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Clinical non-effectiveness of clopidogrel use for peripheral artery disease in patients with CYP2C19 polymorphisms: A systematic review

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Abstract

PURPOSE: To conduct a systematic review to identify studies that assessed the association between CYP2C19 polymorphisms and clinical outcomes in Peripheral artery disease (PAD) patients who took clopidogrel.

METHODS: We systematically searched Ovid EMBASE, PubMed, and Web of Science from November 1997 (inception) to September 2020. We included observational studies evaluating how CYP2C19 polymorphism is associated with clopidogrel's effectiveness and safety among patients with PAD. We extracted relevant information details from eligible studies (e.g., study type, patient population, study outcomes). We used the Risk of Bias in Non-randomised Studies-of Interventions (ROBINS-I) Tool to assess the risk of bias for included observational studies.

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STATEMENTS AND DECLARATIONS

FINANCIAL INTERESTS: The authors declare they have no financial interests.

NON-FINANCIAL INTERESTS: The authors declare they have no non-financial interests.

RESULTS: The outcomes of interest were the effectiveness and safety of clopidogrel. The effectiveness outcomes included clinical ineffectiveness (e.g., restenosis). The safety outcomes included bleeding and death related to the use of clopidogrel. We identified four observational studies with a sample size ranging from 50 to 278. Outcomes and comparison groups of the studies varied. Three studies (75%) had an overall low risk of bias. All included studies demonstrated that carrying CYP2C19 loss of function (LOF) alleles was significantly associated with reduced clinical effectiveness and safety of clopidogrel.

CONCLUSIONS: Our systematic review showed an association between CYP2C19 LOF alleles and reduced functions of clopidogrel. The use of CYP2C19 testing in PAD patients prescribed clopidogrel may help improve the clinical outcomes. However, based on the limited evidence, there is a need for randomized clinical trials in PAD patients to test both the effectiveness and safety outcomes of clopidogrel.

Keywords

Clopidogrel; CYP2C19; peripheral artery disease; effectiveness and safety; restenosis; occlusion

INTRODUCTION

Peripheral artery disease (PAD) affects up to 20% of individuals worldwide.[1] PAD commonly impacts the lower extremities by narrowing vessels that carry blood from the heart to the distal extremities, caused by atherosclerosis, a buildup of fatty plaque in the arteries.[2] PAD can be found in any vessel, but it is more common in lower extremities than upper extremities.[3] Approximately 6.5 million people aged ≥40 years suffered from this disease in the United States (US).[3] Individuals with PAD have a reduced life expectancy by up to 10 years as compared to the general population.

The current American Heart Association / American College of Cardiology (AHA/ACC) guidelines recommend antiplatelet therapy consisting of aspirin (range 75–325mg per day) or clopidogrel alone (75 mg per day) for risk reduction of myocardial infarction, stroke, and vascular death in symptomatic PAD patients.[4] The AHA/ACC guidelines also suggest antiplatelet therapy as a reasonable treatment option to reduce the risk of myocardial infarction, stroke, or vascular death for asymptomatic patients with PAD (i.e., ankle-brachial index ≤ 0.90).[4] Although alternative agents including prasugrel and ticagrelor are available for treating PAD,[5] clopidogrel remains the most commonly used antiplatelet, with over 20 million prescriptions per year.[6]

Clopidogrel is a thienopyridine prodrug that requires hepatic biotransformation to form an active metabolite. Only 15% of the clopidogrel prodrug will be transformed to an active agent. The conversion of clopidogrel to its active metabolite requires two sequential oxidative steps involving several cytochrome P450 (CYP) enzymes, primarily CYP2C19. Therefore, the effectiveness of clopidogrel can be influenced among individuals who are poor and intermediate metabolizers of CYP2C19. In March 2010, the US Food and Drug Administration (FDA) issued a boxed warning that CYP2C19 polymorphism may diminish the clopidogrel's effectiveness in patients with cardiovascular diseases (CVD).[7] There has been an increasing interest in providing pharmacogenetic testing on CYP2C19 to tailor

and personalize clopidogrel therapy in order to improve patient clinical outcomes.[8] The need for pharmacogenomic testing was a central component of the new clopidogrel boxed warning.[9]

Despite the interests, there is a lack of clear recommendations on CYP2C19 genetic testing for clopidogrel use. Existing major guidelines list common CYP2C19 alleles and their clinical relevance, or provide recommendations when pharmacogenomics results are available but none of them explicitly recommend specific CYP2C19 variant alleles testing.[10–12] Most importantly, there is a lack of pharmacogenomic recommendations for PAD population because the associations between CYP2C19 polymorphism and clinical outcomes among clopidogrel users with PAD are not well studied. The objective of this study, therefore, was to systematically review current evidence and evaluate the association between CYP2C19 polymorphisms and effectiveness and safety outcomes in PAD patients who used clopidogrel.

METHODS

Data Sources and Search strategy

This systematic review complied with the internationally accepted gold standard guidelines for systematic reviews as stated in the Cochrane Handbook for Systematic Reviews of Interventions and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13, 14] The study protocol was registered with PROSPERO (i.e., International Prospective Register of Systematic Reviews) website (Registration ID: CRD42020203278). We systematically searched databases including Ovid EMBASE, PubMed, and Web of Science from their inception to September 2020. The search strategy combined database-specific controlled vocabulary truncated and phrase-searched keywords in titles and abstracts as available: (“clopidogrel” OR “plavix”) AND “peripheral vascular disease” AND “Cytochrome P-450 CYP2C19” AND (“pharmacogenetics” OR “loss of function mutation”) AND (“randomized controlled trial” OR “Observational Study”) (Appendix 1).

The intervention or exposure of this study is CYP2C19 genetic variants. The outcomes of interest were the effectiveness and safety of clopidogrel. Effectiveness outcomes included clinical ineffectiveness such as clinical nonresponses, amputation events, restenosis or occlusion, ischemic events, and target limb reintervention. Safety outcomes included bleeding and death related to the use of clopidogrel.

Eligibility criteria

In this systematic review, we restricted our search to randomized clinical trials and prospective or retrospective observational human studies written in English. We included studies focusing on patients with PAD using clopidogrel with any available scientific, generic, or brand name. Eligible studies should assess the effect or association of CYP2C19 polymorphisms with PAD outcomes, and the outcome of the study can be bleeding, clinical ineffectiveness, or death. We excluded case reports, letters to the editor, reviews,

commentaries, editorials, as well as animal or in vitro studies. We also excluded abstracts from conferences with insufficient information about the research are available.

Data extraction and synthesis

After a comprehensive literature search and removal of duplicates, four reviewers (SH, SY, RY, and SL) double screened the articles' titles and abstracts and independently screened for inclusion and exclusion eligibility based on the full text. We used Covidence to assign articles to reviewers and manage the progress of the systematic review.[15] We extracted the study information and details using a standardized data collection sheet (Appendix 2). We summarized key study information from each article, including author names, study year, country/region, study type, patient population (e.g., sample size, conditions, treatments, clinical sites), measuring period, interventions, study outcomes, statistical methods, risk factors, and risk estimates (e.g., point estimate of hazard ratio [HR] or odds ratios [OR], 95% confidence intervals [95%CI], and p-values). We calculated the OR and HR based on the results reported in each included study. Next, four reviewers (SH, SY, RY, and SL) independently assessed the risk of bias of each article using the Risk of Bias in Non-randomized Studies - of Interventions (ROBINS-I) tool and the fifth reviewer (KN) aggregated the results and assigned the overall score.[16] ROBINS-I tool is designed specifically to assess the risk of bias in studies of interventions that did not use randomization to allocate units. Appendix 3 lists bias evaluation from our reviewers using 7 categories from the ROBINS-I tool. Any discrepancies on the relevance between reviewers were consulted with the fifth reviewer (KN). Figure 1 reveals the PRISMA flowchart detailing the selection process.

Risk of bias assessment

We used the ROBINS-I tool to assess the risk of bias for included observational studies.[16] We compared the Newcastle-Ottawa Scale and ROBINS-I and determined the ROBINS-I tool was most appropriate because it has the strength to evaluate the risk of bias in non-randomized studies.[16, 17] The ROBINS-I tool is designed based on the Cochrane Risk of Bias (Cochrane RoB) tool, which is the most frequently used bias assessment tool for randomized control trials (RCT) in systematic reviews.[18] It assesses each result for a specific outcome across seven bias domains: bias due to confounding, bias in selection of participants into the study, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, bias in selection of the reported results.[19] Each domain has a comprehensive list of well documented questions to help reviewers determine the effects of potential confounders. ROBINS-I tool also has separate questions for cohort and case-control studies.

RESULTS

Study Selection and Characteristics

A total of 597 articles were identified by the electronic database search. After removing duplicates, irrelevant articles were screened by abstract review. We retrieved all 104 (17.4%) full-text articles that were neither duplicates nor irrelevant studies. Of these, four (3.8%) met the inclusion criteria for this systematic review. Reasons for exclusion are shown in Figure 1.

The top three reasons for exclusion were: wrong patient population (n=65), wrong outcomes (n=16), and no full text (n=11).

The characteristics of the final four included studies are presented in Table 1. In addition, Figure 2 provides forest plots of outcomes measured. All studies were non-randomized, including two prospective cohort studies,[20, 21] and two retrospective cohort studies.[22, 23] Among the four studies, three evaluated the association between CYP2C19 polymorphisms and clinical ineffectiveness,[20–22] and one focused on the antiplatelet responsiveness to clopidogrel treatment and clinical outcomes.[23] None of the studies reported bleeding as an outcome. The included studies were conducted in China,[20] Greece,[21] Spain,[22] and Taiwan.[23]

Study Summary

Diaz-Villamarin et al. studied the single and combined effect of three genotypes (ABCB1 3435C>T, CYP2C19*2, and CYP2C19*3) on clopidogrel response in 72 patients with peripheral artery disease following percutaneous transluminal angioplasty (PTA) receiving clopidogrel 75 mg for at least three months. Baseline characteristics (e.g. cardiovascular history, smokers, number of treated regions) were balanced between different genotype groups except for low-molecular-weight heparin (LMWH) and β-blockers. The study found that carriers of CYP2C19 loss of function (LOF) alleles and/or ABCB1 TT were at increased risk of restenosis or occlusion of previously treated lesions (adjusted OR: 7.04; 95%CI: 1.80–27.46; p=0.005) (Table 1 and Figure 2). The study concluded that the CYP2C19*2 and ABCB1 TT genotypes were independent determinants of atherothrombotic ischemic events in PAD patients following PTA and treated with clopidogrel.

Guo et al. assessed the association between CYP2C19 genotype and the development of ischemic events (i.e., in-stent restenosis or occlusion) among patients with arteriosclerosis obliterans in the superficial femoral artery receiving DAPT with clopidogrel 75 mg at least five days before endovascular therapy (EVT). Patients without LOF alleles and patients with one or more LOF alleles were compared in the study. Baseline clinical characteristics regarding presentation, lesion severity, and stent use were similar in the two groups. Of the 50 study subjects, 26 (52%) patients had one or more LOF alleles. The study found that LOF allele carriers had an increased risk of ischemic events compared with non-carriers. The percentage of patients with ischemic events was 20.8% in those without LOF alleles, 59.0% in carriers of one LOF allele, and 100% in carriers of two LOF alleles. In addition, the authors measured platelet function using thromboelastography (TEG) platelet mapping and classified the study subjects into high platelet reactivity (HPR) group (ADP- induced inhibition ≤30%) and normal on-treatment platelet reactivity (NPR) group (ADP-induced inhibition >30%). Among the 50 study participants, the authors identified 11 (22.0%) patients in the HPR group. Patients without LOF alleles had a greater platelet inhibition in response to clopidogrel compared to patients with LOF alleles. Furthermore, ischemic event rates at 1-year follow-up were significantly higher in patients with HPR than patients with NPR (log-rank test p=0.012).

Pastromas et al. examined the association between target limb reintervention (TLR) and platelet responsiveness among patients with intermittent claudication (IC) or critical limb

ischemia (CLI) receiving daily treatment of 75 mg clopidogrel after peripheral percutaneous infrainguinal angioplasty (PTA) or stenting. The authors assessed platelet responsiveness using the VerifyNow P2Y12 assay after at least three months of clopidogrel therapy. They defined patients with residual platelet reactivity units (PRUs) ≥ 235 as non-responders (54.0%) and others as responders (46.0%). In this study, confounding by indication was minimized by excluding patients with any hypercoagulation disorders. Of the 113 study subjects, 58.4% were patients with CLI. Non-responders were more likely to have baseline diabetes (68.9% vs. 42.3%, p=0.007), chronic renal failure (21.3% vs. 7.7%, p=0.03), and CLI (70.5% vs. 44.2%, p=0.007). The study found that TLR-free survival rates at 7-year follow-up were 20.7% in responders vs. 1.9% in non-responders, with a significant difference between groups (log-rank test p=0.001). Resistance to clopidogrel was identified as an independent predictor for poor TLR-free survival in a multivariable Cox proportional hazards regression model. As a secondary outcome, amputation-free survival rates at 7-year follow-up was not significantly associated with platelet responsiveness (98.3% in responders vs. 96.7% in non-responders, log-rank test p=0.56)

Lee et al. investigated the association between clinical outcomes indicating ineffectiveness of clopidogrel treatment and CYP2C19 genotype among CLI patients with Rutherford classifications V and VI taking clopidogrel monotherapy after EVT. In this study, patients treated with other antiplatelet agents or anticoagulants at the time of EVT were excluded to avoid confounding bias. The study subjects were classified into three groups by the number of CYP2C19 LOF alleles: 1) extensive metabolizer (EM; no LOF), 2) intermediate metabolizer (IM; one LOF), and 3) poor metabolizer (PM; two LOFs). Among the total of 278 CLI patients, 55.0%, 28.4%, and 16.6% were identified as EM, IM, and PM, respectively. There were no significant differences in baseline demographic and clinical characteristics across groups. The study demonstrated that CYP2C19 genotypes were significantly associated with amputation and all-cause mortality in patients with CLI taking clopidogrel after EVT. Amputation-free survival rates at 1-year follow-up were 82.1% in EM, 66.1% in IM, and 56.6% in PM (log-rank test p=0.0006), and survival rates at 1-year follow-up were 83.7% in EM, 72.2% in IM, and 71.3% in PM (log-rank test p=0.01). The authors identified hemodialysis and the number of LOF alleles as independent predictors for both amputation and all-cause mortality. In this study, platelet aggregation was also measured using the VerifyNow P2Y12 assay. They demonstrated that the EM group had the greatest platelet inhibition after clopidogrel initiation with a mean PRU of 174.6, whereas the PM group had the lowest platelet inhibition with a mean PRU of 245.7.

Bias assessment

The overall quality of the four included studies was high according to the ROBINS-I assessment tool. The bias assessment results are summarized in Table 2.

Diaz-Villamarin et al. received a moderate risk in the domain of bias due to confounding and low risk in all other six domains. The overall risk of bias for this article was low. The authors adjusted clinical variables in the multivariable logistic regression and reported adjusted OR. However, the small sample size from one clinical site could have potential selection bias. In addition, among the 72 patients in the study, the length of clopidogrel treatment varied, with

19 (26.4%) patients treated for six months or less. Current guidelines recommend 12 months of clopidogrel treatment after percutaneous transluminal angioplasty.[24] The duration of clopidogrel treatment may impact the effectiveness; therefore, it should be adjusted for in the regression model.

Guo et al. received a moderate risk in two domains (bias due to missing data and bias in selection of the reported result) and a low risk in all other five domains. The overall risk of bias for this article was moderate. The main concern regarding this study was the high loss to follow-up rate (32.4%), resulting in a reduced power. The authors excluded the 24 patients with missing values in the data analysis which may introduce selection bias.

Pastromas et al. received a low risk in all seven domains. Therefore, the overall risk of bias for this article was low. This study investigated a relatively small number of patients using a single-center observational design, which may lead to inherent bias.

Lee et al. received a moderate risk in the domain of bias due to confounding and a low risk in all other six domains. The overall risk of bias for this article was low. The study included a large sample size from a single clinical site which limits the external validity. Misclassifications could occur due to the controversial methods and timing of platelet function testing.

DISCUSSION

This study is a systematic review that focuses on pharmacogenomics for PAD. Specifically, we evaluated the association between CYP2C19 LOF alleles and adverse events or ineffectiveness in individuals with PAD. Many prior systematic reviews have assessed the effect of CYP2C19 function on clopidogrel effectiveness in CVD. [25, 26] However, this study highlights the importance of CYP2C19 function in PAD, a condition that has garnered significantly less attention. As a result of our rigorous review process, we were able to identify four relevant observational cohort studies. Our analysis revealed a consistent effect of CYP2C19 on clopidogrel effectiveness in PAD patients, suggesting a need to assess recommendations regarding the use of CYP2C19 pharmacogenomic testing in PAD patients prior to prescribing clopidogrel.

All four studies found a higher risk of reduced clinical effectiveness in patients with a CYP2C19 LOF allele. Patients who have LOF alleles for CYP2C19 (*2, *3), especially those with both LOF alleles (also known as poor metabolizers) are at higher risk of low concentrations of the active metabolite of clopidogrel and clinical ineffectiveness.[27] These results suggest the need for CYP2C19 genetic results to evaluate the risk of ineffective clopidogrel use in PAD patients.

In addition to clinical ineffectiveness, bleeding risk is a serious side effects of clopidogrel use. This side effect had been frequently reported in the literature. A recent study by Nguyen et al. for patients with CVD identified two definite risk factors for major bleeding and four risk factors for any bleeding in clopidogrel use.[28] This study also identified CYP2C19 LOF as potential risk factor, but aggregated results showed that CYP2C19 LOF carrier was not a risk factor for either major bleeding or any bleeding (OR = 1.14, 95% CI 0.73–1.80

and OR= 0.65, 95% CI 0.33–1.30, respectively.) In PAD population, all four studies included in this review only focused on the clinical ineffectiveness from LOF alleles. None of these studies evaluated increased function allele (*17), especially in patients with two increased function alleles (*17/*17.) While *17 allele is associated with higher concentration of active metabolites [29, 30], there are fewer studies on its affect to bleeding risk. Li's evaluation of 782 CAD patients from 4 studies showed that the risk of bleeding was 23% increase when compared CYP2C19*17 variants with wild type (OR = 1.25, 95% CI 1.07–1.47.). [31–34] Currently, either FDA label or guidelines have any recommendation regarding bleeding risks with CYP2C19*17 alleles [7, 35], especially in PAD patients. Overall, additional clinical studies are needed to evaluate the bleeding risk of clopidogrel in PAD population and CYP2C19*17 risk alleles.

This systematic review supports the finding that CYP2C19 polymorphisms may affect clinical outcomes in subjects with PAD. Pharmacogenomic testing would allow healthcare providers and others to employ genotype-guided approaches to make clinical decisions. CYP2C19 testing has been implemented across many healthcare settings worldwide for clopidogrel use in CAD patients [36–39], the current data included in this review suggests an opportunity to expand the utilization of CYP2C19 testing results to the PAD population. [40, 41] Genetic testing has the potential to add clinical value. [42–44] In addition, Klarin et al. study provided genetic evidence that therapies targeting specific risk factors could mitigate the rising incidence of PAD.[45] Although the costs of genetic testing vary by laboratory and health system, many health insurance plans currently cover CYP2C19 testing.[46] There are still ethical, and discrimination concerns regarding genetic testing; however, with the development of laws and medical guidelines, patients and health care providers are more and more perceptive to genetic testing given its high accuracy and fast speed to receive the test results.[47]

Preemptive genetic testing for CYP2C19 may help clinicians decide on clopidogrel therapy, alternative antiplatelet agents, or DAPT. In CAD patients, alternatives to replace clopidogrel include prasugrel and ticagrelor.[48] However, these two alternatives (prasugrel and ticagrelor) are not recommended for PAD treatment according to the AHA/ACC guideline. [49] Current recommend treatment include either aspirin alone or clopidogrel alone for PAD patients with symptomatic. Only in patients with both PAD and CAD, ticagrelor can be used concomitant with aspirin to reduce major adverse cardiac events. Alternatives to replace clopidogrel, therefore, are limited in patients with PAD compared to those with CAD. Electronic clinical decision support tools should be developed to assist clinicians with medication choice in patients with CYP2C19 polymorphism. Such tools can help educate clinicians on risks and benefits of clopidogrel use and provide appropriate alternatives to optimize treatment decisions.

Despite the findings, this study had several limitations. We were not able to pool all data due to differences in outcomes, measurement, and stratified cohorts. For instance, Lee et al. stratified the study cohort by metabolism phenotypes (IM, PM, and NM) for clopidogrel treatments, while the other three studies conducted genotype analysis (e.g., CYP2C19 with vs. without LOF alleles). In addition, the study outcomes varied in the four included articles. The primary outcome of Diaz-Villamarin et al. and Guo et al.'s study was restenosis

or occlusion of the treated lesions; the study of Pastromas et al. focused on target limb reintervention free survival time; and Lee et al. measured the risks of amputation and death. Finally, patients included in each study were treated with clopidogrel and followed for different lengths of time which may also affect the study outcomes. Given those challenges and the small sample size we obtained, it was extremely hard to calculate a pooled OR with interpretable clinical meanings. Therefore, we analyzed each study separately.

CONCLUSION

Based on our findings, there is a need for additional RCTs in PAD patients to test the effect of pharmacogenomic testing on effectiveness and safety outcomes. Current evidence demonstrates an association of carrying CYP2C19 LOF alleles with reduced clinical effectiveness and safety of clopidogrel in patients with PAD. We recommend that investigators and clinical practitioners expand the use of CYP2C19 testing beyond CAD patients to include individuals with PAD to improve the effectiveness of clopidogrel therapy in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY:

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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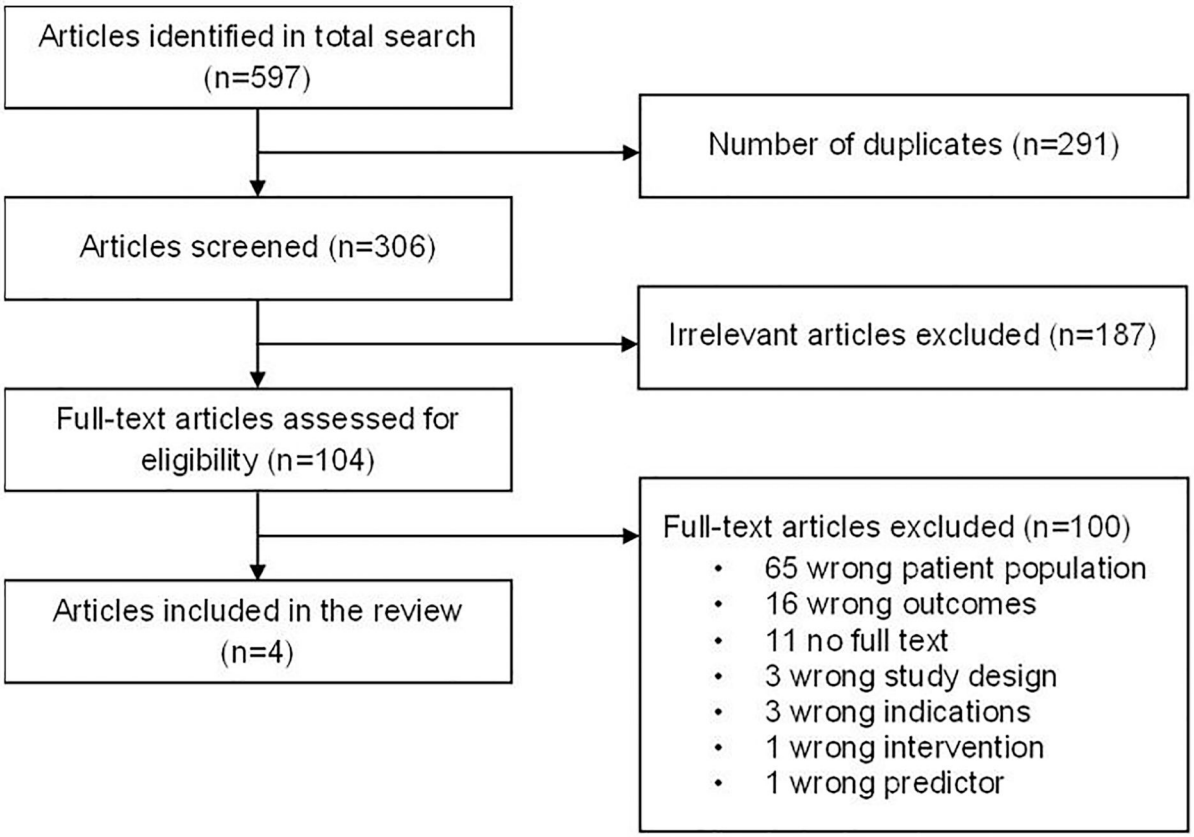

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flowchart of the Systematic Review

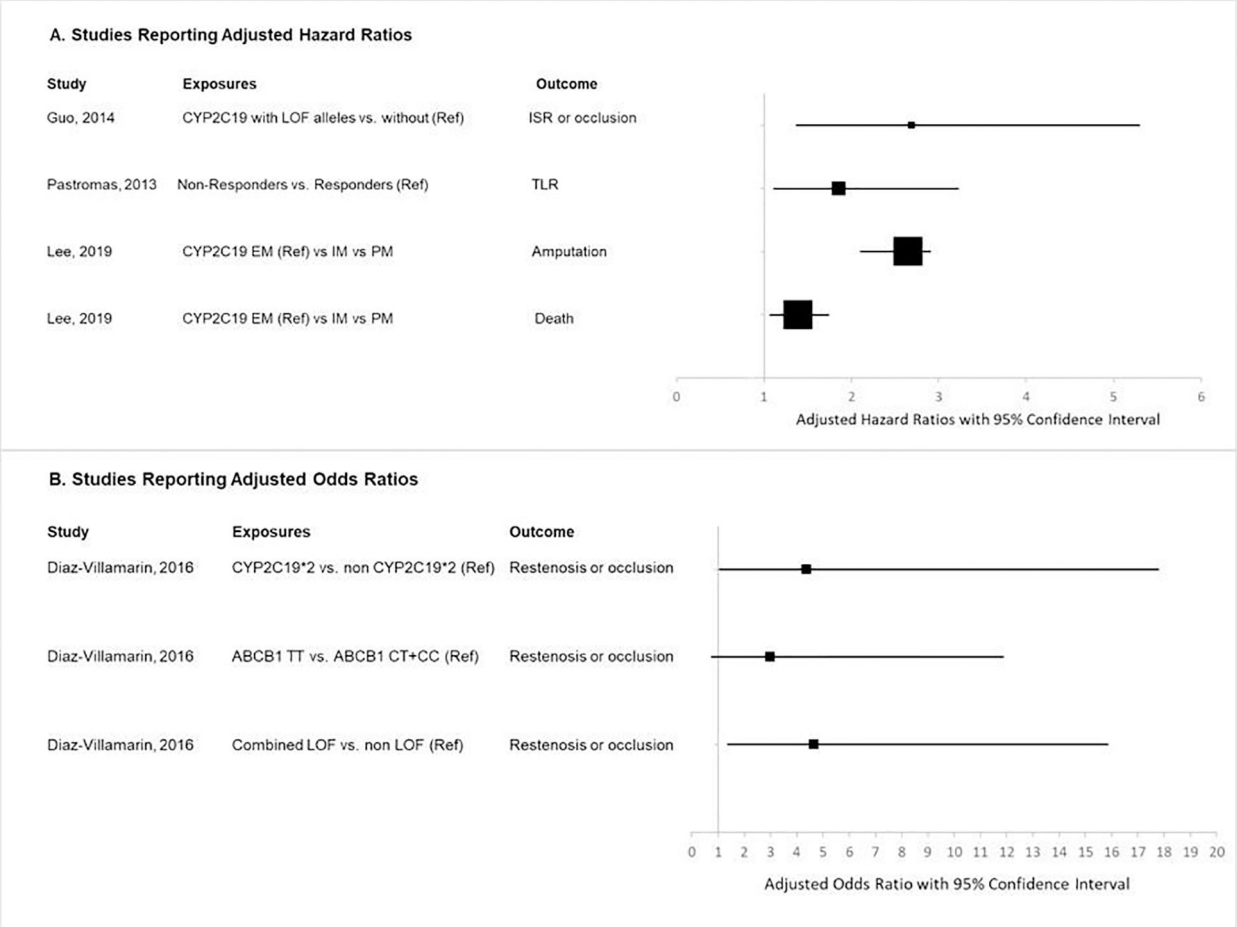

Figure 2. Forest Plots of Included Studies

Abbreviations: **ISR:** in-stent restenosis, **Ref:** reference group, **TLR:** target limb reintervention

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Table 1:

Summary of Included Studies

Author, publication year, country	Study type	Patient population	Sample size	Outcome(s)	Statistical method	Comparison groups	Crude event proportion (# Events by group)	Risk estimate (95% CI), p-value
Diaz-Villamarin, 2016, Spain	Retrospective cohort	PAD pts following PTA treated with clopidogrel 75 mg/day at least 3 months	72	restenosis or occlusion	Multivariable Logistic regression	CYP2C19*2 vs. non CYP2C19*2 (Ref)	14/25 vs. 11/25	aOR: 4.37 (1.07–17.77), p=0.039
						ABCB1 TT vs. ABCB1 CT+CC (Ref)	18/25 vs. 7/25	aOR: 2.98 (0.75–11.86), p=0.12
						Combined LOF vs. non LOF (Ref)	17/25 vs. 8/25	aOR: 4.66 (1.37–15.84), p=0.014
Guo, 2014, China	Prospective cohort	Patients with arteriosclerosis obliterans in the superficial femoral artery receiving dual antiplatelet therapy with clopidogrel 75 mg/day at least 5 days before EVT	50	ISR or occlusion	Multivariable Cox regression	CYP2C19 with LOF alleles vs. without LOF alleles (Ref)	15/26 vs. 4/24	aHR: 2.69 (1.37–5.29), p=0.004
Pastromas, 2013, Greece	Prospective cohort	Patients with IC or CLI receiving dual antiplatelet therapy with clopidogrel 75 mg/day after PTA or stenting	113	TLR	Multivariable Cox regression	Non-Responders vs. Responders (Ref) *	52/73 vs. 21/66	aHR: 1.86 (1.11–3.22), p=0.01
Lee, 2019, Taiwan	Retrospective cohort	Patients with CLI taking clopidogrel after EVT	278	Amputation	Multivariable Cox regression	IM vs. PM vs. CYP2C19 EM (Ref)	24/79 vs. 20/46 vs. 28/153	aHR: 2.65 (2.1–2.9), p=0.009
				Death			18/79 vs. 14/46 vs. 25/153	aHR: 1.39 (1.07–1.74), p=0.037

Abbreviations: **CI:** confidence interval, **CLI:** critical limb ischemia, **EM:** extensive metabolizer, **EVT:** endovascular therapy, **aHR:** adjusted hazard ratio, **IC:** intermittent claudication, **IM:** intermediate metabolizer, **ISR:** in-stent restenosis, **LOF:** loss of function, **aOR:** adjusted odds ratio, **PAD:** peripheral artery disease**, PM:** poor metabolizer, **PTA:** percutaneous transluminal angioplasty, **Ref:** reference group, **TLR:** target limb reintervention

*Converted

Table 2.

Risk of Bias in Non-randomised Studies-of Interventions (ROBINS-I) Bias Assessment Results

Author, year	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	ROBINS-I overall
Diaz-Villamarin, 2016	Moderate	Low	Low	Low	Low	Low	Low	Low
Guo, 2014	Low	Low	Low	Low	Moderate	Low	Moderate	Moderate
Pastromas, 2013	Low	Low	Low	Low	Low	Low	Low	Low
Lee, 2019	Moderate	Low	Low	Low	Low	Low	Low	Low