (%) HTN (%) Diabetes (%)	Treatments Compared	Followup Duration

[s] Mean (standard deviation), unless otherwise stated.

Montalescot[222] 2009 France 20062936 ACAPULCO	Patients with UA or NSTE MI who were to receive clopidogrel 900 mg loading dose	RCT comparing antiplatelet treatments in patients who were assed for platelet reactivity after a run-in period, before randomization	N=56 47 (84%) 61 (12) 9 (16%) 17 (30%) [current smokers] 37 (66%) 11 (20%)	Patients received clopidogrel 900 mg loading dose (single dose or cumulative dose when <900 mg had been previously admitted and aspirin (250-500 mg IV loading dose). They were then randomized to 2 groups: *Prasugrel +aspirin group:* prasugrel 10 mg maintenance dose + aspirin (maximum 100 mg/d) + placebo (instead of clopidogrel) *Clopidogrel + aspirin group:* clopidogrel 150 mg maintenance + aspirin (maximum 100 mg/d) + placebo (instead of prasugrel) After 2 wk patients were switched to the other group (crossover design) for another 2 wk.	Total study duration was 29 d; patients were on each treatment for 2 w (crossover design)

d = day(s); HTN = hypertension; MI = myocardial infarction; NSTE = non–ST-elevation; UA = unstable angina; wk = week(s).

Table 27: Test Information in the Study of Treatment-Effect Modification using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Assay for Phenotypic Testing (agonist) [manufacturer]	Test Timepoint Interval Between Sampling and Genotyping Results Interval Between Collection and DNA extraction	N Tested Test Success Rate (%)	Threshold Used	Rationale for Threshold
Montalescot[222] 2009 France 20062936 ACAPULCO	LTA (ADP 5 and 20 μM) [Model 490-4D aggregometer, Chrono-Log Corporation, Kordia, Netherlands] VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA] VASP assay (VASP kit; Biocytex, Marseilles, France) using flow cytometry (no additional details provided)	Pre-loading dose, 6-18 h after the 900 mg loading dose, and 16-28 h after the last maintenance dose at 2 w and 4 w	54 subjects received maintenance dose; 41 subjects completed the study; test success rate was not estimable	For maximal platelet reactivity by LTA ADP 20 μM:> 50% For residual platelet aggregation by LTA ADP 5 μM: >14% For VASP PRI: ≥50% For VerifyNow PRU: ≥240 units	Based on prior literature (citations provided for each threshold)

ADP = adenosine diphosphate; LTA = light-transmission aggregometry; PRU = platelet reactivity units; VASP = vasodilator-stimulated phosphoprotein; wk = week(s).

Clinical Outcomes

The study did not report patient-relevant clinical outcomes stratified by platelet reactivity status at baseline. One death (due to cancer) was observed and was not considered treatment related.

Intermediate Outcomes: Platelet Reactivity During Followup

The study reported the response rate among “poor responders” to the clopidogrel loading dose during prasugrel-based therapy and during clopidogrel-based therapy. Generally the response rates were higher during prasugrel therapy, regardless of the assay used to assess platelet reactivity. However, the study did not report the response status during followup for patients who were “responders” to the clopidogrel loading dose. Thus, the interaction between post–loading dose response to clopidogrel and treatment assignment could not be assessed.

Assessment of Risk of Bias

The study was considered to have a high risk of bias because incomplete outcome information was reported and information on the generation of the randomized sequence and allocation concealment was unclear. Detailed information for each item considered in the risk-of-bias assessment is given in **Appendix Figure F3** and **Appendix Table F3**.

Studies with Phenotypic Test–Based Selection of Patients

Five studies met our inclusion criteria and reported information on the comparative effectiveness of treatments administered to patients selected on the basis of baseline platelet reactivity. The sample sizes ranged from 52 to more than 2000 participants and all five studies were relatively recent (published in 2008–2011). Four studies were performed mainly or exclusively in the PCI setting; the fifth study included patients with stable coronary artery disease. On-clopidogrel platelet reactivity was used as a selection criterion in all studies; it was assessed using the VerifyNow P2Y12 assay in two studies (in one of them, it was used together with VerifyNow AA, an assay of the platelet response to aspirin), LTA in two studies, and the VASP assay with flow cytometry in one study. The treatment comparisons were between standard-dose clopidogrel-based therapy and high-dose clopidogrel in two studies, addition of a glycoprotein IIb/IIIa inhibitor in two studies, and ticagrelor in the remaining study.

Detailed information on patient selection, study design, patient characteristics, and assays used is summarized in **Table 28**.

Table 28: Descriptive Characteristics of the Studies with Phenotypic Test–Based Selection of Patients

Author, Year Country PMID Study name (if available)	Selected Population	Study Design Treatment Strategies Compared	Assay (agonist) [model, manufacturer]	Total N Enrolled Male (%) ,Age (SD), Dyslipidemia (%), Smokers (%), HTN (%), Diabetes (%)
Price[170] 2011 Multinational 21406646 GRAVITAS	Patients who had PCI with ≥1 drug-eluting stent for treating stable CAD or NSTE ACS; after protocol amendment, enrollment was expanded to include patients with STE MI. Patients on IIb/IIIa inhibitors were excluded. In patients with no prior exposure to clopidogrel, a dose of 600 mg had to have been administered ≤2 h post-PCI; patients already on clopidogrel must have received 75 mg/d for ≥7 d, or, if <7 d, a loading dose ≥300 mg when clopidogrel was started.	Double-blind, placebo-controlled RCT, multicenter Platelet reactivity was measured 12-24 h post-PCI. Patients with high-on treatment reactivity (PRU≥230) were randomized (central randomization, double-blind design) to two groups (standard- and high-dose clopidogrel). Also included a nonrandomized comparator arm of randomly selected patients among those without high on-clopidogrel reactivity (PRU<230); information on this group of patients isin the report section on Key Question 2b. *High-dose clopidogrel:* total 1st day dose 600 mg, followed by 6-mo maintenance dose 150 mg/d *Standard-dose clopidogrel:* loading dose of placebo, followed by a dose of 75 mg/d + a placebo tablet Aspirin treatment (75-162 mg/d) was required for both groups.	For selecting patients and for assessing reactivity as an outcome: VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA]	2214 (with PRU≥230) in the randomized groups; 586 (with PRU<230) in the randomly selected comparator group[a] 1441 (65%) 64.1 (10.5) 1934 (87%) 313 (14%) [current smokers] 1886 (85%) 1004 (45%)
Palmerini[186] 2010 Italy 19604542 DOUBLE	Patients with STE MI referred to a single center for PCI within 12 h of symptom onset	Randomized, single-center All patients received a clopidogrel loading dose of 300 mg; peri-procedural abciximab and BMS were mandatory. Platelet reactivity was measured at baseline (after a loading dose of 300 mg and the first maintenance dose of 75 mg) and patients with PRI VAS<30% were excluded. Patients had been randomized before the first reactivity assessment into two groups (high- and standard-dose maintenance clopidogrel) *High-dose clopidogrel maintenance:* 150 mg/d for 1 mo *Standard-dose clopidogrel maintenance:* 75 mg/d for 1 mo All patients were on aspirin treatment (160 mg/d)	*For selecting patients:* Platelet VASP kit (ADP) [Biocytex, Marseille, France] *For assessing reactivity as an outcome:* Platelet VASP kit (ADP) [Biocytex, Marseille, France], LTA (NR) [Chronolog 700; no additional details provided], VerifyNow P2Y12 (NR) [NR], whole blood impedance aggregometry (NR) [NR]	52 eligible (with VASP PRI ≥30%); 48 included in analyses (7.7% excluded) 43 (90%) 63 (12.2) 21 (44%) 32 (67%) [current or former smokers] 23 (48%) 8 (17%)
Valgimigli[173] 2009 Multinational (Italy, Belgium, France, Spain) 19528337 3T/2R	Patients scheduled for coronary angiography, PCI, or both; presenting with stable or troponin-negative NSTE ACS were screened for inclusion; patients with evidence of myocardial damage or ongoing MI were excluded.	Patients were eligible for aspirin response evaluation (cut-off 550 units) if they were taking aspirin orally at doses of ≥80 mg/d for ≥5 days or received intravenous 500 mg aspirin ≥15 minutes before and did not receive clopidogrel or ticlopidine in the previous 7 days. Patients with aspirin poor responsiveness qualified for randomization before PCI only when clopidogrel screening requirements were met. Screening for clopidogrel response (cut-off: 40% inhibition) was undertaken in patients at steady state for aspirin provided ≥1 of the following requirements was fulfilled: the patient received a 600- or 300-mg loading dose ≥2 or 6 hours before, respectively, or the patient received a 75-mg maintenance clopidogrel dose for ≥7 consecutive days. Patients who were poor responders to both aspirin and clopidogrel followed the aspirin poor-responders randomization scheme. *IIb/IIIa inhibitor group:* tirofiban 50 mL diluted in 200 mL of 0.9% NaCl solution *Placebo group:* 50 mL of 0.9% NaCl was injected in 200 mL of 0.9% NaCl solution by an unblinded research study nurse (all other personnel were blinded to treatment assignment). Tirofiban was given as a bolus of 25 μg kg^{-1} 3 min^{-1}, followed by an infusion of 0.15 μg kg^{-1} min^{-1} for 14 to 24 h. Intravenous aspirin at the time of PCI in patients at steady state for the treatment was allowed and left to the discretion of the treating physician. Anticoagulation during PCI was with heparin or	VerifyNow P2Y12 (ADP) (Accumetrics, San Diego, CA) VerifyNow Aspirin (AA) (Accumetrics, San Diego, CA)	337 patients were poor responders to aspirin or clopidogrel; 174 were poor responders to clopidogrel alone; 26 were poor responders to both; 73 patients were excluded pre-randomization; 263 patients were randomized (147 were exclusively clopidogrel poor responders). For all randomized patients: 193 (73%) 68.2 (9.7)

[a] Patient characteristics extracted only for patients in the randomized groups (i.e., patients with PRU≥230 randomly assigned to high or low-dose clopidogrel strategies).

Author, Year Country PMID Study name (if available)	Selected Population	Study Design Treatment Strategies Compared	Assay (agonist) [model, manufacturer]	Total N Enrolled Male (%) ,Age (SD), Dyslipidemia (%), Smokers (%), HTN (%), Diabetes (%)
		bivalirudin. Rescue use of tirofiban was allowed (without unblinding).		141 (54%) 40 (15%) [current smokers] 188 ((71%) 69 (26%)
Cuisset[223] 2008 France 19463379	Patients with stable angina or a positive functional study with planned PCI with stent implantation of a de novo lesion in a native coronary artery; all patients were on chronic aspirin therapy (75 mg/d) and none were on clopidogrel	Blood samples were obtained ≥12 h after the loading dose of aspirin and clopidogrel; clopidogrel nonresponders (>70% on-clopidogrel reactivity used as the cut-off) were randomized to a IIb/IIIa inhibitor group or a conventional treatment group. Loading dose of clopidogrel (600 mg) and aspirin (250 mg) were administered the day before the procedure. Response was assessed ≥12 h after the loading dose of aspirin and clopidogrel; nonresponders were then randomized into the following groups: *IIb/IIIa inhibitor group:* systematic administration of abciximab to all patients. *Conventional treatment group:* use of IIb/IIIa agonists left at the discretion of physicians. Abciximab was administered with a 0.25 mg/kg of body weight bolus, followed by a 0.125 µg/kg/min [maximum, 10 µg/min] infusion for 12 h).Anticoagulation during the procedure was with heparin (50 U/kg in the IIb/IIIa group vs. 70 U/kg in the conventional treatment group). After discharge all patients received aspirin (75 mg) and clopidogrel (150 mg) maintenance doses for at least 1 mo.	LTA (ADP) [PAP4 aggregometer; Biodata Corporation; Wellcome, Paris, France]	149 nonresponders to clopidogrel 113 (76%) 65 (8.6) 111 (74%) 58 (39%) 93 (62%) 56 (38%)
Gurbe[137][b] 2010 Multinational (N. America and Europe) 20194878 RESPOND	Patients with documented stable CAD who were on aspirin therapy (75-100 mg/d)	All patients received a loading dose of clopidogrel (300 mg) and response was assessed at 6-8 h post-loading. Nonresponse was defined as absolute change post-loading ≤10%; response status was confirmed 2-4 w before the first dose of study drug Both responders and nonresponders were randomized to 2 groups during period 1: *Clopidogrel:* clopidogrel 600 mg loading dose, followed by clopidogrel 75 mg/d maintenance for 14 d *Ticagrelor:* ticagrelor 180 mg loading dose, followed ticagrelor 90 mg BID maintenance dose In period 2, all nonresponders switched treatment, whereas half of the responders continued the same treatment, and the other half of the responders switched to the other treatment; both groups were treated for an additional 14 d. Patients continuing on the same treatments in both study periods did not receive loading doses of study drug during period 2. Switching patients received loading doses of the new drug. In addition to study drug, all patients received concomitant aspirin therapy (75 to 100 mg/d).	*For assigning patients to randomized groups:* LTA (ADP 20 µmol/L) [Chronolog Optical Aggregometer, model 490-4D; no other details] *For response assessment:* LTA (ADP 5 and 20 µmol/L, and collagen 2 µg/mL) [Chronolog Optical Aggregometer, model 490-4D; no other details], VerifyNow P2Y12 (ADP) [Accumetrics, San Diego, CA], Platelet VASP kit (ADP) [Biocytex, Marseille, France], ADP-stimulated glycoprotein IIb/IIIa and P-selectin expression by flow cytometry [no other details]	98 76 (78%) 65 (8) 92 (94%) 17 (17%) [current smokers] 79 (81%) 25 (26%)

AA = arachidonic acid; ADP = adenosine diphosphate; BMS = bare-metal stent; d = day; h = hour; HTN = hypertension; LTA = light-transmission aggregometry; mo = month; NSTE = non–ST-elevation; PCI = percutaneous coronary intervention; PMID = PubMed identification number; PRI = platelet reactivity index; PRU = platelet reactivity units; RCT = randomized controlled trial; SD = standard deviation; STE = ST-elevation; VASP = vasodilator-stimulated phosphoprotein; w = week(s).

[b] Despite including both responders and nonresponders, this study was categorized as a study of randomized treatment after test-based selection because patients were assigned to different treatments during the course of the study based on their original response status.

Clinical Outcomes

Clinical outcome comparisons between the randomized treatment groups were reported in three of the five studies. We discuss here the key results; more detailed information on clinical outcomes is presented in **Table 29**.

One study—the large GRAVITAS[Gauging Responsiveness with a VerifyNow Assay—Impact on Thrombosis and Safety] trial,[170] enrolling 2214 randomized patients—included patients who had undergone PCI for stable coronary artery disease or non–ST-elevation acute coronary syndrome and showed increased on-clopidogrel reactivity on the VerifyNow P2Y12 assay. The patients were randomized to high-dose clopidogrel or standard-dose clopidogrel, both in combination with aspirin. After 6 months of followup, there was no statistically significant difference between the randomized groups in the rate of cardiovascular death, nonfatal myocardial infarction, stent thrombosis, all-cause mortality, or composite cardiovascular outcomes (either cardiovascular death or nonfatal myocardial infarction, or cardiovascular death, nonfatal myocardial infarction, or stent thrombosis). The study also included followup information for a randomly selected group of patients with low platelet reactivity at baseline who were treated with standard-dose clopidogrel (see the Results section for Key Question 1b for details).

Two smaller studies (of <150 patients each) with 1-month followup assessed the impact of treatment with a glycoprotein IIb/IIIa inhibitor in addition to standard treatment. Both studies used the VerifyNow P2Y12 assay to assess platelet reactivity. One demonstrated a statistically significant improvement in survival (freedom from cardiovascular death) in a time-to-event analysis and a statistically significant reduction in the risk of the composite outcome of death, periprocedural myonecrosis, stent thrombosis, or recurrent acute coronary syndrome. Other comparisons were either not statistically significant or the data reported were inadequate for estimating an effect size (and the associated P value).

Detailed information on the outcomes reported in all three studies is presented in **Table 29**. Because the patient populations were heterogeneous, selected on the basis of different inclusion criteria, and assessed using different therapeutic regimens, we did not perform meta-analyses for any of the outcomes reported.

Table 29: Key Findings of Studies with Phenotypic Test–Based Selection of Patients and Clinical Outcomes

Author Year Country PMID Study Name (if available)	Treatment Groups (sample size)	Followup Duration	Summary of Findings
Price[170] 2011 Multinational 21406646 GRAVITAS	*High-dose clopidogrel (n=1109):* total first day dose 600 mg, followed by a maintenance dose of 150 mg/d for 6 mo *Standard-dose clopidogrel (n=1105):* loading dose of placebo, followed by a dose of 75	6 mo	Randomized comparison[a]: *CV death, nonfatal MI, or stent thrombosis:* HR=1.01 (95% CI 0.58, 1.76); P=0.97 CV death: HR=0.38 (95% CI 0.10, 1.43); P=0.14 Nonfatal MI: HR=1.12 (95% CI 0.59, 2.12); P=0.72 Stent thrombosis: HR=0.63 (95% CI 0.21, 1.93); P=0.42 CV death or nonfatal MI: HR=0.93 (95% CI 0.53, 1.64); P=0.80 All-cause death: HR=0.70 (95% CI 0.27, 1.85); P=0.48 Absolute difference in change of platelet reactivity from baseline at 30 d: -22% (95% CI -26%, -18%); P<0.001

[a] Detailed results on the comparison of patients with high- versus low-platelet reactivity receiving standard dose clopidogrel (i.e. the nonrandomized comparison) has been summarized in the results section on Key Question 2b.

	mg/d + a placebo tablet		Absolute difference in change of platelet reactivity at 6 mo: -24% (95% CI -28%, -20%); P<0.001
Valgimigl[173j][b] 2009 Italy 19528337 3T/2R	*IIb/IIIa inhibitor group (n=68, clopidogrel-only nonresponders):* tirofiban 50 mL diluted in 200 mL of 0.9% NaCl solution *Placebo group (n=79, clopidogrel-only nonresponders):* 50 mL of 0.9% NaCl was injected in 200 mL of 0.9% NaCl solution by an unblinded research study nurse (all other personnel were blinded to treatment assignment).	1 mo	Periprocedural MI (based on "the universal definition" of MI[224]) among clopidogrel-only nonresponders: 16.5% (11 events) in the tirofiban group vs. 30.8% in the placebo group (24 events); OR=0.44 (95% CI 0.2, 0.99); P= 0.05 [calculated results] Periprocedural MI (based on "the universal definition" of MI[224]) among clopidogrel and aspirin nonresponders: 0% (0 events) in the tirofiban group vs. 25.0% (event count and denominator NR) in the placebo group [no effect size could be calculated] Among clopidogrel-only nonresponders no deaths were observed. No other outcome was reported separately for clopidogrel nonresponders. Among clopidogrel-only nonresponders a subgroup analysis by inhibition status (≥21% vs. <21%) on the effect of treatment on periprocedural MI was performed. The p-value for the interaction test was 0.35. The sample size of the compared groups was not extractable.
Cuisset[223] 2008 France 19463379	*IIb/IIIa inhibitor group (n=74):* systematic administration of abciximab to all patient *Conventional treatment group (n=75):* use of IIb/IIIa agonists left at the discretion of physicians	1 mo	*Time-to-event analysis for CV-event free survival:* log-rank statistic=7.5; p=0.006 *Death from any cause, periprocedural myonecrosis, acute or subacute definite or probable stent thrombosis and recurrent ACS:* 14 events in the IIb/IIIa inhibitor group vs. 30 events in the conventional treatment group; OR=0.35 (95% CI, 0.17 – 0.74); P=0.006 [calculated results] *Death:* 0 events in the IIb/IIIa inhibitor group vs. 1 event in the conventional treatment group; OR=0.33 (95% CI, 0.01– 8.32); P=0.50 [calculated results] *Stent thrombosis:* 0 events in the IIb/IIIa inhibitor group vs. 1 events in the conventional treatment group; OR=0.33 (95% CI, 0.01 – 8.32); P=0.50 [calculated results] *Recurrent ACS:* 1 event in the IIb/IIIa inhibitor group vs. 2 events in the conventional treatment group; OR=0.50 (95% CI, 0.04 – 5.64); P=0.57 [calculated results] *Periprocedural myonecrosis:* 13 events in the IIb/IIIa inhibitor group vs. 26 events in the conventional treatment group; OR=0.40 (95% CI, 0.19 – 0.86); P=0.02 [calculated results]

ACS = acute coronary syndrome; CI = confidence interval; CV = cardiovascular; d = day; HR = hazard ratio; LTA = light-transmission aggregometry; MI = myocardial infarction; mo = month; OR = odds ratio; PMID = PubMed identification number; PRI = platelet reactivity index; TIMI = Thrombolysis In Myocardial Infarction Study Group.

Intermediate Outcome: Platelet Reactivity During Followup

Two small studies, each including fewer than 100 patients, reported information on intermediate outcomes during 1 month of followup. The outcomes were assessed using different assays and were heterogeneously reported. The key findings are summarized in **Table 30**.

Table 30: Key Findings of Studies with Phenotypic Test–Based Selection of Patients and Intermediate Outcomes

Author Year Country PMID Study Name (if available)	Treatment Groups (sample size)	Followup Duration	Summary of Findings
Palmerini[186] 2010 Italy 19604542 DOUBLE	*High-dose clopidogrel maintenance (n=24):* 150 mg/d for 1 mo	1 mo, assessments performed at 1 we	Mean change of PRI from baseline based on a random effects linear model in the high-dose group at 1 w: -27.8 (95% CI -33.7, -21.9); P=0.0001 Mean change of PRI from baseline based on a random effects linear model in the low-dose group at 1 w: -7.9 (95% CI -14.1, 1.8); P=0.11

[b] Outcome data were extracted only for patients with poor-response to clopidogrel or clopidogrel and aspirin (i.e., we did not extract data on patients who were poor responders to aspirin alone).

	Standard-dose clopidogrel maintenance (n=24): 75 mg/d for 1 mo	Mean change of PRI from baseline based on a random effects linear model in the high-dose group at 30 d: -31.6 (95% CI, -37.6, -25.7); P=0.0001 Mean change of PRI from baseline based on a random effects linear model in the low-dose group at 30 d: -18.7 (95% CI -24.8, -12.6); P=0.0001 Overall mean difference of PRI between-groups (from the random effects linear model)=-15.4 (95% CI -23.0, -7.8); P=0.0001 Rate of poor response (defined as PRI>50%) in the high-dose group at 1 mo: 21% Rate of poor response (defined as PRI>50%) in the high-dose group at 1 mo: 71% (P=0.001 for the difference between groups) Mean difference between-groups by LTA (from the random effects linear model; unclear timepoint)=-13.1 (95% CI -19.8, -6.4); P=0.0001 Mean difference between-groups by VerifyNow (from the random effects linear model; unclear timepoint)=-57.1 (95% CI -97.1, -17.1); P=0.005 Mean difference between-groups by whole blood aggregometry (from the random effects linear model; unclear timepoint)=-1.3 (95% CI -2.6, -0.1); P=0.04 Adverse events (study-defined as such): 2 in the high dose group (1 epistaxis; 1 thrombocytopenia); 2 in the low-dose group (1 subacute stent thrombosis; 1 thrombocytopenia) Additional results are presented in Figures 1-3 of the paper.
Gurbe[137][c] 2010 Multinational (N. America and Europe) 20194878 RESPOND	*Clopidogrel:* clopidogrel 600 mg loading dose, followed by clopidogrel 75 mg/d maintenance *Ticagrelor:* ticagrelor 180 mg loading dose, followed ticagrelor 90 mg BID maintenance dose Nonresponders=41 Responders=57 (the study had a crossover trial)	*NONRESPONDER COHORT* The proportion of patients who responded to ticagrelor was higher than those who responded to clopidogrel - using a cut-off of 10% absolute change; risk difference 0.25 (95% CI 0.08, 0.41); P=0.005 [McNemar test] - using a cut-off of 30% absolute change; risk difference 0.62 (95% CI 0.42, 0.79); P<0.001 [McNemar test] - using a cut-off of 50% absolute change; risk difference 0.13 (95% CI 0.01, 0.23); P=0.046[McNemar test] Inhibition of platelet aggregation (LTA ADP 20 µmol/L) was higher at all times with ticagrelor, compared to clopidogrel (P≤0.05); similar results were observed with LTA ADP 5 µmol/L and LTA collagen 2 µg/mL VerifyNow PRU was significantly lower with ticagrelor versus clopidogrel at all timepoints except the initial crossover-period (P≤0.05) During both treatment periods, VASP PRI was significantly lower in ticagrelor-treated patients. ADP-induced IIb/IIIa and P-selectin expression were lower at steady state in ticagrelor-treated patients. *RESPONDER COHORT* Platelet aggregation (LTA ADP 20 µmol/L) was lower after ticagrelor compared with clopidogrel therapy in period 1 and after crossing over in period 2. In patients who continued on the same therapy platelet aggregation was significantly lower at all timepoints after steady state with ticagrelor (P<0.05) Inhibition of platelet aggregation (LTA ADP 20 µmol/L) was higher after loading and maintenance ticagrelor therapy (P<0.05) except at period 2, day 15, 0 hours. Loading with ticagrelor after patients switched from clopidogrel was similar to the inhibition in patients treated with ticagrelor in period 1. In patients who did not switch, inhibition was greater with ticagrelor after steady state was reached (P<0.05). Similar results at steady state were obtained with LTA ADP 5 µmol/L and collagen 2 µg/mL (P<0.001). VerifyNow PRU was significantly lower with ticagrelor versus clopidogrel at all timepoints except the initial crossover-period up to 1 h (P≤0.05). Similar results were observed in patients who continued on the same therapy and those who switched. For comparisons at steady state VASP PRI was lower during ticagrelor treatment compared to

[c] Despite including both responders and nonresponders, this study was categorized as a study of randomized treatment after test-based selection because patients were assigned to different treatments during the course of the study based on their original response status.

	clopidogrel. After treatments were switched, PRI was significantly lower with ticagrelor at all timepoints except on day 15 (in patients who crossed over; presumably due to carryover). PRI in patients who did not switch was lower during steady state ticagrelor therapy at "nearly all timepoints". ADP-induced IIb/IIIa and P-selectin expression were lower at steady state in ticagrelor-treated patients (P<0.001).

ADP = adenosine diphosphate; d = day; LTA = light-transmission aggregometry; mo = month; PRI = platelet reactivity index; PRU = platelet reactivity units; VASP = vasodilator-stimulated phosphoprotein; wk = week(s).

Assessment of Risk of Bias of Individual Studies

A detailed assessment of 17 risk-of-bias items for studies comparing treatment effects in patients selected on the basis of phenotypic testing for platelet reactivity is presented in **Appendix Table F4** and is summarized in **Appendix Figure F4**. Overall, the risk of bias varied across the randomized studies. The large, multicenter GRAVITAS trial had low risk of bias, both regarding aspects related to the index test of interest as well as general aspects of randomized trial design (e.g., generation of the randomization sequence and allocation concealment). In contrast, smaller studies with short-term followup were generally considered to have a higher risk of bias, owing to problems in the application of the tests of interest (e.g., an unclear rationale for the thresholds used) or incomplete reporting of outcomes. Furthermore, these studies often did not provide information sufficient to judge their risk of bias regarding general aspects of randomized trial design.

Key Question 3b: How Do Modifying Factors Affect the Association of Alternative Test-and-Treat Strategies and Patient Outcomes?

Only two of the studies considered relevant to Key Question 3a provided information about the use of testing for clinical decision-making with data stratified by patient characteristics.

The first study reported results from the genetic substudy of the CURE trial comparing clopidogrel plus aspirin (dual-antiplatelet) and placebo plus aspirin in patients with non–ST-elevation myocardial infarction.[91] The study reported modification of the treatment effect by genotype grouping stratified by ancestry. There was no significant interaction between genotype group and treatment (P=0.12 for the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or stroke; P=0.28 for the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, stroke, recurrent ischemia, or hospitalization for unstable angina) among individuals of "European ancestry" and individuals of "Latin American ancestry" (P=0.81 and P=0.71, respectively, for the same outcomes).

The second study reporting relevant information compared conventional dual-antiplatelet therapy (aspirin plus clopidogrel) with adjunctive use of a glycoprotein IIb/IIIa inhibitor (aspirin plus clopidogrel plus abciximab) in 149 clopidogrel nonresponders (by LTA) undergoing PCI, angiography, or both.[173] Treatment-effect modification by baseline percent inhibition (≥21% vs. <21%) was assessed for the outcome of periprocedural myocardial infarction. An interaction test indicated that effect modification was not statistically significant (P=0.35); the reported data did not allow for the calculation of treatment-effect size stratified by baseline platelet reactivity.

Information on the risk of bias of these studies is summarized in the preceding section, because they were also included in Key Question 3a. We caution that the studies did not use any procedure to adjust for multiple statistical testing in these subgroup analyses of effect modification.

Key Question 4: What Are the Potential Adverse Effects or Harms from Genetic or Phenotypic Testing *per se* or from Test-Directed Treatments?

Key Question 4 aimed to address the potential harms of test-guided treatment as well as the harms of the testing process itself.

Harms of Test-Directed Treatment

For harms of test-directed treatment, **Table 31** summarizes the number of studies and adverse events or harms assessed for each of the designs we reviewed for Key Question 4. We discuss studies belonging to each of three designs—studies of test-and-treat strategies, studies of treatment-effect modification, and studies with test-based selection—separately for genetic testing (for CYP2C19 variants) and for phenotypic testing (of platelet reactivity). (Please see the Methods section for more information.)

Table 31: Number of Studies Relevant to Key Question 4 and Harms Assessed, by Study Design and Test Used

Study Design	Genetic Testing for CYP2C19 Variants	Phenotypic Testing for Platelet Reactivity
RCTs of test-and-treat strategies	0	5 (bleeding events)
RCTs of treatment-effect modification by test results	5 (bleeding events)	1 (bleeding events and treatment discontinuations)
RCTs with test-based selection	0	3 (bleeding events)

See the Methods section for details on the relevant study designs.
RCT = randomized controlled trial.

Genetic Testing for CYP2C19 Variants

Studies of Test-and-Treat Strategies

No studies compared testing for CYP2C19 variants with alternative testing strategies (including a no-testing strategy) to guide treatment decisionmaking.

Studies of Treatment-Effect Modification by CYP2C19 Genotype Status

Five studies (reported in four publications[85,90,91,207,209]) provided information on treatment-effect modification of bleeding outcomes by CYP2C19 status. Four were based on large randomized trials of clopidogrel-based therapy that included more than 1000 patients in their genetic substudies. Of these, one study enrolled patients with non–ST-elevation acute coronary syndromes, one enrolled those with acute coronary syndromes undergoing PCI, one involved patients with ST-elevation or non–ST-elevation acute coronary syndromes,[90] and one enrolled persons with atrial fibrillation who were not appropriate candidates for vitamin K–antagonist treatment.[91] The fifth study was a small genetic substudy of 126 patients with myocardial infarction who were randomized to either aspirin plus high-dose maintenance clopidogrel or to triple-antiplatelet therapy (aspirin, clopidogrel, and cilostazol).[209] With a followup of only30 days, the study reported that no major bleeding events by TIMI criteria were observed in either group. Details about the selection criteria, patient characteristics, and study design are under Key Question 3a results.

Results from the four larger studies comparing the effect of alternative treatment strategies (stratified by CYP2C19 genotype) on safety outcomes (in all four studies, bleeding events). Treatment effects were not statistically significant within genotype groups, with a single exception (for major hemorrhagic events among patients classified as "intermediate metabolizers" in the study of patients with atrial fibrillation). The test for interaction (i.e., a test for heterogeneity of treatment effects across genotype groups) was not statistically significant for any of the reported comparisons. This indicates that the impact of the compared treatments on bleeding events was not significantly different across patient groups defined by CYP2C19 genotype.

Table 32: Bleeding Events in Studies of Treatment-Effect Modification by CYP2C19 Genotype Status

Author Year Country UID Study name (if available)	Outcome Definition (timing)	Subgroup defined by genotype	Treatment group	Number with outcome/total number within phenotype-treatment group	Comparison between treatments, within genotype groups ES (95% CI); P-value [statistical test]	Comparison of treatment effects across genotype groups
Pare[91d] 2010 Multinational 20979470 CURE	Major bleeding (definition NR)	Poor metabolizers	Dual antiplatelet therapy	0/61	NA	P=0.64
			Aspirin monotherapy	1/55		
		Intermediate metabolizers	Dual antiplatelet therapy	19/437	HR=1.61 (0.79 – 3.28) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	13/442		
		Extensive metabolizers	Dual antiplatelet therapy	42/1033	HR=1.43 (0.89 – 2.30) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	29/987		
		Ultra metabolizers	Dual antiplatelet therapy	39/847	HR=1.19 (0.74 – 1.91) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	31/826		
		Unknown metabolizers	Dual antiplatelet therapy	2/152	HR=1.77 (0.15 – 20.33) [Cox proportional hazards model; adjusted for age, sex, ancestry]	
			Aspirin monotherapy	1/176		

[d] The authors reported extensive subgroup and sensitivity analyses across different genotype grouping and after including or excluding subgroups of patients by ethnicity. The results relevant to treatment effect heterogeneity from these analyses are presented in the text of this section.

Pare[91] 2010 Multinational 20979470 ACTIVE A	Major hemorrhage=overt bleeding requiring transfusion of at least 2 units of blood or any overt bleeding meeting the criteria for severe hemorrhage	Poor metabolizers	Dual antiplatelet therapy	0/9	NA	P=0.08
			Aspirin monotherapy	2/12		
		Intermediate metabolizers	Dual antiplatelet therapy	10/93	HR=5.84 (1.26 – 27.06) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	2/92		
		Extensive metabolizers	Dual antiplatelet therapy	10/199	HR=0.91 (0.38 – 2.18) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	11/235		
		Ultra metabolizers	Dual antiplatelet therapy	8/222	HR=1.24 (0.43 – 3.59) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	6/200		
		Unknown metabolizers	Dual antiplatelet therapy	4/37	HR=1.84 (0.34 – 10.08) [Cox proportional hazards model; adjusted for age, sex]	
			Aspirin monotherapy	2/35		
Mega[85,207e] 2009 Multinational 19106084 19414633 TRITON-TIMI 38	Safety outcome=major or minor bleeding (TIMI criteria); analysis for this outcome was performed on an "as-treated" basis	Carriers of a reduced function allele	Clopidogrel + aspirin	11/393	OR=0.66 (0.30 – 1.42) [calculated values]	P=0.73 [calculated from z-test between odds ratios]
			Prasugrel + aspirin	17/405		
		Noncarriers	Clopidogrel + aspirin	30/1061	OR=0.77 (0.48 – 1.26) [calculated values]	
			Prasugrel + aspirin	38/1047		
Kim[209] 2011 S. Korea 21511217 ACCELAMI2C19	Major bleeding events (based on TIMI criteria)	Carriers	Triple therapy (adjunctive cilostazol)	0/39	OR not estimable	NA
			High-maintenance dose clopidogrel	0/38		
		Noncarriers	Triple therapy (adjunctive cilostazol)	0/25		
			High-maintenance dose clopidogrel	0/24		

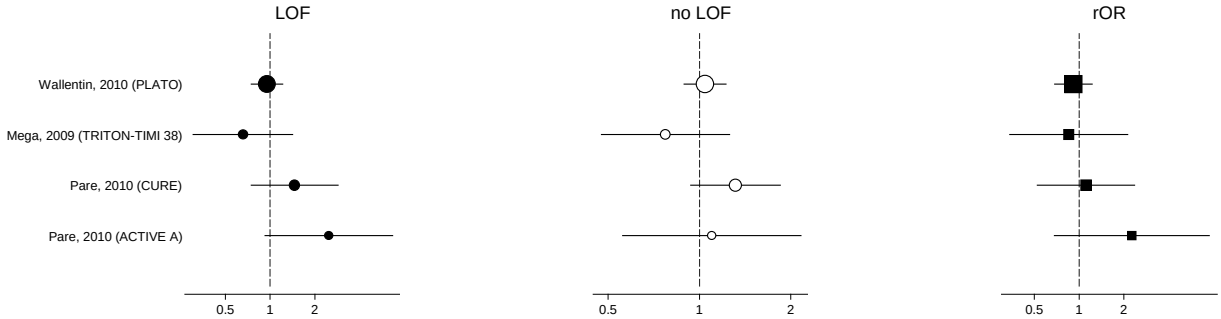
[e] Patients with genotypes of "unclear" functional significance were excluded.

Wallentin[90] 2010 Multinational 20801498 PLATO	Major bleeding	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	149/1380	HR =1.04 (0.82–1.30) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.60
			Clopidogrel+ aspirin	143/1380		
		No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	331/3547	HR =0.96 (0.83–1.12) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	340/3506		
		No CYP2C19 LOF or GOF allele (*1)	Ticagrelor + aspirin	176/1846	HR =1.12 (0.90–1.38) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.19
			Clopidogrel+ aspirin	161/1856		
		Any CYP2C19 LOF allele (*2–*8) but no GOF allele (not *17)	Ticagrelor + aspirin	108/1011	HR =1.03 (0.79–1.34) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	108/1053		
		Any CYP2C19 GOF allele (*17)	Ticagrelor + aspirin	196/2070	HR =0.86 (0.71–1.05) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	214/1977		
	Major bleeding not related to CABG	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	56/1380	HR =1.39 (0.93–2.08) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.31
			Clopidogrel+ aspirin	41/1380		
		No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	121/3547	HR =1.08 (0.84–1.40) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	110/3506		
	Major bleeding related to CABG	Any CYP2C19 LOF allele (*2–*8)	Ticagrelor + aspirin	96/1380	HR =0.87 (0.66–1.14) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	0.93
			Clopidogrel+ aspirin	107/1380		
		No CYP2C19 LOF allele (*1 or *17)	Ticagrelor + aspirin	218/3547	HR =0.88 (0.73–1.05) [Cox regression with adjustment for ethnic group, sex, use of PPI, aspirin dose, smoking status, and diabetes]	
			Clopidogrel+ aspirin	246/3506		

ACTIVE A Trial = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A trial; CABG = coronary artery bypass grafting; GOF = gain-of-function; HR = hazard ratio; LOF = loss-of-function; OR = odds ratio; PLATO = PLATelet inhibition and patient Outcomes trial; PPI = proton-pump inhibitors; TIMI = Thrombolysis In Myocardial Infarction; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38.

Because of the large differences in populations included, treatments compared, and exposure and outcome definitions among studies reporting on treatment-effect modification by CYP2C19 variants, we did not perform a meta-analysis. For purposes of illustration, we used the counts reported in the studies to compare the treatment effect among carriers of CYP2C19 loss-of-function alleles versus noncarriers (i.e., carriers of CYP2C19*1 or *17 [normal and gain-of-function alleles, respectively]). **Figure 34** presents odds ratios for the treatment effect within each genotype subgroup and relative odds ratios comparing the treatment effect across genotype groups. This relative treatment effect is equivalent to a test of genotype × treatment interaction (see **Table 32** for detailed results on treatment-effect heterogeneity by genotype status).

Figure 34: Bleeding Events in Large Randomized Trials Reporting Information on Effect Modification by CYP2C19 Variants



Forest plots for treatment effects (odds ratios) on bleeding outcomes among carriers of at least one loss-of-function (LOF) allele (left panel); treatment effects (odds ratios) among noncarriers of LOF alleles (middle panel); and for relative effects (relative odds ratios [rOR]) comparing the treatment effect among LOF carriers and LOF noncarriers (right panel). The CURE and ACTIVE A trials compared aspirin plus clopidogrel versus aspirin monotherapy; the TRITON-TIMI 38 trial compared aspirin plus clopidogrel versus aspirin plus prasugrel; the PLATO trial compared aspirin plus clopidogrel versus aspirin plus ticagrelor. Point estimates for treatment effects are shown as black circles (carriers) or white circles (noncarriers); point estimates for relative treatment effects are shown as black squares. Horizontal extending lines denote 95 percent confidence intervals for all estimates. Vertical dashed lines denote no effect. Please see **Table 32** for definitions of the genotype categories and outcomes reported by each study.
ACTIVE A = Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A; CURE = Clopidogrel in Unstable Angina to Prevent Recurrent Events trial; PLATO = PLATelet inhibition and patient Outcomes trial; TRITON-TIMI 38 = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction.

Studies with Genetic Test–Based Selection of Patients

No studies fulfilling our inclusion criteria selected patients on the basis of CYP2C19 genotype to compare alternative treatment strategies selecting patients for inclusion on the basis of CYP2C19 genotype.

Assessment of Risk of Bias of Individual Studies

All studies addressing Key Question 4 were also included in Key Question 3a. As such, detailed information on the 17 risk-of-bias items we assessed is not presented here but in the preceding section.

Phenotypic Testing for Platelet Reactivity

Studies of Test-and-Treat Strategies

Five studies[217-221] comparing alternative test-and-treat strategies provided information on harms of test-directed treatment. The study design, patient characteristics, and phenotypic assays used in these studies have been presented in **Tables 33** under Key Question 3a. Briefly, the studies randomized patients into groups receiving clopidogrel at a dose guided by phenotypic testing or into groups receiving standard-dose clopidogrel. Four of the studies used the VASP assay to monitor response and modify treatment accordingly; the fifth study used the VerifyNow P2Y12 assay. No study identified a statistically significant difference between any study arms in the risk of bleeding events (the only treatment-related harms reported in the included studies; **Figure 35**). Outcome definitions and frequency of bleeding events are given in **Table 33**. The studies had short followup durations (1 year in one study; 6 months in another; and 30 days in the remaining three) and few events were observed, particularly severe or major bleeding outcomes. Consequently, data were sparse and CIs around effect estimates were wide, indicating substantial uncertainty.

Table 33: Bleeding Events in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity

Author Year Country PMID Study Name (if available)	Treatment Group Sample Size	Outcome Definition	Time Point	No. with Outcome (%)	Comparative Metric	95% CI	P Value [statistical test]
Wang[217] 2011 China 21538380	VASP-guided treatment N=150	TIMI major bleeding: intracranial bleeding, or clinically overt bleeding associated with a decrease in hemoglobin of 50 g/L	1 yr	0 (0%)	Not estimable	Not estimable	P>0.99[f] [unclear]
	Control N=156			0 (0%)			
	VASP-guided treatment N=150	TIMI minor bleeding	1 yr	19 (12.7%)	OR=0.76 (calculated)	0.40 to 1.45 (calculated)	P=0.06[g] [unclear]
	Control N=156			25 (16.6%)			
Bonello[218] 2009 France 19101221	VASP-guided treatment N=215	TIMI major bleeding	30 d	2 (0.9%)	OR=1.00 (calculated)	0.14 to 7.13 (calculated)	P>0.99 [Fisher exact test]
	Control N=214			2 (0.9%)			
	VASP-guided treatment N=215	TIMI minor bleeding	30 d	6 (2.8%)	OR=1.51 (calculated)	0.42 to 5.42 (calculated)	P=0.8 [chi-square]
	Control N=214			4 (1.9%)			
Bonello[219] 2008 France 18387444	VASP-guided treatment N=78	TIMI major ajor bleeding: intracranial bleeding, or clinically overt bleeding associated with a decrease in hemoglobin of 5 g/dL	30 d	1 (1.3%)	OR=1.08 (calculated)	0.07 to 17.53 (calculated)	P>0.99 [Fisher exact test; calculated]

[f] It is unclear how the reported P value of 1.0 was calculated. The P value here is nonestimable by the chi-square test or Fisher exact test.

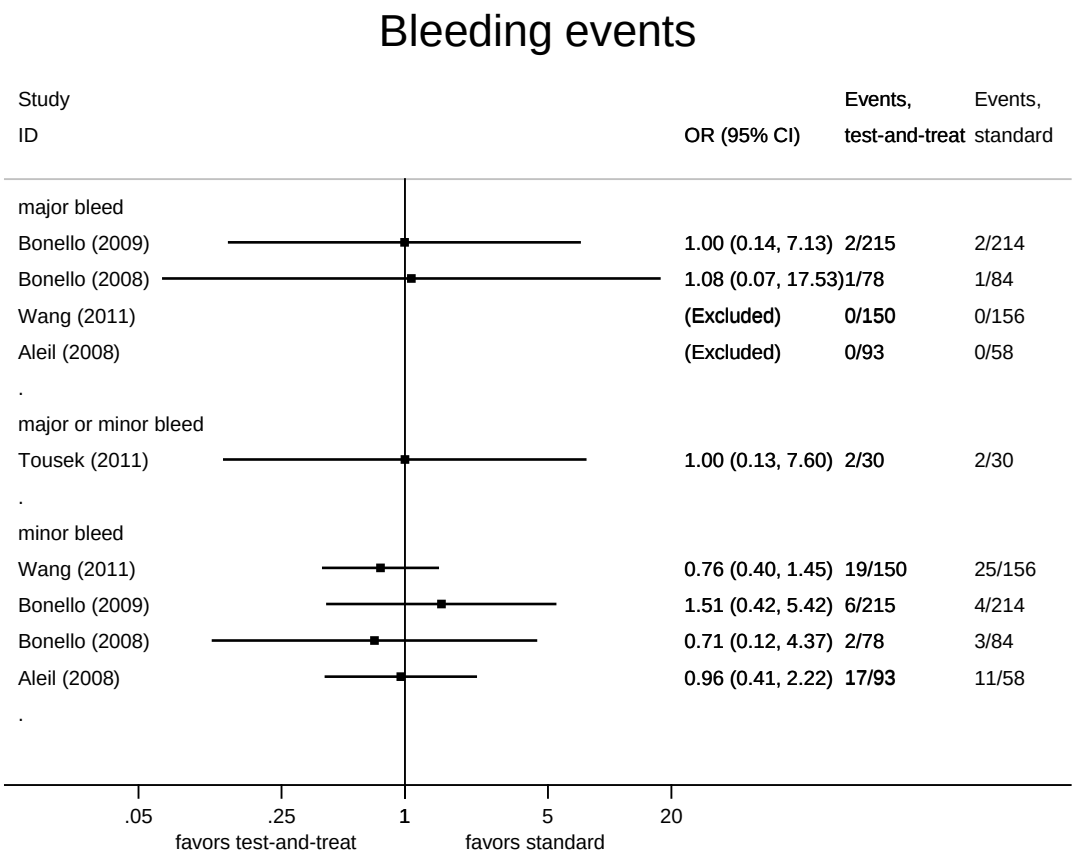
[g] Although P=0.06 was the reported P value, recalculation using a chi-square test gives a P value of 0.403 and recalculation using the Fisher exact test gives a P value of 0.420. It is unclear how the reported P value was calculated.

	Control N=84			1 (1.2%)			
	VASP-guided treatment N=78	TIMI minor bleeding	30 d	2 (2.6%)	OR=0.71 (calculated)	0.12 to 4.37 (calculated)	P>0.99 [Fisher exact test; calculated]
	Control N=84			3 (3.6%)			
Tousek[220] 2011 Czech Republic 21663983	VerifyNow-guided treatment N=30	TIMI major or minor bleeding	6 mo	2 (6.7%)	OR=1.00 (calculated)	0.13 to 7.60 (calculated)	P>0.99 [Fisher exact test; calculated]
	Control N=30			2 (6.7%)			
Aleil[221] 2008 France 19463377 VASP-02	VASP-guided treatment N=95 (93 included in analyses)	Major bleeding=intracranial bleeding or decrease in hemoglobin requiring transfusion	30 d	0 (0%)	Not defined	Not defined	NA
	Control N=58			0 (0%)			
	VASP-guided treatment N=95 (93 included in analyses)	Minor bleed=increase in skin bleeding, appearance of epistaxis, gum bleeding about which the patient complains	30 d	17 (18%)	OR=0.96 (calculated)	0.41 to 2.22 (calculated)	P=0.92 [chi-square test; calculated]
	Control N=58			11 (19%)			

CI = confidence interval; d = days; mo = months; NA = not applicable; NR = not reported; OR = odds ratio; PCI = percutaneous coronary intervention; TIMI = Thrombolysis in Myocardial Infarction; VASP = vasodilator-stimulated phosphoprotein; yr = year.

"Calculated" indicates that the result was not reported by the article's authors; rather, we derived the result from reported raw data.

Figure 35: Bleeding Events in Studies of Test-and-Treat Strategies using Phenotypic Testing of Platelet Reactivity



CI = confidence interval; OR = odds ratio. See **Table 33** for details on the strategies compared and the definitions of outcomes employed in each study.

Studies of Treatment-Effect Modification by Baseline Platelet Reactivity

The single study reporting on treatment-effect modification by baseline on-clopidogrel platelet reactivity reported that no treatment discontinuations, non–CABG-related major bleeding events (by T Thrombolysis in Myocardial Infarction [TIMI] criteria), or severe or life-threatening bleeding events (by GUSTO [Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries] criteria) were observed during the trial. Additional information on minor bleeding events or deaths (one death, due to cancer) was not stratified by baseline reactivity status.

Studies with Phenotypic-Test–Based Selection of Patients

Of the five studies that reported results from randomized trials with phenotypic testing–based patient selection, three reported information on treatment-related harms. [170,173,223] Detailed information about the study design, patient characteristics, and assays used in these studies is presented under Key Question 3a.

The large randomized GRAVITAS trial compared high-dose clopidogrel (in 1109 patients) versus standard-dose clopidogrel (in 1105 patients) ; all the patients had high on-clopidogrel platelet reactivity as measured by the VerifyNow P2Y12 assay. The study found no statistically significant difference between the two treatment groups for any bleeding event (hazard ratio for high-dose vs. standard-dose therapy, 1.19; 95 percent CI 0.93, 1.53) (P = 0.18) after 6 months of followup.

Two other smaller studies with short followup durations (1 month in both cases) reported that no major bleeding events were observed in the whole study population (regardless of baseline platelet reactivity status). Results from all studies are summarized in **Table 34**.

Table 34: Key Findings of Studies with Phenotypic Test–Based Selection of Patients Reporting Information on Harms

Author Year Country PMID Study Name (if available)	Treatment Groups (sample size)	Followup Duration	Summary of Findings
Price[170] 2011 Multinational 21406646 GRAVITAS	*High-dose clopidogrel (n=1109):* total first day dose 600 mg, followed by a maintenance dose of 150 mg/d for 6 mo *Standard-dose clopidogrel (n=1105):* loading dose of placebo, followed by a dose of 75 mg/d + a placebo tablet	6 mo	Randomized comparison[h]: Severe or moderate bleeding events (GUSTO criteria): HR=0.59 (95% CI 0.31, 1.11); P=0.10 Any bleeding: HR=1.19 (95% CI 0.93, 1.53); P=0.18
Valgimigli[173i] 2009 Italy 19528337 3T/2R	*IIb/IIIa inhibitor group (n=68, clopidogrel-only nonresponders):* tirofiban 50 mL diluted in 200 mL of 0.9% NaCl solution *Placebo group (n=79, clopidogrel-only nonresponders):* 50 mL of 0.9% NaCl was injected in 200 mL of 0.9% NaCl solution by an unblinded research study nurse (all other personnel were blinded to treatment assignment).	1 mo	No major bleeding events occurred in the study.
Cuisset[223] 2008 France 19463379	*IIb/IIIa inhibitor group (n=74):* systematic administration of abciximab to all patient *Conventional treatment group (n=75):* use of IIb/IIIa agonists left at the discretion of physicians	1 mo	No patients had a major bleeding event (TIMI criteria). No patients required transfusions.

ACS = acute coronary syndrome; CI = confidence interval; CV = cardiovascular; d = day; HR = hazard ratio; LTA = light-transmission aggregometry; MI = myocardial infarction; mo = month; OR = odds ratio; PMID = PubMed identification number; PRI = platelet reactivity index; TIMI = Thrombolysis In Myocardial Infarction Study Group.

Assessment of Risk of Bias of Individual Studies

All studies addressing Key Question 4 also addressed Key Question 3a. As such, detailed information on the 17 risk-of-bias items we assessed are not presented here but in the preceding section.

Harms of Testing *per se*

We found no studies reporting on the harms of the testing process for CYP2C19 genotyping or measuring platelet reactivity in the populations of interest. A review of the extensive literature on potential harms of prognostic or predictive testing in general (potential for discrimination,

[h] Detailed results on the comparison of patients with high- versus low-platelet reactivity receiving standard dose clopidogrel (i.e. the nonrandomized comparison) has been summarized in the report's section on Key Question 2b.
[i] Outcome data were extracted only for patients with poor-response to clopidogrel or clopidogrel and aspirin (i.e., we did not extract data on patients who were poor responders to aspirin alone).

privacy concerns, or other ethical, legal, or social issues) was considered beyond the scope of this review. Regarding platelet reactivity testing, in many patients (e.g., those undergoing PCI), blood samples can be obtained as part of standard procedures (e.g., after placement of the arterial sheath). In such cases, obtaining samples for analysis would not be expected to cause additional harm.

One study[219] comparing VASP-guided therapy with standard clopidogrel dosing in the PCI setting noted that patients in the test-guided arm had a longer time from clopidogrel loading to PCI than patients in the non–test-guided treatment arm (mean±SD, 26 ± 3 hours vs. 65 ± 24 hours; $P<0.001$). The delay was due to the need for repeat testing and treatment modification until a predefined reactivity threshold was reached in the test-guided group. It is unclear whether this delay resulted in harm to patients.

Discussion

Antiplatelet therapy is used for a number of ischemic cardiovascular conditions. Clopidogrel is among the most commonly prescribed antiplatelet drugs. It is used extensively in the interventional management of coronary artery disease and the treatment and secondary prevention of acute coronary syndromes.[225] Furthermore, it is used for the management of patients undergoing neurointervention (with stent placement), for the prevention of stroke in patients with atrial fibrillation who are not candidates for vitamin K antagonist therapy, and for the management of selected patients with peripheral arterial disease. However, response to clopidogrel therapy—as assessed by *ex vivo* studies of platelet function— is variable among patients and over time (within a patient). Some patients experience little suppression of platelet reactivity (despite being compliant to treatment) while others experience more profound suppression that may increase their risk of bleeding. Given the availability of several therapeutic options for antiplatelet treatment (e.g., increasing the loading or daily maintenance dose of clopidogrel or using adjunctive or replacement therapies such as prasugrel, ticagrelor, or cilostazol), there is interest in reliably identifying patients who are less likely to respond to standard clopidogrel treatment, as well as those who are most likely to respond to alternative treatments. This report reviewed the evidence of the effectiveness and comparative effectiveness of two types of tests that have been extensively evaluated as biomarkers for outcome prognosis for patients receiving clopidogrel therapy and as biomarkers of treatment response: genetic testing for CYP2C19 variants and phenotypic testing for on-clopidogrel platelet reactivity.

Key Findings and Assessment of the Strength of Evidence

Table 35 presents a summary of the report's key findings for each Key Question. When appropriate, results are presented separately for each of the populations and outcomes of interest. We did not assess the strength of evidence for evidence on analytic validity (because analytic validity is a prerequisite for the clinical use of the tests and because no framework exists for assessing the strength of evidence for analytic validity studies) or for studies exclusively assessing platelet reactivity as an outcome (because platelet reactivity measurements during followup are not usually performed as part of clinical care and because platelet reactivity is not a patient-relevant outcome). Instead, we focus here on the body of evidence pertaining to predictive effects, treatment decisionmaking, and harms as related to patient-relevant clinical outcomes. Please see the Methods section for a detailed discussion of our approach to rating the strength of evidence.

Table 35: Key Findings from This Review

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
1a: What it the analytic validity of tests for genotyping CYP2C19 variants?	NA	Genotyping for any CYP2C19 variant	NA	SOE = NA • Few studies provided information on analytic validity specifically using samples obtained from patient populations relevant to this review. • When available, data were limited to test–retest reliability. • There was limited information comparing the validity of different genetic testing assays. • However, based on data on healthy volunteers (not reviewed in this report), the analytic validity of genotyping assays can be considered robust.
1b: What is the predictive value of genetic testing for CYP2C19 variants?	Ischemic heart disease	Genotyping for LOF CYP2C19 variants	Stent thrombosis	SOE = *Low* • Meta-analysis of 13 studies found a statistically significant association. • RR=1.56 (95 percent CI 1.15, 2.11) • There was little evidence of heterogeneity (I^2=0%); but the test for small-study effects was statistically significant. • There was some concern about selective outcome reporting. • Studies reported few outcome events and the summary estimate was imprecise.
			MACE	SOE = *Low (for lack of association)* • Meta-analysis of 15 studies did not identify a statistically significant association. • RR=1.09 (95 percent CI 0.93, 1.29) • There was some evidence of heterogeneity (I^2=33%) and small-study effects.
			All other clinical outcomes	SOE = *Insufficient* • Few studies reported information for noncomposite clinical outcomes other than stent thrombosis. • There was substantial concern about selective outcome reporting. • Study-specific and meta-analysis estimates (when performed) indicated substantial uncertainty.
		Genotyping for GOF CYP2C19 variants	MACE	SOE = *Low* • Meta-analysis of 4 studies found a statistically significant protective effect (for carriers vs. noncarriers). • RR=0.80 (95 percent CI 0.70, 0.90) • There was substantial concern about selective outcome reporting.
			All other clinical outcomes	SOE = *Insufficient* • Few studies provided relevant information. • There was substantial concern about selective outcome reporting. • Study-specific and meta-analysis estimates (when performed) indicated substantial uncertainty.
	Patients undergoing neurointervention	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • Only a single small study was available; it did not report information on clinical outcomes.
	Patients with atrial fibrillation	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • Only a single study was available. • Estimates were imprecise owing to the small number of outcome events observed.
1c: What factors affect the predictive value of genetic testing for CYP2C19 variants?	All patient populations	Genotyping for any CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • 10 studies provided information on effect modification; no factor affected predictive performance statistically significantly regarding clinical outcomes; no factor was assessed by more than 3 studies, giving rise to concerns about selective outcome reporting.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• Effect modification by study-level factors was not statistically significant and CIs were wide for all genotype–outcome pairs assessed.
2a: What is the analytic validity of tests for on-clopidogrel platelet reactivity?	NA	All assays used to measure on-clopidogrel platelet reactivity	NA	SOE = NA • Few studies reported information on analytic sensitivity and specificity, possibly reflecting the research community's belief that there is no good reference standard assay for platelet reactivity. • Agreement ranged from poor to moderate and was variable between tests. The highest agreement was observed between applications of the same assay with different concentrations of agonists, rather than between different assays.
2b: What is the predictive ability of phenotypic testing for platelet reactivity?	Ischemic heart disease	LTA	All-cause mortality	SOE = *Low* • 10 studies using heterogeneous methods to define increased reactivity were available. • These studies support an association between increased platelet reactivity measured by LTA and mortality.
			Cardiovascular mortality	SOE = *Low* • 8 studies using heterogeneous methods to define increased reactivity were available. • Studies provided evidence of an association between increased reactivity and cardiovascular mortality; however, clinical heterogeneity precluded firm conclusions. • There was some concern about selective outcome reporting.
			Acute coronary syndromes	SOE = *Low* • 17 studies using heterogeneous methods to define increased reactivity were available. • Studies often found statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions. • There was some concern about selective outcome reporting.
			Stent thrombosis	• 18 studies using heterogeneous methods to define increased reactivity were available. • Studies often found statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions. • There was some concern about selective outcome reporting.
			Stroke	SOE = *Low (for lack of association)* • 11 studies using heterogeneous methods to define increased reactivity were available. • Studies generally did not report statistically significant associations between increased reactivity as measured by LTA and clinical events; however, clinical heterogeneity did not allow for stronger conclusions or quantitative synthesis to increase precision.
			MACE	SOE = *Low* • 33 studies using heterogeneous methods to define increased reactivity were available. • The majority of reviewed studies suggested a statistically significant association between increased platelet reactivity measured by LTA and composite cardiovascular events. • Definitions of composite outcomes where often heterogeneous.
			All other clinical outcomes**	SOE = *Insufficient* • Clinical and population heterogeneity or small number of studies limited our ability to draw conclusions
		VerifyNow	All cause mortality	SOE = *Low (for lack of association)* • 5 studies were available. Meta-analysis of 3 studies did not find an association between increased reactivity measured by VerifyNow and all-cause mortality.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• RR=1.39 (95 percent CI 0.73, 2.66) • The summary estimate was imprecise and 95% CI did not rule out clinically meaningful effects.
			Cardiovascular mortality	SOE = *Low* • 6 studies were available. Meta-analysis of 4 studies found a statistically significant association with little evidence of heterogeneity. • RR=2.50 (95 percent CI 1.28, 4.87) • The CI of the summary estimate indicated substantial uncertainty. • There was some concern about selective outcome reporting.
			Acute coronary syndromes	SOE = *Low* • 14 studies using heterogeneous methods to define increased reactivity were available. • Studies generally suggested an association between increased reactivity as measured by VerifyNow and acute coronary syndromes, both periprocedurally and during longer followup.
			Bleeding events (major and all levels of severity combined)	SOE = *Low (for lack of association)* • 8 studies were available. • Meta-analysis of 3 studies with data on any bleeding event did not find an association between increased reactivity measured by VerifyNow. • RR=1.02 (95 percent CI 0.79, 1.31). There was little evidence of heterogeneity (I^2=0 percent). • Meta-analysis of 4 studies with data on major bleeding events did not find an association between increased reactivity measured by VerifyNow. • RR=0.85 (95 percent CI 0.47, 1.52). There was substantial evidence of heterogeneity (I^2=62 percent). • For major bleeding events the summary estimate was imprecise and the 95% CI did not rule out clinically meaningful effects.
			MACE	SOE = *Low* • 17 studies were available. Meta-analysis of 11 studies identified a statistically significant association. • RR=2.46 (95 percent CI 1.88, 3.22) • There was little evidence of statistical heterogeneity (I^2=21 percent) and studies used fairly similar definitions of increased reactivity. • The test for small-study effects was statistically significant.
			All other clinical outcomes**	SOE = *Insufficient* • Clinical heterogeneity or small number of studies limited our ability to draw conclusions
		VASP assay	Cardiovascular mortality	SOE = *Low (for lack of association)* • 6 studies were available. Meta-analysis of 4 studies did not identify a statistically significant association. • RR=2.09 (95 percent CI 0.74, 5.88) • Although the test for heterogeneity was nonsignificant, point estimates from individual studies ranged from strong protective effects to strong harmful effects. • The meta-analytic summary point estimate was far from the null and its CI was wide (imprecise). • Clinically significant effects could not be ruled out.
			Acute coronary syndromes	SOE = *Low (for lack of association)* • 6 studies were available. Meta-analysis of 3 studies did not identify a statistically significant association. • RR=1.47 (95 percent CI 0.77, 2.79)

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
				• The test for heterogeneity was non-significant but point estimates from individual studies were highly variable. • The meta-analytic summary point estimate was far from the null and its CI was wide (imprecise). • Clinically significant effects could not be ruled out.
			Stent thrombosis	SOE = *Low* • 7 studies were available. Meta-analysis of 4 studies identified a statistically significant association. • RR=3.37 (95 percent CI 1.59, 7.11) • There was little evidence of statistical heterogeneity and studies used fairly similar definitions of increased reactivity. • The summary estimate was imprecise but the lower bound was consistent with a 59% increase in risk in the high-reactivity group. • There was some concern about selective outcome reporting.
			MACE	SOE = *Low* • 7 studies were available. Meta-analysis of 6 studies identified a statistically significant association. • RR=2.57 (95 percent CI 1.21, 5.47) • There was evidence of statistical heterogeneity. • The summary estimate was imprecise but the lower bound was consistent with a 21% increase in risk in the high-reactivity group. • There was some concern about selective outcome reporting.
			All other clinical outcomes	SOE = *Insufficient* • Few studies reported information. • Clinical heterogeneity or small number of studies limited our ability to draw conclusions .
		PFA-100	MACE	SOE = *Low* • 7 of the 8 studies on this assay reported information on composite clinical outcomes. • Heterogeneity in the methods used to define increased reactivity precluded definitive conclusions; however, studies generally indicated an association between increase platelet reactivity as measured by the PFA-100 assay and composite clinical outcomes.
			All other clinical outcomes	SOE = *Insufficient* • Few of the available studies reported information on other outcomes. • There was concern about selective outcome reporting.
		All other assays	All clinical outcomes	SOE = *Insufficient* • Few studies were available. • When ≥2 studies were available for the same outcome they used heterogeneous metrics or thresholds to define increased reactivity or used different agonists for *ex vivo* stimulation of platelets.
	Patients with cerebrovascular disease	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • Only 4 studies, using diverse assays to measure reactivity, were available. • Studies were fairly small.
	Patients with peripheral arterial disease	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • Only a single small study was available.

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
2c: What factors affect the predictive value of phenotypic testing for platelet reactivity?	All patient populations	All assays used to measure on-clopidogrel platelet reactivity	All clinical outcomes	SOE = *Insufficient* • 5 studies provided information on effect modification; no factor was assessed by more than 2 studies. • Effect modification by study-level factors could not be assessed for most assay–outcome pairs; when such analysis was possible (for VerifyNow MACE), results indicated substantial uncertainty.
3a: What is the comparative effectiveness of alternative test-and-treat strategies	Ischemic heart disease	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • 3 studies provided information on treatment effect modification; no studies directly compared testing strategies; no studies randomized patients selected on the basis of genotype status. • Studies compared different antiplatelet treatments and produced heterogeneous results. • Study-specific estimates were imprecise.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • The 5 randomized studies of testing strategies were small, had different designs, and produced extreme results with considerable statistical uncertainty. • Only a single study of effect modification was identified; the study had been terminated early and did report information on clinical events. • Studies of test-based patient selection assessed different treatments.
	Atrial fibrillation	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • Only one study providing information on effect modification by CYP2C19 status was identified. • The study did not find evidence of effect modification by genotype status but there was considerable statistical uncertainty in the study estimates.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
	Other patient populations	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
		Phenotypic testing for platelet reactivity	All clinical outcomes	SOE = *Insufficient* • No studies were identified.
3b: What factors modify the comparative effectiveness of alternative test-and-treat strategies?	All patient populations	Genetic testing for CYP2C19 variants or phenotypic testing for platelet reactivity (all assays assessed)	All clinical outcomes	SOE = *Insufficient* • 2 studies provided information on effect modification; each assessed different effect modifiers; no statistically significant interactions were reported.
4: What are the harms of testing? What are the harms of test-directed treatment?	All patient populations	Genetic testing for CYP2C19 variants	All clinical outcomes	SOE = *Insufficient* • 4 studies assessed treatment effect modification by genotype status; no studies directly compared testing strategies; no studies randomized patients selected on the basis of genotype status. • Studies compared different antiplatelet treatments and had heterogeneous results. • No studies provided direct information on the harms of testing *per se*.
		Phenotypic testing for	All clinical	SOE = *Insufficient*

Key Question	Population	Test/Assay	Outcome	SOE Summary and Comments
		platelet reactivity (all assays assessed)	outcomes	• The 5 randomized studies of testing strategies were small, had different designs, and produced extreme results with considerable statistical uncertainty (and in several cases simply did not report any outcome events). • Only a single study of effect modification was identified; safety outcomes either did not occur (regardless of reactivity status) or results were not stratified by reactivity group. • Studies of test-based patient selection assessed different treatments.

CI = confidence interval; GOF = gain-of-function; LOF = loss-of-function; MACE = major adverse cardiovascular events; NA = not applicable; RR = relative risk; SOE = strength of evidence.

Analytic Validity

Overall, we found limited evidence for the analytic validity of assays for genotyping CYP2C19 alleles in the populations of interest. However, the available studies reported almost perfect test–retest reliability and there is general consensus that genotyping methods have adequate analytic performance for clinical application (given the nature of genetic testing, we believe that supportive evidence can be drawn from the application of genetic tests to other patient populations and for the application of genotyping methods to other genes).

In contrast, numerous studies on the analytic validity of assays for measuring platelet reactivity in the populations of interest were available. Platelet reactivity can be affected by environmental exposures (e.g., drugs received by the patient) and patient characteristics (because, e.g., patients with diabetes have higher reactivity than nondiabetic individuals) and is variable within a person over time. Many of the studies we reviewed performed inappropriate analyses for comparing measurements obtained by reactivity assays, and often did not report information adequate to allow for a complete evaluation of their experimental design and analyses. In the subset of studies using appropriate designs and analyses to assess agreement or analytic performance, tests considered as "analytic gold standards" in some publications were considered as index tests (with measurement error that cannot be ignored) in other publications, indicating the lack of consensus on the optimal method for reactivity measurement. In the studies we reviewed, assays had low to moderate levels of interassay agreement. Agreement was generally higher between applications of the same assay across different concentrations of agonists (e.g., LTA with 5 μM ADP concentration vs. 20 μM) than between different assays. The Working Group on High On-Treatment Platelet Reactivity identified the lack of a universally accepted reference assay as a key reason for the limited implementation of platelet reactivity testing in clinical settings.[37]

Predictive Value of Genetic and Phenotypic Testing for Patients on Clopidogrel-based Antiplatelet Therapy

Studies assessing the predictive value of CYP2C19 variants and platelet reactivity for patients receiving clopidogrel-based antiplatelet therapy represented the majority of the studies included in this report. It is interesting to compare our findings with previous systematic reviews and meta-analyses assessing CYP2C19 polymorphisms (**Table 36**) and those assessing assays for measuring platelet reactivity (**Table 37**).

Genetic Testing for CYP2C19 Variants

In general, In our comparative effectiveness review, we have synthesized more publications than previous reviews (with the sole exception of the study by Holmes et al.,[39] which included studies of CYP2C19 variants available only in abstract form),[a] with generally similar findings.

[a] The EPC recognizes that several studies relevant to the topic have been published since the conduct of the literature search conducted as the basis for this report. Per our protocol, we plan to update our literature searches (through the first quarter of 2012) and include any additional relevant studies in our analyses. For example, we anticipate that the updated review will include the results from the recently published genetic substudy of the CHARISMA trial (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance, reporting on effect modification by CYP2C19 variants) and the RAPID GENE randomized trial (Reassessment of Anti-Platelet Therapy Using an Individualized Strategy Based on Genetic Evaluation, comparing genetic testing for CYP2C19 variants with standard care for patients undergoing PCI).

We found evidence supporting an association between loss-of-function CYP2C19 alleles and increased risk of stent thrombosis and cardiovascular death, but not the composite of adverse cardiovascular outcomes, all-cause mortality, or cardiovascular mortality. Furthermore, we found an association between gain-of-function alleles and reduced risk of MACE (composite). The strength of these findings is limited by concerns about selective reporting bias, because only a subset of the available studies contributed results to the polymorphism–outcome pairs of interest. Furthermore, studies reported on a relatively limited number of events, leading to substantial uncertainty in their effect-size estimates. In addition, data were reported almost exclusively under a dominant genetic model (carriers of at least one allele vs. noncarriers). It is unclear if this is the true underlying genetic model; the reported data precluded further analyses to address this issue. Studies did not genotype the same CYP2C19 alleles—for example, loss-of-function alleles other than *2 were not commonly genotyped. However, most loss-of-function variants other than *2 are relatively rare and as such we believe that any effect of exposure misclassification due to incomplete genotyping is likely to be limited. Finally, we found some evidence of statistically significant small-study effects for the two outcomes assessed by most studies (MACE and stent thrombosis).

Generally, regarding the predictive effects of CYP2C19 genotype status, existing systematic reviews have reached similar conclusions to ours, both in magnitude and direction. Also consistent with our findings, previous analyses have suggested that selective outcome reporting and publication bias may have affected meta-analytic estimates.[26,39] Meta-regression analyses did not any study-level factors that could explain the differences between smaller and larger studies, and between-study heterogeneity was relatively limited across all comparisons we performed. However, tests for funnel plot asymmetry (such as tests used in our report and by others) cannot differentiate "publication bias" from true heterogeneity among smaller and larger studies. As such, significant results from these tests should not be overinterpreted as proof of the presence of bias. Other explanations, such as true heterogeneity among larger and smaller studies, as well as chance, may explain these results.[55]

Table 36: Results of Systematic Reviews Assessing the Effect of CYP2C19 Variants on Clinical Outcomes Among Patients Receiving Clopidogrel Treatment

Author Year PMID	No. of Studies	Selection Criteria	Outcome [no. of studies/events]: Effect Size (95% CI)
Sofi[30] 2011 20351750	7	Prospective studies; no language restriction	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* Cardiovascular events [7 studies]: RR 1.96 (1.14, 3.37) ST [4 studies]: RR 3.82 (2.23, 6.54)
Jin[28] 2011 20845077	8	Prospective cohort studies; English language only	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* Adverse clinical events [8 studies]: OR 1.46 (1.01, 2.13) Cardiac mortality [5 studies]: OR 2.07 (1.22, 3.52) MI [5 studies]: OR 1.69 (1.09, 2.61) ST [5 studies]: OR 3.81 (2.27, 6.40)
Mega[29] 2010 20978260	9	Prospective cohort studies or clopidogrel-treated arms from RCTs; summary data for meta-analysis were provided by the primary investigators	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* MACE [9 studies]: HR 1.57 (1.13, 2.16) Stent thrombosis [6 studies]: HR 2.81 (1.81, 4.37) Cardiovascular death [9 studies]: HR 1.84 (1.03, 3.28) Nonfatal MI [9 studies]: HR 1.45 (1.09, 1.92) Nonfatal stroke [9 studies]: HR 1.73 (0.68, 4.38)

Hulot[27] 2010 20620727	10	Original epidemiological studies; no language restriction	MACE [10 studies, 1003 events]: 1OR .29 (1.12, 1.49) Stent thrombosis [4 studies]: OR 3.45 (2.14, 5.57) Mortality [5 studies]: OR 1.79 (1.10, 2.91)
Bauer[26] 2011 21816733	15	Observational studies and clinical trials; no language restriction	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* MACE [12 studies]: OR 1.11 (0.89,1.39) Stent thrombosis [9 studies]: OR 1.77 (1.31, 2.40) *Carriers of at least one CYP2C19 GOF allele vs. noncarriers* MACE [5 studies]: OR 0.93 (0.75, 1.14) Stent thrombosis [3 studies]: OR 0.99 (0.60, 1.62)
Liu[226] 2011 21794898	20	Articles, conference abstracts and presentation slides; no language restriction	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* Stent thrombosis [18 studies]: OR 2.58 (1.77, 3.77) MACE [18 studies]: OR 1.26 (1.06, 1.50) *Carriers of at least one CYP2C19 GOF allele versus noncarriers* MACE [6 studies]: OR 0.82 (0.69, 0.98)
Holmes[39] 2011 22203539	32	Articles reporting original data without language restriction	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* MACE [24 studies]: RR 1.18 (1.09, 1.28) MI-fatal and nonfatal [9 studies]: RR 1.37 (1.13, 1.65) MI-nonfatal [3 studies]: RR 1.48 (1.05, 2.07) Stent thrombosis [14 studies]: RR 1.75 (1.50, 2.03) Stroke-fatal and nonfatal [4 studies]: RR 1.98 (0.77, 5.09) Bleeding-all [3 studies]: RR 0.84 (0.75, 0.94) Bleeding-severe [4 studies]: RR 1.07 (0.92, 1.25)
Li[227] 2012 22123356	11	Randomized or cohort studies; no information on language reported	*Carriers of at least one CYP2C19 GOF allele vs. noncarriers* MACE [8 studies]: OR 0.82 (0.72, 0.94) Bleeding [4 studies]: OR 1.25 (1.07, 1.47)
Zabalza[228] 2012 21693476	13	Case–control or prospective cohort; English language	*Carriers of at least one CYP2C19 LOF allele vs. noncarriers* MACE [11 studies]: HR 1.23 (0.97, 1.55) ST [7 studies]: HR 2.24 (1.52, 3.30) *Carriers of at least one CYP2C19 GOF allele vs. noncarriers* MACE [4 studies]: HR 0.75 (0.66, 0.87) Major bleeding [4 studies]: HR 1.26 (1.05, 1.50)

CI = confidence interval; GOF = gain-of-function; HR = hazard ratio; LOF = loss-of-function; MACE = major adverse cardiovascular events; MI = myocardial infarction OR = odds ratio; RCT = randomized controlled trial; RR = relative risk; ST = stent thrombosis.
Effect-size values higher than 1 indicate a detrimental effect (increased frequency of outcomes) among patients carrying the LOF allele. Not all studies reported all outcomes. Reviews are listed by number of included studies. When available, meta-analysis results are from analyses using random effects models. For consistency, data are presented only from dominant genetic models.

Phenotypic Testing for Platelet Reactivity

A large number of studies assessed the predictive value of various assays for measuring platelet reactivity, mostly in patients with ischemic heart disease. Studies used different assays, definitions, and cutoff values to define increased platelet reactivity. Cutoff values were often identified through analyses of receiver-operating-characteristic curves conducted on the same datasets used to assess the phenotypic test's predictive ability, suggesting that estimates of predictive performance (i.e., estimates of the prediction error) may be overly optimistic. Similar to the situation with genetic testing for CYP2C19 variants, only a subset of the studies evaluating each assay contributed data to each of the outcomes of interest, raising concerns about selective outcome reporting.

Compared to previous systematic reviews, our review includes a much larger number of studies and considers multiple assays assessing on-clopidogrel platelet reactivity using agonists to stimulate platelets *ex vivo*. In contrast to previous meta-analyses, our review does not combine results across different assays (i.e., across tests using different measurement principles), different agonist concentrations, or different calculation methods or cutoff values for defining high

reactivity. We believe that this choice is supported by our review of analytic validity that found low-to-moderate agreement between different assays. We found statistically significant associations between platelet reactivity as measured by various assays (particularly LTA, VerifyNow P2Y12, and the VASP assay) and adverse cardiovascular outcomes. Of note, our analyses include almost double the number of studies included in a recently published meta-analysis of individual data on the VerifyNow assay.[229] Despite differences in selection criteria and analysis methods, our findings were similar, identifying a fairly strong association between platelet reactivity measured by this assay and ischemic events.

We found that comparative studies of test performance (defined as studies assessing the predictive value of two or more assays) were generally rare and almost universally did not perform analyses (or report data) to inform about the relative predictive ability of tests. Nonetheless, a qualitative comparison of point estimates and CIs from the available studies suggested that the tests assessed had comparable predictive performance.

Table 37: Results of Systematic Reviews Assessing the Predictive Ability of Phenotypic Testing on Clinical Outcomes among Patients Receiving Clopidogrel Treatment.

Author Year PMID	No. of Studies	Selection Criteria	Outcome [no. of studies/events]: Effect Size (95% CI)
Aradi[230] 2010 20826265	20	Observational studies; no language restriction	*High vs. normal on-clopidogrel platelet reactivity* Nonfatal MI [11 studies]: OR 3.00 (2.26, 3.99) ST [11 studies]: OR 4.14 (2.74, 6.25) Composite ischemic events [17 studies]: OR 4.95 (3.34, 7.34) CV death [11 studies]: OR 3.35 (2.39, 4.70)
Brar[229] 2011 22032704	6	Individual patient data meta-analysis of studies of the VerifyNow P2Y12 assay; no language restriction	*PRU ≥230 vs. <230* Composite primary endpoint [6 studies]: HR 2.10 (1.62, 2.73) Death [6 studies]: HR 1.66(1.03, 2.68) MI [6 studies]: HR 2.04(1.51, 2.76) Stent thrombosis [6 studies]: HR 3.11 (1.50, 6.46)
Combescure[231] 2010 20156305	15	Prospective cohort or RCTs; no language restriction	*Clopidogrel nonresponder vs. responder* Recurrent ischemic events [15 studies]: RR 3.53 (2.39, 5.20) *ADP cutoff >65% for clopidogrel nonresponse* Recurrent ischemic events [3 studies]: RR 5.8 (3.2, 10.3) *ADP lower cutoff for clopidogrel nonresponse* Recurrent ischemic events [7 studies]: RR 2.9 (2.2, 3.7)
Snoep[232] 2007 17643570	25	Prospective cohort or case–control studies, and RCTs; no language restriction	*Clopidogrel resistance vs. nonresistance* MACE [9 studies] OR 8.0 (3.36, 19.05) Subacute ST [3 studies] OR 7.02 (0.63, 79.01) Composite endpoint of clinical ischemic events [4 studies]: OR 12.02 (5.91, 24.42) Myonecrosis (elevated CK-MB levels) [1 study]: OR 2.20 (0.93, 5.22)
Sofi[233] 2010 20135063	14	Prospective cohort studies; no language restriction	*Clopidogrel responder vs. nonresponder* MACE [14 studies]: OR 5.67 (2.97, 10.84)

CI = confidence interval; CK-MB = creatine kinase (MB fraction); HR = hazard ratio; MACE = major adverse cardiovascular events; MI = myocardial infarction; OR = odds ratio; PRU = platelet reactivity unit; RCT = randomized controlled trial; RR = relative risk; ST = stent thrombosis.
Effect size values higher than 1 indicate a detrimental effect (increased frequency of outcomes) among patients with increased on-clopidogrel platelet reactivity. Not all studies reported all outcomes. Reviews are listed by the number of included studies. When available, meta-analysis results are from analyses using random effects models.

Use of Testing to Guide Treatment Decisionmaking

To our knowledge, this is the first review to comprehensively evaluate the use of genetic and phenotypic testing for guiding antiplatelet therapy with a focus on patient-relevant benefits and harms. Only a small subset of the reviewed studies provided information that can be used to directly inform therapeutic decisionmaking. Ideally, directly comparative studies of test-guided treatment versus no testing (or alternative testing strategies) would be used to inform clinical decisions on the benefits and harms of testing. However, because such studies are rarely available, we also considered studies of effect modification based on RCTs of non–test based treatments as well as RCTs of non–test-based therapies using the tests of interest to select "nonresponsive" patients (e.g., patients with high on-clopidogrel reactivity at baseline).

Genetic Testing for CYP2C19 Variants

Regarding genetic testing for platelet reactivity, we reviewed several studies assessing effect modification by CYP2C19 variants; of these, 4 were based on large RCTs comparing antiplatelet treatments within patient groups defined by CYP2C19 genotype. To our knowledge, only one previously published review evaluated such studies of effect modification by CYP2C19 variants and quantitatively synthesized relevant data from large randomized trials. The authors concluded that there is "no clinically significant interaction of CYP2C19 genotype with the association of clopidogrel therapy and cardiovascular events."[39] On the basis of population and treatment heterogeneity, we refrained from quantitatively synthesizing studies of effect modification (two studies compared clopidogrel vs. placebo; one study compared clopidogrel vs. prasugrel; and one study compared clopidogrel vs. ticagrelor; populations were also heterogeneous: 1 study enrolled patients with atrial fibrillation patients; and three with ischemic heart disease). Given that the drugs compared have different mechanisms of action, it is plausible that that the true interaction effects (treatment × CYP2C19 genotype) being estimated in the studies may have different magnitudes or directions. However, none of the four studies provided strong evidence that the CYP2C19 genotype modifies the effectiveness of clopidogrel therapy. Despite the studies having included several thousand randomized participants, CIs for relative treatment effects (by genotype status) were fairly wide (with the exception of the CI for the PLATO study) and we could not rule out clinically meaningful differences with regard to relative treatment effectiveness.

Currently, the United States FDA-approved label for clopidogrel[b] states that poor metabolizers (as identified by CYP2C19 genotype) treated with clopidogrel "exhibit higher cardiovascular event rates following myocardial infarction than do patients with normal CYP2C19 function." The label also states that CYP2C19 genotyping "can be used as an aid in determining therapeutic strategy", and that alternative treatment strategies should be considered "in patients identified as CYP2C19 poor metabolizers." Although our review of observational (mostly cohort) studies identified some evidence to support the association between loss-of-function CYP2C19 variants and increased rates of cardiovascular events, no evidence of clopidogrel effect modification was found, and the evidence is thus insufficient to support the use of genetic testing to guide antiplatelet treatment selection. However, that the statistical power to detect effect modification was limited in the included studies.

[b] Available at http://products.sanofi.us/PLAVIX/PLAVIX.html; last accessed May 10, 2012.

Phenotypic Testing for Platelet Reactivity

Regarding phenotypic testing for platelet reactivity, we identified a small number of RCTs comparing alternative test-and-treatment strategies. The studies were small and produced extreme results (with very wide CIs) for all clinical outcomes reported (both benefits and harms). Furthermore, studies had heterogeneous designs (e.g., they differed in the number of measurements performed for monitoring and in the treatment modifications applied for nonresponding patients) and relatively short followup periods (all less than 1 year). In addition, we identified only a single small study (terminated early) of effect modification by baseline platelet reactivity with a short followup duration. This study did not provide compelling evidence of effect modification by baseline platelet reactivity. Finally, although we identified a number of studies selecting patients on the basis of high baseline platelet reactivity and then randomizing them into non–test based comparator groups, these studies inform us about the value of testing to guide treatment selection only if it is assumed that the randomized treatments have the same effect (or at least a relative effect known with certainty) in patients with low baseline platelet reactivity. Overall, we believe there is insufficient evidence to support (or refute) the use of use of phenotypic testing for platelet reactivity to guide antiplatelet treatment selection. Our findings do support current clinical practice guidelines, which in general do not advocate the use of phenotypic testing for platelet reactivity for guiding choice of treatment. A recent consensus document from the Working Group on High On-Treatment Platelet Reactivity also noted that there are "limited data to support that alteration of therapy based on platelet function measurements actually improves outcomes." [37]

Modifiers of the Impact of Test Results

We found little evidence that patient-level characteristics, disease features, cointerventions (including PPIs), or system-level factors impact on the predictive effect of genetic or phenotypic tests of interest. No modifier was assessed in more than three studies for any test–outcome pair of interest to this review. Even when considering information across studies (meta-regression) we did not identify any study-level characteristics that significantly modified the predictive effect of genetic testing (meta-regression analyses were undertaken for CYP2C19 variants only, owing to the small number of available studies for other outcomes). However, both within-study comparisons and meta-regression analyses (across studies) identified substantial uncertainty regarding the magnitude and direction of modifier effects, indicating that further research is needed to identify situations where the tests may be most informative.

Harms of Testing and Test-Directed Treatment

Direct evidence on the harms of test-directed treatment was reported by a limited number of comparative studies. Although the studies did not raise concerns about an increased rate of harms, they were generally small and had followup periods too short to permit conclusions. We found no evidence on harms of testing *per se*, possibly reflecting the fact that testing procedures, both for genetic and phenotypic testing, are associated with minimal physical harm (e.g., minor complications of phlebotomy to obtain blood samples). Other potential harms of testing—unnecessary exposure to higher-risk treatment or ethical and social issues, such as discrimination on the basis of genetic information and the possibility that the prognostic value of genetic information may be overestimated by patients[234]—were not considered in the studies we reviewed.

Applicability

The vast majority of included studies enrolled patients with ischemic heart disease (acute or chronic coronary disease represented almost all available studies for all Key Questions). Other populations who are potential candidates for antiplatelet therapy (e.g., patients with cerebrovascular disease, peripheral arterial disease, or atrial fibrillation) were included in a minority of studies only. This imbalance is not unexpected, given that clopidogrel's primary indications pertain to ischemic heart disease populations. However, it is probably not prudent to extrapolate findings from studies of ischemic heart disease to other patient populations. Given that a large number of studies included patients undergoing PCI, applicability may also be limited to noninterventional settings.

Particularly for genetic testing for CYP2C19 variants, patient race or ethnicity may be an important effect modifier, because the prevalence of variant alleles is substantially different among racial and ethnic groups (e.g., *2 variants are much more common in East Asian populations than others). We found little evidence that prognostic effects were different in our subgroup analysis by ethnicity; although this finding is consistent with evidence on other gene-disease associations[235], more evidence is needed for patient populations underrepresented in this review (e.g., blacks and East Asians).

The majority of studies were conducted in tertiary (usually academic) medical centers. Studies of treatment-effect modification by CYP2C19 genotype were based on large randomized trials, and findings may not be generalizable to everyday care settings. Because patient information on preexisting vascular disease in studies of predictive effects was generally incompletely reported, it is unclear whether patients in the included studies are representative of those seen in clinical practice. Nonetheless, the distribution of risk factors for ischemic vascular disease (male sex, hyperlipidemia, diabetes, hypertension, smoking, etc.) appeared to be representative of contemporary patient populations, and the majority of studies were conducted in recent years.

Implications for Clinical and Policy Decisionmaking

Despite the availability of a large literature on the use of genetic testing of CYP2C19 variants and phenotypic testing of platelet reactivity for predicting outcomes in patients receiving clopidogrel-based therapy, studies provided limited information on the value added by these tests over ascertainment of conventional risk factors in the populations of interest (e.g., clinical or laboratory information or disease-specific predictive scores). Furthermore, there was little comparative evidence that could be used to identify the most informative test or combination of tests for predicting clinical outcomes. This and other limitations of the existing literature may reduce the potential for clinical application of the tests reviewed herein as predictive markers for patients on clopidogrel-based antiplatelet therapy. The available evidence was even more limited regarding the use of either type of testing to guide the choice of antiplatelet therapy.

Additional research seems warranted for both genetic and phenotypic tests. Genetic testing methods appear to be fairly standardized and, owing to Mendelian randomization (i.e., the random assortment of alleles during meiosis), observational studies of genetic tests are less susceptible to confounding bias than observational studies of environmental exposures. As such, future research may provide useful information on the predictive value of CYP2C19 variants. In contrast, phenotypic testing with platelet reactivity can be done using a number of different assays and principles of measurement. Identifying the optimal assay, agonist concentration, and

reactivity cutoff values for predicting clinical events is a significant challenge for future research. This challenge needs to be addressed in order to prioritize which reactivity assays should be evaluated as methods for guiding treatment choice.

Limitations of the Evidence

On the basis of the large number of reviewed studies, we believe that the evidence regarding genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity (for guiding antiplatelet treatment and predicting outcomes in patients who receive it) is limited in the following ways.

- *Lack of comparative studies of alternative testing strategies evaluating the relative predictive ability of the tests of interest.* No studies reported valid direct comparisons between the tests of interest in terms of predictive performance; studies either assessed a single test or simply provided information on test performance separately for each test applied to the study population.
- *Lack of separation of development ("training") and assessment ("test") samples when developing prognostic markers.* Many studies of phenotypic tests used receiver-operating-characteristic methods to define an "optimal" cutoff value of reactivity (typically, one that maximized the sum of sensitivity and specificity), without using any methods to limit overfitting.[236] In most cases these cutoffs were then applied to the same population sample that was used to derive them, in order to calculate predictive performance. This approach leads to overly optimistic estimates of test performance (i.e., inflated sensitivity and specificity) that are unlikely to generalize to the target population.[237]
- *Paucity of studies evaluating the impact of test-guided treatment selection on the basis of the tests of interest.* The literature we reviewed included few studies directly comparing alternative testing strategies or assessing effect modification by test results. Available studies were small, had short followup periods, or did not report information on clinical outcomes. The four large studies reporting information on treatment effect modification by CYP2C19 status based on RCTs of antiplatelet regimens were conducted in heterogeneous populations and compared different treatment regimens. No studies used quantitative methods to assess the intermediate outcomes of impact of testing on predictive probabilities or treatment decisions.
- *Heterogeneity in exposure definitions.* Our ability to synthesize findings across studies was limited by the use of heterogeneous methods to define exposure status. This was particularly true for studies of platelet reactivity assays because of the large variability in measurement methods (existence of multiple assays) and in cutoff values for defining positive results (among studies using the same assay). Heterogeneity of exposure definitions was also present in studies of CYP2C19 variants, because studies genotyped different subsets of known CYP2C19 alleles (e.g., loss-of-function alleles other than *2 were not commonly genotyped). Furthermore, data were reported in a way that only allowed for analyses under a dominant genetic model (carriers vs. noncarriers), which may not be the true genetic model.
- *Potential for selective outcome reporting.* There was high likelihood that several of the outcomes we reviewed were selectively reported. For example, for both types of tests, studies reporting on MACE often did not report information on the individual component

outcomes, leading to a much smaller number of studies contributing data to analyses of noncomposite outcomes.

Ongoing Research

A search on May 3, 2012, in the ClinicalTrials.gov registry identified 173 potentially relevant records. After full text review, 28 records of studies that can be expected to provide information relevant to Key Questions 3 and 4 of this report were identified. **Appendix G** summarizes information from these studies. None of these studies provided results in the ClinicalTrials.gov database; however, two of the studies have recently published results in peer reviewed journals and we anticipate including them in the update of the present report.

Evidence Gaps

Table 38 summarizes the evidence gaps with regards to the five Key Questions of this systematic review.

Table 38: Evidence gaps

Key Question	Category	Evidence Gap
1. Analytic validity and predictive value of genetic testing for CYP2C19 variants	Population	• Evidence on the analytic validity of testing in the populations relevant to this report was limited; however, it appears that the analytic validity of genetic testing assays in common use is considered adequate by the research community. • Studies of the predictive value of CYP2C19 variants were almost exclusively limited to patients with ischemic heart disease. Additional evidence is needed on the predictive value of tests in other patient populations who are candidates for antiplatelet therapy.
	Intervention and exposure definition	• Additional studies comprehensively genotyping CYP2C19 variants (i.e,. genotyping all variants prevalent in the population of interest) and reporting on long-term clinical outcomes are needed. • Combinations of types of tests (e.g., genetic and phenotypic testing) or combinations of genetic tests were rarely assessed.
	Comparator	• No studies reporting valid direct comparisons of the predictive value of different tests were available.
	Outcome	• Many studies assessed platelet reactivity (i.e., an intermediate outcome of unclear clinical significance) exclusively. • Studies reporting clinical outcomes often reported only composite outcomes (without providing information on other component outcomes separately).
2. Analytic validity and predictive value of phenotypic testing for platelet reactivity	Population	• Evidence on the analytic validity of testing for populations other than those with ischemic heart disease was limited. • Evidence on the predictive value of phenotypic testing for populations other than those with ischemic heart was limited.
	Intervention and exposure definition	• No single assay for measuring platelet reactivity can be considered a “gold standard” test. • Combinations of tests (e.g., genetic and phenotypic testing) or combinations of genetic tests were rarely assessed.
	Comparator	• No studies reporting valid direct comparisons the predictive value of different tests were available.
	Outcome	• Studies reporting clinical outcomes often reported only composite outcomes (without providing information on the component outcomes separately).
3. Use of testing to guide treatment choice	General evidence gap	• Directly comparative studies of alternative test strategies using phenotypic tests were few and had short followup durations and heterogeneous designs. • No studies comparing genetic testing for CYP2C19 variants versus alternative testing strategies were available. • Studies of effect modification were few, pertained to heterogeneous populations, reported information on intermediate outcomes only, or compared different antiplatelet treatments.
4. Harms of testing or test-directed	General evidence gap	• Few studies provided direct comparative information on the impact of alternative test-and-treat strategies on patient safety.

Key Question	Category	Evidence Gap
treatment		• No studies on the harms of testing *per se* were identified. • More evidence is needed regarding monitoring strategies that involve repeat testing that may lead to delays in invasive interventional procedures.

Future Research

This review has identified substantial gaps in the literature on genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity, both as biomarkers of future outcomes among patients who are receiving clopidogrel therapy and—more importantly—as tests for guiding treatment selection for patients who are candidates for antiplatelet treatment. We believe that the following evidence gaps may represent fruitful areas for future research (i.e., that they represent areas in need of future research):

- *Analytic validity of phenotypic testing:* Future studies using rigorous methods to inform the analytic validity of tests for measuring platelet reactivity are needed, particularly with regard to test–retest reliability, interassay agreement, and analytic performance. Ideally studies should evaluate multiple assays applied to the same samples, enroll a large number of patients, and be conducted in a multilaboratory setting. Despite the small number of available studies, we do not believe that studies of the analytic validity of conventional genotyping assays for CYP2C19 alleles should be considered a research priority, since (for various reasons discussed above) the existing assays are considered robust. However, studies of predictive effects or clinical utility of CYP2C19 assays should report details of their genotyping quality control methods.[238]
- *Predictive accuracy, with a focus on comparative predictive performance:* Although we identified several studies reporting on the predictive value of the tests of interest, studies had several limitations in their design and analysis methods (see the Limitations of the Evidence Base section above). Thus, large-scale prospective studies of the tests of interest are needed to derive reliable estimates of predictive performance. Because the major source of costs for conducting such studies stems from the need to follow up with large numbers of individuals over time, it may be worthwhile to perform measurements of on-clopidogrel platelet reactivity at baseline using multiple assays as well as obtain genetic material for genotyping CYP2C19 variants or other emerging genetic biomarkers. Such a design would provide information on multiple tests simultaneously; for conducting informative direct comparisons between assays, however, such studies would have to be large. This is because the results of different reactivity assays are correlated (although interassay agreement is not perfect, correlations higher than 0.5 were common in the reviewed studies). Studies of CYP2C19 variants need to assess all polymorphisms with adequate prevalence in the populations of interest; considerations should also be given to genotyping other genes involved in clopidogrel metabolism (e.g., ABCB1) and the assessment of gene–gene interactions. Studies will need to predefine cutoff values and genotype groupings and compare the classification performance of individual tests, as well as the reclassification performance of each assay compared to established clinical risk scores or results of other assays.[239-244]
- *Direct comparisons of methods for test-guided treatment selection:* Even if the predictive value of tests were established, this information is often inadequate as a basis for

treatment decisionmaking. Once the predictive performance of the tests of interest is established, the most promising tests could be prioritized for assessment in directly comparative studies of testing versus no testing. Although costly and time-consuming, such studies can provide unconfounded estimates of the relative benefits and harms of the compared strategies.[245] In addition, there are several alternative randomized designs that can be used to assess the clinical impact of medical tests on clinical outcomes (randomization with respect to testing or treatment).[246] For example, one design that may be particularly appealing for the tests of interest to this review is the use of a test in all patients who are candidates for antiplatelet treatment, followed by randomization of only test-"positive patients to receive one of several different treatments. This design can provide information on the relative harms and benefits of a treatment among patients whose treatment would be expected to change on the basis of test results. A third comparator arm of standard treatment in the test-negative patients can be included as an additional (nonrandomized) "control" group (such an approach was taken by the randomized GRAVITAS trial considered under Key Questions 2 and 3 of this report). A more detailed discussion of efficient biomarker study designs and the conditions under which they can lead to increases in statistical power are out of the scope of this report but have been discussed extensively elsewhere (e.g., Scher et al. 2011,[247] Simon 2010[248]).

- *"Repurposing" completed randomized trials to assess effect modification:* An approach to assessing effect modification by genotype status is to repurpose already completed randomized trials, in which the drugs of interest were tested against a suitable comparator, by genotyping tissue samples from enrollees. Results of genetic analyses in archival samples can be associated with the prospectively recorded clinical outcomes.[32,33] When samples are available from all participants in randomized controlled trials (or a random sample thereof), associations between genotype status with treatment outcomes are unconfounded. Provided that statistical corrections for repeated testing are used (if more than one association is tested) and that associations of interest are prespecified (to avoid selective reporting), such studies can support valid inference on treatment effect heterogeneity across genotype groups.[33] Such analyses are probably less applicable to phenotypic testing because of the nature of the measurements needed.
- *Using monitoring of platelet reactivity to guide treatment:* Strategies of monitoring platelet reactivity can be conceptualized as "dynamic treatment regimes"[249-251] (i.e., rules for sequential decisionmaking based on the evolution of reactivity measurements over time; obviously such methods are not applicable to CYP2C19 testing). Such approaches formalize the process of choosing between competing monitoring strategies, based on expected responses to treatment and related intermediate and long-term outcomes, using appropriate statistical models. Compared to standard research methods (e.g., directly comparing two monitoring strategies in a parallel-group study), dynamic treatment modeling may be better at identifying the optimal monitoring regime while accounting for multiple monitoring visits and the fact that treatment decisions at each visit are determined by the measurements (e.g., platelet reactivity, other laboratory measurements, etc.) obtained in previous visits. Indeed, statistical methods exist that can use observational or randomized study data to determine the factors that should be considered as triggers for intervention, as well as the optimal cutoff values of these factors. These approaches may increase the efficiency of future research because it is impossible to

conduct randomized trials comparing all alternative test-and-treat strategies for different assays, thresholds of reactivity, and alternative therapeutic interventions.

Conclusions

In summary, we found limited evidence on the analytic validity of genetic testing for platelet reactivity. However, using evidence from other populations and genetic variants, we believe that the available assays for CYP2C19 genotyping have adequate technical test performance. In contrast, we found a large body of evidence on the analytic validity of assays for measuring platelet reactivity, suggesting that interassay agreement is poor to moderate. No phenotypic assays can be considered a "gold standard" test.

We found some evidence supporting a significant association between loss-of-function CYP2C19 variants and increased risk of stent thrombosis and cardiovascular mortality. We also found a significant association between gain-of-function alleles and reduced risk of MACE. The interpretation of these associations should be cautious, given the potential for selective reporting and small-study effects to have affected study results. Furthermore, the applicability of findings to patient populations other than those with ischemic coronary artery disease (particularly those undergoing revascularization procedures) was limited. We also found evidence supporting an association between high on-clopidogrel platelet reactivity as measured by various assays (particularly LTA, VerifyNow P2Y12, and the VASP assay) and adverse cardiovascular events. Our confidence in these findings is limited by the relatively small number of studies available for each test–outcome combination, the potential for selective outcome reporting, and the common lack of separation between the populations used to derive test thresholds of optimal predictive value and those used to assess predictive value at these thresholds.

Regarding the use of genetic testing for CYP2C19 variants to guide treatment choice, we reviewed four large randomized trials reporting information on effect modification on clinical outcomes by CYP2C19 alleles and several smaller trials mostly reporting on platelet reactivity as the outcome of interest. All but one study did not find evidence of statistically significant effect modification by genotype status. Given the heterogeneity of patient populations, treatments, and outcomes assessed, as well as the lack of studies directly comparing alternative testing strategies, we believe there is insufficient evidence regarding the use of CYP2C19 genotyping for guiding treatment choice.

Finally, regarding the use of platelet reactivity measurement to for guiding antiplatelet treatment choice, we found five randomized studies directly comparing alternative test and treat strategies, one study assessing effect modification by baseline on-clopidogrel reactivity, and three randomized trials of antiplatelet therapy using platelet reactivity to select patients with high reactivity. Taken together, these studies provided insufficient evidence for the utility of platelet reactivity testing to guide antiplatelet therapy because short follow-up periods and low numbers of outcome events resulted in imprecise and often implausibly extreme estimates of benefit.

Additional research is needed to better establish the predictive value and clinical utility for treatment decisionmaking, both for genetic testing for CYP2C19 variants and phenotypic testing for platelet reactivity, focusing on standardizing testing methods and assessing the relative impact of testing strategies on patient-relevant clinical outcomes in large, well-conducted clinical trials.

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Acronyms

ACTIVE A	Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A trial
ADP	Adenosine diphosphate
AHRQ	Agency for Healthcare Research and Quality
CABG	Coronary artery bypass graft
CI	Confidence interval
CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Events trial
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EPC	Evidence-based Practice Center
FDA	Food and Drug Administration
GRAVITAS trial	Gauging Responsiveness with a VerifyNow Assay—Impact on Thrombosis and Safety trial
GUSTO criteria	Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries criteria
LTA	Light-transmission aggregometry
MACE	Major adverse cardiovascular events
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
PFA	Platelet Function Analyzer
PLATO trial	PLATelet inhibition and patient Outcomes trial
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies 2
RFLP	Restriction fragment length polymorphism
RR	Relative risk
TEP	Technical Expert Panel
TOO	Task Order Officer
TRITON-TIMI 38 trial	Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis In Myocardial Infarction 38
VASP	Vasodilator-stimulated phosphoprotein