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Collette, Sabine L; Bokkers, Reinoud P H; Dierckx, Rudi A J O; van der Laan, Maarten J; Zeebregts, Clark J; Uyttenboogaart, Maarten

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# Clinical importance of testing for clopidogrel resistance in patients undergoing carotid artery stenting—a systematic review

Sabine L. Collette<sup>1</sup>, Reinoud P. H. Bokkers<sup>1</sup>, Rudi A. J. O. Dierckx<sup>1,2</sup>, Maarten J. van der Laan<sup>3</sup>, Clark J. Zeebregts<sup>3</sup>, Maarten Uyttenboogaart<sup>1,4</sup>

<sup>1</sup>Department of Radiology, Medical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;

<sup>2</sup>Department of Nuclear Medicine and Molecular Imaging, Medical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; <sup>3</sup>Division of Vascular Surgery, Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; <sup>4</sup>Department of Neurology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

*Contributions:* (I) Conception and design: All authors; (II) Administrative support: SL Collette; (III) Provision of study materials or patients: SL Collette, M Uyttenboogaart; (IV) Collection and assembly of data: SL Collette; (V) Data analysis and interpretation: SL Collette, M Uyttenboogaart, RPH Bokkers; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Sabine L. Collette, MD. University Medical Center Groningen, Huispostcode AB51, Hanzeplein 1, Postbus 30001, 9700 RB Groningen, the Netherlands. Email: s.l.collette@umcg.nl.

**Abstract:** Dual antiplatelet therapy is frequently prescribed for patients undergoing carotid artery stenting (CAS), however clopidogrel resistance might cause thromboembolic complications. The role of testing for clopidogrel resistance in patients undergoing CAS is unclear. In this study, we aimed to review the periprocedural thromboembolic outcomes in clopidogrel resistant patients who underwent CAS. We conducted a review of PubMed, EMBASE, and the Cochrane Library up to October 7, 2020. Studies were included that investigated at least ten patients aged 18 years or older with a symptomatic carotid artery stenosis requiring CAS. Studies were excluded that investigated patients with a carotid artery dissection, case reports, case series of less than ten patients, reviews, commentaries, letters to the editors, and conference abstracts. The primary endpoint was the incidence of thromboembolic events. One hundred seventy-seven unique articles were identified of which three studies were included in our systematic review. The sample sizes ranged from 76 to 449 patients and the follow-up duration from 24 hours to 2 years postprocedural. Two retrospective observational studies determined clopidogrel resistance using measurement of P2Y<sub>12</sub> reaction units, and one historical cohort study used genetic testing. Two studies concluded that clopidogrel resistance was a risk factor for thromboembolic complications, the other found higher values of P2Y<sub>12</sub> reaction units in patients with thromboembolic events compared to those without. In conclusion, current literature supports a possible relationship between clopidogrel resistance and thromboembolic complications in patients who underwent CAS. Preprocedural testing for clopidogrel resistance might therefore be of additional value. Randomized studies using a valid, reliable clopidogrel resistance test and clinical endpoints, are however required to make a definitive statement and to determine the impact of the thromboembolic complications. This study was registered within PROSPERO (CRD42020197318).

**Keywords:** Carotid artery stenting (CAS); clopidogrel resistance; CYP2C19; pharmacogenetics; thromboembolic outcomes

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## Introduction

Carotid artery stenting (CAS) is a widely used treatment method for patients with a symptomatic or asymptomatic carotid artery stenosis (1). In the peri- and postprocedural phase, patients are at increased risk for thromboembolic complications, such as in-stent thrombosis or recurrent stroke (2-5). To reduce the risk of such events, treatment with clopidogrel and aspirin is frequently prescribed from approximately three days prior to CAS until at least thirty days postprocedural (1). Nevertheless, the intended treatment effect of this dual antiplatelet therapy is not always achieved despite patient adherence. This might be due to clopidogrel resistance (6,7).

Clopidogrel is a pro-drug that is converted to an active metabolite in the liver by cytochrome P450 enzymes, in which CYP2C19 plays an important role (8,9). The active metabolite binds irreversibly to the P2Y12 receptor and consequently inhibits the binding of adenosine diphosphate. This leads to a decrease in platelet activation, platelet aggregation, and clot formation (9). In clopidogrel resistance, a relevant change in platelet function is absent despite adequate clopidogrel dosage. This is mostly caused by a CYP2C19 polymorphism (10,11). From a pharmacological point of view, clopidogrel resistance can be delineated with a P2Y12 reaction units (PRU) value  $\geq 235$  or percentage platelet inhibition  $\leq 20\%$  (7,12). Clinically, it can be observed by the development of thromboembolic complications despite clopidogrel treatment (13).

Associations between clopidogrel resistance and thromboembolic complications have been found repeatedly in patients who underwent a percutaneous coronary intervention and received clopidogrel (14). The role of clopidogrel resistance testing in patients undergoing CAS is however unclear (1). We therefore aimed to review the periprocedural thromboembolic outcomes in clopidogrel resistant patients who underwent CAS.

We present the following article in accordance with the PRISMA reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-7153>).

## Methods

### *Search strategy*

To identify studies investigating the relationship between clopidogrel resistance and thromboembolic complications during and after CAS, we conducted a review of PubMed, EMBASE, and the Cochrane Library up to October 7,

2020. Our search string consisted of the following, fixed, structure: ‘carotid artery stenting’ AND ‘clopidogrel’ AND (‘clopidogrel resistance’ OR ‘platelet function tests’). The search was completed with relevant synonyms (details can be found online in the [Appendix 1](#)).

The articles found by the search string were screened for eligibility by reading the title and abstract. If an article was considered potentially relevant, the full text version was assessed. Additional relevant publications were identified in two ways. First, for each article found on PubMed, the first 40 similar articles were screened for relevance after they were filtered on ‘best match’. Second, references of the articles that were screened in whole were reviewed.

The selection of studies was performed independently by two authors (SC and MU). The electronic searches, identification of additional publications, and removal of duplicates was executed by one author (SC). Discrepancies were resolved by discussion.

The identification and removal of duplicates were performed using Endnote X9 software (Clarivate Analytics, Philadelphia, Pennsylvania, USA). Studies were screened using Covidence software (Veritas Health Innovation, Melbourne, Australia; available at [www.covidence.org](http://www.covidence.org)). This study is registered within PROSPERO (CRD42020197318).

### *Study selection*

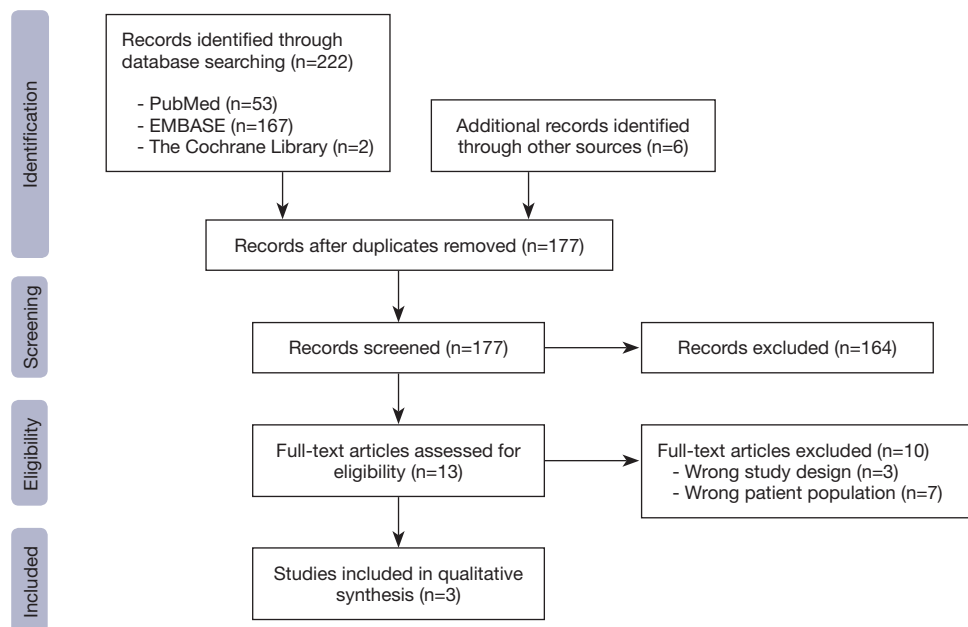
Studies were included that investigated at least ten patients aged 18 years or older with a symptomatic carotid artery stenosis requiring CAS. Excluded were studies investigating patients with a carotid artery dissection, case reports, case series of less than ten patients, reviews, commentaries, letters to the editors, and conference abstracts.

### *Data collection*

After a study was included, the following data was collected: first author, year of publication, country of study, study design, sample size, mean age, comparison, type of clopidogrel resistance test, threshold used for the clopidogrel resistance test, number of clopidogrel resistant patients, duration of follow-up, primary endpoint, and number of patients with the primary endpoint.

### *Quality assessment*

The risk of bias was determined using the ‘Risk Of Bias In Non-randomized Studies of Interventions’ (ROBINS-I)



**Figure 1** Flowchart of included studies.

tool. The assessment took place across seven domains, followed by an overall judgment (low, moderate, serious or critical risk of bias, or no information). A study with a low risk of bias was considered to be comparable to a randomized trial, and a study with a critical risk of bias was too questionable to include in the systematic review (15).

The strengths of the research methods and results were determined using ‘Methodological Index for Non-Randomized Studies’ (MINORS). This tool consists of twelve items that are scored with 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate) points. A total score of 16 points was considered ideal for non-comparative studies, whereas a total score of 24 points was considered ideal for comparative studies (16).

Two authors (SC and MU) independently assessed the quality of the studies. Disagreement was solved by discussion.

### Outcomes

The primary endpoint was the incidence of thromboembolic events.

## Results

### Search results

The search yielded 53 articles from PubMed, 167 articles

from EMBASE, 2 articles from the Cochrane Library, and 6 articles through other sources. Following the removal of duplicates, 177 unique articles remained. One hundred sixty-four were excluded during screening for title and abstract, and 10 during the full text review. This left 3 articles to be included for our systematic review (Figure 1).

### Quality assessment

All studies were included in the systematic review as their risk of bias was not considered to be critical (Table 1). In the assessment of methodological quality, none of the studies received the maximum MINORS score. For the non-comparative study (18), the MINORS score was 8. The items that were not adequately reported, and consequently received a score of 0 points, include a prospective collection of the data, unbiased assessment of the study endpoints, loss to follow up less than 5%, and prospective calculation of the study size. For the comparative studies, the MINORS scores were 17 (17) and 18 (19). The items of the weakest reporting were almost consistent with those of the non-comparative study. The main difference was the sufficient documentation of the loss to follow-up in the comparative studies (Table 2).

### Characteristics

Two of the eligible studies were retrospective observational

**Table 1** 'Risk Of Bias In Non-randomized Studies of Interventions' (ROBINS-I) assessment of the included studies

Study	Baseline confounding	Selection of participants	Classification of interventions	Deviation from intended interventions	Missing data	Measurement of outcomes	Selection of the reported result	Overall
Song <i>et al.</i> (17) [2013]	Moderate	Moderate	Low	Low	Low	Low	Low	Moderate
Sorkin <i>et al.</i> (18) [2014]	Serious	Moderate	Moderate	Low	Moderate	Low	Serious	Serious
Zhu <i>et al.</i> (19) [2016]	Moderate	Moderate	Low	Low	Moderate	Low	Moderate	Moderate

Critical, critical risk of bias; low, low risk of bias; moderate, moderate risk of bias; serious, serious risk of bias.

**Table 2** 'Methodological Index for Non-Randomized Studies' (MINORS) scores of the included studies

Items	Song <i>et al.</i> (18) [2013]	Sorkin <i>et al.</i> (17) [2014]	Zhu <i>et al.</i> (19) [2016]
1. A clearly stated aim	2	2	2
2. Inclusion of consecutive patients	2	2	2
3. Prospective collection of data	0	0	0
4. Endpoints appropriate to the aim of the study	2	2	2
5. Unbiased assessment of the study endpoints	0	0	0
6. Follow-up period appropriate to the aim of the study	1	2	2
7. Loss to follow-up less than 5%	2	0	2
8. Prospective calculation of the study size	0	0	0
<i>Additional criteria for comparative studies</i>			
9. An adequate control group	2		2
10. Contemporary groups	2		2
11. Baseline equivalence of groups	2		2
12. Adequate statistical analyses	2		2
Total MINORS score [maximum possible score]	17 [24]	8 [16]	18 [24]

Each item is scored with 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate) points. The total score that was considered ideal was 16 points for non-comparative studies and 24 points for comparative studies.

studies (17,18) and one was a historical cohort study (19). They were performed in South Korea (17), United States of America (18), and China (19). Sample sizes ranged from 76 to 449 patients. Overall, the studies aimed at investigating the relationship of clopidogrel resistance or PRU values with thromboembolic outcomes after CAS. The primary endpoints included new cerebral ischemic lesions observed on brain magnetic resonance imaging (MRI) (17), (ipsilateral) ischemic stroke, (ipsilateral) transient ischemic attack (18,19), previous ischemic symptom recurrence, stent thrombosis, and death (19) (Table 3).

### ***Clopidogrel resistance testing and outcome***

Tests that were used to determine clopidogrel resistance varied among the included studies. Measurement of PRU values was used in the studies of Song *et al.* (17) and Sorkin *et al.* (18); genetic testing was performed in the article of Zhu *et al.* (19).

### ***Measurement of PRU values***

PRU values can be measured with a point-of-care assay

**Table 3** Study characteristics, methods and results

Study [year]	Study design	Sample size	Mean age (years)	Comparison	Type of clopidogrel resistance test, threshold	Number (%) of clopidogrel resistant patients	Duration of follow-up	Number (%) of patients with primary endpoint
Song <i>et al.</i> (17) [2013]	Retrospective observational study	76	68.1 (SD, 7.7)	PRU $\geq$ 240 versus PRU $<$ 240	PRU measurement, PRU $\geq$ 240	50 (65.8)	24 hours	New lesions on brain MRI PRU $\geq$ 240: 37 (74.0); PRU $<$ 240: 8 (30.8)
Sorkin <i>et al.</i> (18) [2014]	Retrospective observational study	449	70 (range, 27–94)	Not applicable	PRU measurement, PRU $\geq$ 238	Unknown	30 days, 1 year, and 2 years	Ipsilateral ischemic events 30 days: 11 (2.4); 1 year: 22 (4.9); 2 years: 24 (5.3)
Zhu <i>et al.</i> (19) [2016]	Historical cohort study	241	64.3 (SD, 9.3)	CYP2C19*2 and *3 versus *1/*1	Genetic analysis	152 (63.1)	1 year	Ischemic events *2 and *3: 27 (17.8); *1/*1: 7 (7.9)

MRI, magnetic resonance imaging; PRU, P2Y12 reaction units; SD, standard deviation.

able to rapidly test the platelet activity of patients who are treated with clopidogrel. To perform the test, a small amount of whole-blood is required. In the test device, the blood sample is mixed with adenosine diphosphate and prostaglandin E1 to induce platelet aggregation. During the process of platelet aggregation, an amount of light transmitted through the sample is measured. If platelet aggregation is not adequately inhibited by clopidogrel, the sample will be clearer which results in a higher absorbance of light. The amount of platelet aggregation mediated by the P2Y12 receptor is expressed as PRU, with high PRU values indicating more reactive platelets (20,21). Although an optimal threshold is under debate (22–24), a PRU value  $\geq$ 235 or percentage platelet inhibition  $\leq$ 20% are generally considered clopidogrel resistant (7,12). In the studies of Song *et al.* (17) and Sorkin *et al.* (18), PRU values were determined using VerifyNow<sup>®</sup> (Accumetrics, San Diego, California, USA).

Song *et al.* (17) analyzed the relationship between clopidogrel resistance and new cerebral ischemic lesions detected on postprocedural brain MRI. A total of 50 out of the 76 (65.8%) included patients had an increased PRU value. At 24 hours after CAS, the images showed that 45 (59.2%) patients had newly developed cerebral ischemic lesions. Thirty-seven (82.2%) of the patients with new ischemic lesions were clopidogrel resistant. In multivariate analysis, clopidogrel resistance was found to be associated with new ischemic events (OR, 6.804; 95% CI, 2.225–20.806).

Sorkin *et al.* (18) investigated whether ipsilateral cerebral ischemic events after CAS were related to PRU values. During follow-up at one and two years after CAS, the mean PRU values were found to be significantly higher in patients with an ipsilateral ischemic event than in patients without [PRU value at one year: 252 and 202 (P=0.008), respectively; PRU at two years: 244 and 203 (P=0.047), respectively]. The ischemic free survival did not differ between patients under and above the initial PRU threshold of  $\geq$ 238. When it was compared with a lower threshold, the ischemic free survival was longer for patients with a PRU value  $\leq$ 198 than for patients  $\leq$ 237 (P=0.027).

### Genetic testing

Important loss-of-function alleles are CYP2C19\*2 and CYP2C19\*3. These allelic variants are associated with a reduced activity of the CYP2C19 enzyme, which keeps the amount of active clopidogrel metabolite low (11,25,26).

Therefore, the risk of thromboembolic complications persists. In the study of Zhu *et al.* (19), CYP2C19\*2 and CYP2C19\*3 polymorphisms were detected by analyzing the images of the hybrid DNA sections obtained through polymerase chain reaction. The genetic analysis was performed using a commercially available kit (BaiO Technology Co, Ltd., Shanghai, China).

Zhu *et al.* (19) aimed to determine the relationship between the loss-of-function alleles CYP2C19\*2 and \*3 and the clinical efficacy of clopidogrel. A total of 152 out of the 241 (63.1%) included patients had the CYP2C19\*2 and \*3 alleles. Ischemic events occurred in 27 (17.8%) carriers of these alleles. Compared with the non-carriers, patients with the CYP2C19\*2 and \*3 alleles were found to be at increased risk of ischemic complications (hazard ratio, 2.131; 95% CI, 1.067–4.255). No difference was observed between CYP2C19\*2 and CYP2C19\*3 carriers related to number of ischemic events. The risk of cumulative ischemic events was increased for patients with two CYP2C19\*2 or CYP2C19\*3 alleles compared to carriers of one allele (hazard ratio, 6.176; 95% CI, 1.145–33.32).

## Discussion

This systematic review included two retrospective observational studies and one historical cohort study, which investigated the relationship of clopidogrel resistance or PRU values with thromboembolic outcomes after CAS. Although different tests were used to diagnose clopidogrel resistance, two studies concluded that patients with clopidogrel resistance are more likely to experience thromboembolic events (18,19). The other study found higher PRU values in patients with ischemic events at one and two years of follow-up, and a longer ischemic free survival in patients with a PRU value under the threshold of  $\leq 198$  instead of the initial PRU threshold of  $\leq 237$  (18).

These results are in line with other neuro-interventional studies in different populations (7,27). In a 4.5-year prospective study among 96 patients who underwent extracranial or intracranial neurovascular stenting, clopidogrel resistance (defined as percentage platelet inhibition  $\leq 20\%$ , determined using VerifyNow<sup>®</sup>) was associated with periprocedural thromboembolic complications. In addition, patients were divided in two groups: one group consisted of 49 patients of whom 33% had a percentage platelet inhibition  $\leq 20\%$ , the other group consisted of 47 patients of whom 40% had a percentage platelet inhibition  $\leq 20\%$ . In the group of 47 patients,

additional clopidogrel was given to the clopidogrel resistant patients. This did however not result in a lower complication rate compared with the clopidogrel resistant patients whose dose of clopidogrel was not adjusted (7). A prospective study analyzed the mean percentage platelet inhibition (determined using VerifyNow<sup>®</sup>) of 5 patients with and 48 patients without thromboembolic complications within six months after neurovascular stenting. A threshold of  $<40\%$  was defined as clopidogrel resistance. The mean platelet inhibition percentages differed significantly among both groups [patients with complications, 23% (range, 21–27); without complications, 35% (range, 16–91);  $P=0.01$ ] (27).

The clopidogrel metabolism was investigated by measuring PRU values or genetic testing. The advantages of measuring PRU values are that it is easy to perform, patients can be rapidly tested at bedside, and blood samples do not need any preparation before testing. Disadvantages are the relatively high costs compared with other platelet function assays and the variability of test results within an individual. The advantages of genetic testing are the consistent test results as a patient its genotype does not change over time and that testing can take place before treatment with clopidogrel. Disadvantages are the process of testing takes time, qualified personnel is needed to prepare the blood samples, the high costs in comparison with PRU measurement, and the impossibility to test patient adherence (insufficient clopidogrel intake would lead to abnormal PRU values, while genetic testing would not show any abnormalities). In clinical practice, measurement of PRU values might be more convenient than genetic testing because it can be performed in a short amount of time by a variety of health care workers.

There is an ongoing debate regarding the optimal threshold for the PRU values (22–24). This is reflected by the different PRU values ( $\geq 240$ ,  $\geq 238$ , and  $\geq 199$ ) that were maintained in two of the included studies (17,18). The most commonly used PRU value to define clopidogrel resistance,  $\geq 235$ , could be questioned as it is based on a relatively small sample size. In addition, the possible individual variability of PRU values during clopidogrel treatment was not taken into account during the determination of this threshold (12,23,28). Among patients who underwent percutaneous coronary intervention, it was found that, even with a clopidogrel loading dose of 600 mg, PRU levels decreased during the first month of clopidogrel treatment. This result can be considered clinically relevant as the classification of the majority of patients with a PRU decrease changed from poor (PRU value  $\geq 235$ ) to full responder (PRU value

<235). The PRU values did not differ between the next five months of follow-up (23). As in the included studies, clopidogrel treatment is usually started within three to seven days before the CAS, or in some cases only one day in advance, and continued for, preferably, at least thirty days (1,17-19). For the measurement of PRU values, blood samples are mostly drawn once and within two days before or after the CAS (7,17,18,27). As PRU values can still lower within the first month of clopidogrel treatment, it could be hypothesized that treatment should start at least one month before CAS. Whether this early start of clopidogrel treatment outweighs the risk of bleeding in all types of responders and symptomatic patients should be investigated.

In addition to clopidogrel, patients of the included studies received aspirin from approximately seven days before the CAS until preferably thirty days postprocedural. As aspirin inhibits platelet activity through a different pathway than clopidogrel, it might have lowered the risk of thromboembolic complications (29). As consequence, a possible relationship of clopidogrel resistance or PRU values with thromboembolic complications could have been missed. The included studies showed however still significant results. Even if no significant results were found, the clinical implication could be questioned because dual antiplatelet therapy is prescribed to the majority of patients (1). Furthermore, two of the included studies also investigated the influence of aspirin resistance or assays and found no relationship with thromboembolic outcomes (17,18). Although it has been found that aspirin resistant patients do respond less to clopidogrel than aspirin sensitive patients, which was also observed by Song *et al.* (17), it does not apply the other way around (30).

Although the incidence of clopidogrel resistance could not be precisely determined due to the use of various tests and thresholds, it appears to be common in patients undergoing CAS [65.8% in the study of Song *et al.* (17) and 63.1% in the study of Zhu *et al.* (19)]. Follow-up research into optimization of antiplatelet treatment for this population seems therefore to be warranted. In an observational study amongst 77 patients, the percentage platelet inhibition was compared between patients who were treated with clopidogrel and cilostazol (15 patients) or clopidogrel alone (62 patients) for CAS, acute ischemic stroke or transient ischemic attack. Clopidogrel resistance (defined as percentage platelet inhibition <20%, determined using VerifyNow<sup>®</sup>) was found in none of the patients treated with clopidogrel and cilostazol, and in 18 out of the

62 (29%) patients treated with clopidogrel alone. Moreover, the percentage platelet inhibition was significantly higher in patients who received additionally cilostazol [cilostazol and clopidogrel: mean, 64.9%; standard deviation (SD), 22.7; clopidogrel only: mean, 41.7%; SD, 28.0, P=0.005]. This might indicate that the addition of cilostazol increased the percentage platelet inhibition, however, no definite conclusions could be drawn due to potential selection bias and inclusion of a limited number of cilostazol-treated patients (31). A similar effect of cilostazol was found in a prospective non-randomized study amongst 137 patients who underwent CAS. A significantly lower PRU value (determined using VerifyNow<sup>®</sup>) was detected in patients treated with cilostazol and clopidogrel than in patients treated with aspirin and clopidogrel (mean, 147.0; SD, 64.3, and mean, 235.4; SD 71.2, respectively). Moreover, the incidence of new ischemic ipsilateral lesions was significantly lower in patients who received cilostazol and the incidence of hemorrhagic events did not significantly differ between both groups. This might suggest that cilostazol is effective and safe, however, as for the previous study, the results might have been affected by selection bias due to the non-randomized design (32). Another treatment option that has been investigated is ticlopidine/ginkgo biloba. In a multicenter, randomized, controlled trial, clopidogrel resistant patients (defined as percentage platelet inhibition <20%, determined using VerifyNow<sup>®</sup>) who underwent CAS were randomized into the ticlopidine/ginkgo biloba arm or clopidogrel arm. During the first interim analysis amongst 42 patients (20 and 22 patients, respectively), it was decided to terminate the trial early due to an impractical recalculated sample size. At this stage, the incidence of new ischemic lesions at 24 hours after CAS did not differ between both groups, although treatment with ticlopidine/ginkgo biloba increased the PRU values significantly [difference, 21.0 (6.0–35.0) and 0.0 (–3.0–3.0), P<0.001] (33). Based on the aforementioned studies, a combination of cilostazol and clopidogrel might be promising in prevention of thromboembolic complications in clopidogrel resistant patients who undergo CAS. Randomized clinical trials are needed to further elaborate on the effectiveness and safety of cilostazol.

This systematic review has some limitations. First, only three studies were identified due to the limited availability of publications. In addition, the studies that were included used different tests for clopidogrel resistance and various thresholds. Although the results pointed in the same direction, no definitive statement could be made



about the possible relationship clopidogrel resistance and thromboembolic complications in patients who underwent CAS. Second, the included studies were (partly) retrospective in design, which makes them susceptible to baseline confounding and missing data. This may have affected the results in unpredictable ways. Third, the in- and exclusion criteria of all studies might have caused selection bias with the likely direction in favor of the patients without complications. Nevertheless, the studies identified clopidogrel resistance or higher PRU values as a risk factor for thromboembolic complications. In addition, two studies were performed among Asians, who are known to have the highest prevalence of clopidogrel resistance (34-36). This could have resulted in a stronger significance compared with similar studies in patients with a different racial background. Fourth, the outcome assessors of the included studies were not blinded to the intervention and outcomes. The risk of bias was however expected to be low because both test results and thromboembolic complications were objective measurements. Final, the impact of thromboembolic events could not be adequately determined as only one clinical endpoint (death) was examined.

## Conclusions

Current literature supports a possible relationship between clopidogrel resistance and thromboembolic complications in patients who underwent CAS. Preprocedural testing for clopidogrel resistance might therefore be of additional value. Randomized studies using a valid, reliable clopidogrel resistance test and clinical endpoints, are however required to make a definitive statement and to determine the impact of the thromboembolic complications.

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