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Citation
Goncalves, L. N., Velze, V. van, Klok, F. A., Gal, P., Vos, R. C., Hamming, J. F., & Bogt, K. E. A. van der. (2023). High on-treatment platelet reactivity in peripheral arterial disease: a systematic review. *Vascular*. doi:10.1177/17085381231214324

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Downloaded from:	https://hdl.handle.net/1887/3947627

Note: To cite this publication please use the final published version (if applicable).

Review Article

Vascular

Vascular
2023, Vol. 0(0) 1–14
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DOI: 10.1177/17085381231214324
journals.sagepub.com/home/vas
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High on-treatment platelet reactivity in peripheral arterial disease: A systematic review

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Abstract

Objectives: To highlight current evidence pertaining to the measurement methods and prevalence of high on-treatment platelet reactivity (HTPR) in patients with PAD, as well as to evaluate the relationship between HTPR and recurrent adverse cardiovascular and limb events in PAD patients.

Methods: A systematic review of English-language literature on HTPR in patients with PAD. An electronic literature search of PubMed and Medline was performed in May 2021.

Results: A total of 29 studies with a total number of 11,201 patients with PAD were identified. HTPR during clopidogrel treatment ranges from 9.8 to 77%, and during aspirin treatment ranges from 4.1 to 50% of PAD patients. HTPR was associated with adverse clinical outcomes. The need for limb revascularisation was higher in patients with HTPR during clopidogrel use. Similarly, HTPR during aspirin use in the PAD population was predictive of adverse cardiovascular events (HR 3.73; 95% CI, 1.43–9.81; p = .007). A wide range of techniques were applied to measure platelet resistance, without consensus on cut-off values. Furthermore, differing patient populations, a variety of antiplatelet regimens, and differing clinical endpoints highlight the high degree of heterogeneity in the studies included in this review.

Conclusion: No consensus on technique or cut-off values for HTPR testing has been reached. Patients with HTPR are potentially at a greater risk of adverse limb-related and cardiovascular events than patients sensitive to antiplatelet therapy illustrating the need for clinical implementation of HTPR testing. Future research must aim for consistent methodology. Adaptation of antiplatelet therapy based on HTPR results requires further exploration.

Keywords

Platelet resistance, high on-treatment platelet reactivity, clopidogrel, aspirin, CYP2C19, peripheral arterial disease

Introduction

Antiplatelet therapy plays an important role in the treatment of patients with atherosclerotic disease such as coronary artery disease (CAD), ischemic cerebrovascular disease (CVD), and peripheral arterial disease (PAD), assisting in the prevention of vascular-related morbidity and mortality.[1] The European Society of Vascular Surgery (ESVS) and the European Society of Cardiology (ESC) guidelines recommend clopidogrel, or aspirin as an alternative, as either monotherapy or dual antiplatelet therapy in patients with PAD.[1] Despite adequate adherence to antiplatelet therapy, a large proportion of patients develop thrombotic complications or progression of disease. One of the possible causes of the aforementioned adverse events is nonresponsiveness to antiplatelet therapy. Figure 1 demonstrates the proposed

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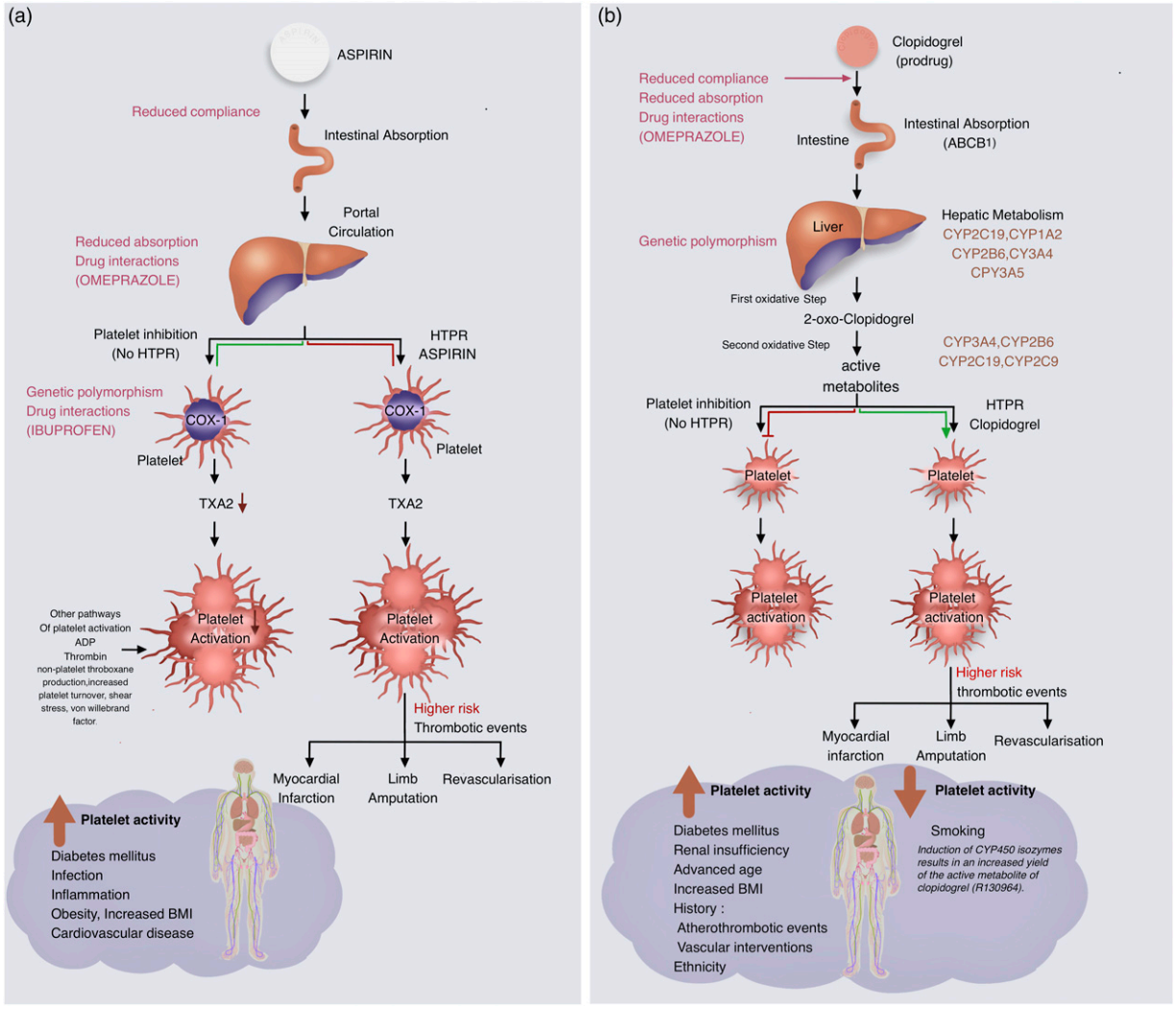

Figure 1. (a) Proposed effects of HTPR during aspirin therapy in PAD patients. HTPR during aspirin treatment is suspected to involve insufficient COX-1 inhibition and thus inhibition of platelet thromboxane A2. Other inhibition pathways independent of aspirin inhibition, and involving thrombin and ADP, for example, must also be considered. (b) Proposed effects of HTPR during clopidogrel therapy in PAD patients. The suspected mechanism for HTPR during clopidogrel treatment is variation in CYP2C19 activity. Patients who are homozygous for non-functional CYP2C19 alleles are unable to convert clopidogrel into its active metabolite and the efficacy of clopidogrel is thus markedly reduced.

effect of HTPR on clinical outcomes during aspirin and clopidogrel therapy.

The measurement of HTPR in clinical research is heterogenous in the selection of measurement technique and in the determined cut-off for HTPR. Light transmission aggregometry (LTA) is the gold standard for evaluation for platelet aggregation, but it is both a time-consuming and cumbersome process. Platelet function testing for clopidogrel and aspirin has been developed to include point-of-care testing and semi- and automated testing systems. Platelet function analysers such as VerifyNow, platelet function analyser 100 (PFA-100), vasodilator-stimulated phosphoprotein (VASP) phosphorylation, and flow cytometry are frequently implemented in clinical research.[2]

HTPR during clopidogrel treatment has been extensively explored in CAD and CVD patients, with evidence of increased risk of vascular events if present.[3–5] A recent meta-analysis reported a five-fold increased risk of cardiovascular morbidity and mortality in clopidogrel resistant CAD patients compared to responders.[6] Similarly, a systematic review and meta-analysis of 20 studies of CAD patients reported a four-fold increased risk of cardiovascular, cerebrovascular, and vascular events when resistant to aspirin.[7] While a growing body of research highlights the impact of HTPR in patients with CAD and CVD, this field has been explored to a lesser degree in patients with PAD.[8] PAD is frequently the initial presenting disease process in patients with CVD and requires long-term antiplatelet therapy,

highlighting the importance of extending HTPR research to PAD patients.

The aim of this systematic review is to examine current knowledge on the measurement of HTPR and the impact on both limb-related and cardiovascular outcomes in PAD patients, in order to suggest a feasible clinical application of this diagnostic tool.

Methods

Search strategy

A comprehensive electronic search was conducted using Pubmed and Medline from inception to May 2021 to identify relevant literature. The search strategy was designed and conducted by an expert reference librarian with input from the study's principal investigators (L.G. and K.B.). Controlled vocabulary supplemented with keywords was used to define the Medical Subject Headings (MeSH), and included 'peripheral arterial disease', 'resistance', 'platelet reactivity', 'high on treatment', 'clopidogrel', and 'aspirin'. The detailed search strategy applied can be found in the Supplementary Appendix. Articles were systematically screened according to the PRISMA guidelines with a two-stage method. Stage one included screening by title and abstract, followed by stage two with full-text screening. The systematic review of the literature and results conducted in this article are not part of a registered study.

Article selection

Studies were considered eligible for inclusion if they met the following criteria: (a) reported on participants with PAD, defined as patients with claudication symptoms or ankle-brachial index <0.9; (b) participants were receiving clopidogrel or aspirin therapy or both; and (c) antiplatelet resistance was evaluated. Studies were included regardless of study design, size, or length of follow-up. Only full-text articles in English were included. During abstract review, we excluded review articles, conference abstracts, and case-reports. Articles reporting experimentation in animal studies were also excluded. We excluded papers that did not contain a direct measure of platelet function. Titles and abstracts were screened by two reviewers (L.G. and V.V.) to identify potentially relevant articles. Discrepancies in judgment were resolved after discussion with a third referee (K.B.). When we found duplicate reports of the same study in preliminary abstracts and articles, we analysed data from the most complete data set.

Quality assessment

The quality and the risk of bias of the selected and included articles were independently evaluated by two reviewers (L.G. and V.V.) according to the revised Quality Assessment of Diagnostic Accuracy Studies.[9] In instances of discrepancy in interpretation, a third independent reviewer (KB) was asked to adjudicate.

Outcome measures

Outcomes of interest were the prevalence of clopidogrel resistance, aspirin resistance or dual therapy resistance. Platelet function analysis technique applies and specifically the cut-off values selected and the timing of testing were explored. Genetic testing of the CYP2C19 loss-of-function allele and the impact on clopidogrel effectiveness was studied. Clinical outcomes can be divided into mortality; major adverse cardiovascular events (MACE) such as myocardial infarction or stroke; and major adverse limb-related events (MALE) such as amputation, stent thrombosis, and target limb revascularisation.

Data extraction

Data were extracted by the main author using a Microsoft Excel 2016 spreadsheet designed for this review; this information included hospital location, study time frame and design, specific inclusion or exclusion criteria, type of antiplatelet therapy, high-on treatment testing apparatus, and stage of PAD. Quantitative data collected included total number of patients and antiplatelet dosage. A meta-analysis could not be performed due to the heterogenicity in study design and outcome measures of the studies included.

Results

Overview of studies

An overview of the article selection process for this systematic review is reported in a flow diagram in Figure 2, according to the PRISMA guidelines.[10] A total of 222 articles were found based on the search terms in Supplementary Appendix 1, of which 29 articles were selected for review. Table 1 presents study characteristics of the 29 selected publications. All studies were published during the period from 2009 to 2021. The results of the quality assessment are illustrated in Supplementary Appendix 2.

Patient characteristics

Twenty-four studies included patients exclusively with PAD, and 16 studies detailed the degree of PAD according to the Fontaine Classification, from intermittent claudication (II) to critical limb ischemia (IV). Five of the 29 studies included in this review assessed patients with PAD, CAD, or cerebrovascular disease; these studies did not subdivide the PAD population according to a PAD classification system.[11–15] Nineteen studies performed antiplatelet

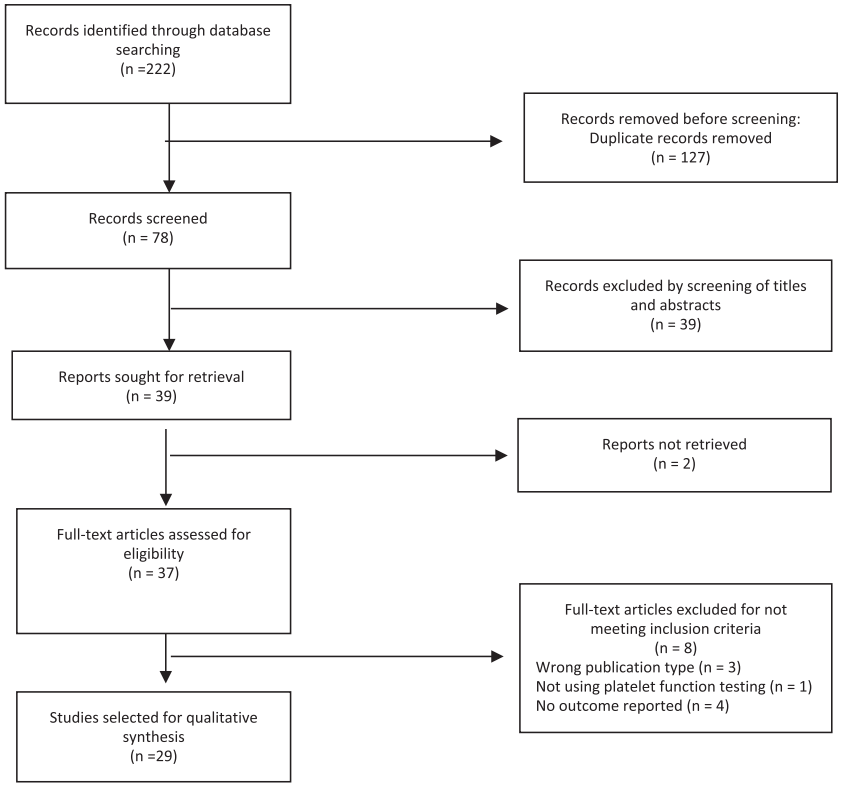

Figure 2. Preferred reporting items for systematic review and meta-analysis protocols flow chart for the selection of included studies.

resistance testing in patients receiving revascularisation procedures such as percutaneous transluminal angioplasty with- or without stenting, bypass operations, thrombectomy, thromboendoarterectomy, or percutaneous coronary intervention, as shown in Table 1.[12,15–32]

Antiplatelet therapy

Of the studies included five evaluated monotherapy with clopidogrel, four evaluated monotherapy with aspirin, 17 evaluated dual antiplatelet therapy, and four studies also included other antiplatelet regimes, as shown in Table 1. The clopidogrel dosage regime was 75 mg per day, sometimes preceded by a loading dose of 300–600 mg prior to intervention. Aspirin dosage regimes varied from 75 to 100 mg per day or a personalised dose of 162 or 325 mg, sometimes preceded by a loading dose of 300–500 mg. Three studies included patients receiving clopidogrel 75 mg once daily or aspirin 81–100 mg once daily at baseline, with a switch to ticagrelor 90–180 mg twice daily following antiplatelet resistance testing.[30,33,34]

Measurement of antiplatelet resistance

Table 2 highlights the various assays used to assess platelet resistance. The included studies selected LTA; whole blood flow cytometry or multiplate, vasodilator-stimulated protein phosphorylation assay (VASP), platelet function analyser 100 (PFA-100; PFA-200); platelet activation assay (PACT); VerifyNow; and thromboelastrography platelet mapping (TEG). The most frequently adopted platelet resistance assay was the VerifyNow system, mentioned in 16 of the 29 studies.[11–13,21–24,27,29,30,32–37] Various definitions of HTPR were described, and the timing of testing in relation to initiation of a (loading dose of) antiplatelet therapy or intervention differed per study (Table 2).

HTPR to clopidogrel was mentioned in 25 studies, as described in Table 2.[11–13,15–20,22–27,29–36,38,39] Clavijo et al. tested platelet resistance with the VerifyNow and VASP techniques.[33] The VerifyNow assay detected 36% of patients with HTPR before clopidogrel daily dose, and 30% HTPR 6 h after the clopidogrel dose. The VASP assay showed 44% HTPR and 42% HTPR in the aforementioned patients, respectively. Leunissen et al. tested platelet resistance with various techniques and cut-off values.[24] HTPR for clopidogrel, as assessed by the VerifyNow assay, was 43.3% at a platelet reaction unit (PRU) >235, 60% at a PRU >208 and 83.3% for >40% inhibition. Platelet resistance at baseline and after 17.5 months follow-up was studied by Linnemann et al., with a change in HTPR for clopidogrel from 35.2% at baseline to 17.6% at follow-up.[38] According to a receiver-operating characteristic curve analysis performed by Spiliopoulous et al., the optimal VerifyNow cut-off for the detection of a

Table 1. Characteristics of included studies.

Study	Design	Patient population	No of patients	Treatment tested	Fontaine classification	Intervention
Akinosoglou, 2018[11]	Cohort	PAD/CAD/ Stroke PAD	22 7	Clopidogrel 75 mg	-	N/A
Borowski, 2020[16]	Cohort	PAD	72	Clopidogrel 75 mg + ASA 75 mg; or ASA 75 mg	IIB, III, and IV	EVT
Busch, 2021[17]	Cohort	PAD	102	DAPT: 600 mg clopidogrel post-intervention, 75 mg clopidogrel/day; 100 mg ASA	IIB	EVT
Cassar, 2006[18]	Retrospective case-control	PAD	67	75 mg clopidogrel +75 mg ASA 30 days; or 300 mg clopidogrel loading dose +75 mg ASA	-	EVT
Clavijo, 2018[33]	Cohort	PAD	50	DAPT: clopidogrel 75 mg + ASA 81 mg; switch ticagrelor 90 mg 2dd	III and IV	N//A
Clavijo, 2018[35]	Cohort	PAD	100	DAPT: ASA + clopidogrel	III and IV	N/A
Dhillon, 2020[34]	Retrospective cohort	PAD	50	DAPT: clopidogrel 75 mg + ASA 81 mg; switch ticagrelor 90 mg 2dd	III and IV	N/A
Grifoni, 2018[19]	Cohort	PAD	177	DAPT: ASA 100-325 mg + P2Y12 inhibitor (clopidogrel, prasugrel, and ticagrelor)	I–IV	EVT
Guo, 2014[20]	Cohort	PAD	74	DAPT: clopidogrel 75 mg, ASA 100 mg	II, III, and IV	EVT
Gupta, 2017[12]	Cohort	PAD/CAD PAD	8582 876	DAPT: clopidogrel 600 mg, 300 mg, 75 mg; ASA 300 mg oral, 324 mg oral/250 mg intravenous	-	PCI
Hartinger, 2018[21]	Cohort	PAD	21	ASA 100 mg; or ASA + clopidogrel	-	Bypass, patch, EVT, and thrombectomy amputation
Hernandez-Suarez, 2018[36]	Cross-sectional	PAD	46	Clopidogrel 75 mg; ASA + clopidogrel; clopidogrel + cilostazol	-	N/A
Hernandez-Suarez, 2017[13]	Cross-sectional	PAD/CAD/ cerebral arterial disease/ stroke PAD	100 32	Clopidogrel 75 mg; Clopidogrel + ASA	-	N/A
Karnabatidis, 2014[22]	Cohort	PAD	145	ASA 100 mg; DAPT: ASA 100 mg + clopidogrel 75 mg	IIB, III, and IV	EVT
Khan, 2020[14]	Cohort	PAD/CAS PAD	64 52	Baseline ASA 81 mg; personalised dosage of 162 mg or 325 mg in resistant patients	-	N/A
Lee, 2019[23]	Retrospective cohort	PAD	278	Clopidogrel	III and IV	EVT
Leunissen, 2016[24]	Cohort	PAD	30	ASA; DAPT post-procedure ASA + clopidogrel LD 300 mg	IIa–IV	EVT

(continued)

Table 1. (continued)

Study	Design	Patient population	No of patients	Treatment tested	Fontaine classification	Intervention
Linnemann, 2009[25]	Cohort	PAD	98	ASA 100 mg; DAPT ASA 100 mg + clopidogrel 75 mg at FU	-	EVT, Bypass, TEA
Linnemann, 2010[26]	Cohort	PAD	40	Group 1: clopidogrel 75 mg Group 2: ASA 100 mg + clopidogrel 300 mg LD	-	EVT
Linnemann, 2010[38]	Cohort	PAD	54	Clopidogrel 75 mg	-	-
Madsen, 2011[39]	Cohort	PAD	267	Group 1: ASA Group 2: no treatment Group 3: clopidogrel Group 4: DAPT ASA + clopidogrel	I–III	-
Mazur, 2013[15]	Cohort	PAD/CAD PAD	37 26	Clopidogrel 75 mg +/− ASA 75 mg or 150 mg	-	EVT
Pasala, 2016[37]	Cohort	PAD	120	ASA ≥75 mg	-	-
Pastromas, 2013[27]	Cohort	PAD	113	Clopidogrel 75 mg; post-intervention clopidogrel 75 mg + ASA 100 mg	II, III, and IV	EVT
Saunders, 2011[28]	Cohort	PAD	80	Aspirin; or aspirin + clopidogrel	-	EVT
Spiliopoulos, 2013[29]	Cohort	PAD	100	DAPT with clopidogrel 75 mg; DAPT clopidogrel 75 mg + ASA 100 mg 6 months post-intervention	III and IV	EVT
Spiliopoulos, 2014[30]	Retrospective cohort	PAD	37	Clopidogrel 75 mg; switch ticagrelor 180/90 mg 2dd + ASA 100 mg for 6 months, thereafter ticagrelor indefinitely	III and IV	EVT
Tepe, 2012[31]	Prospective case-control	PAD	80	ASA + placebo; or ASA + clopidogrel. Clopidogrel 300 mg LD or 75 mg, ASA 500 mg LD or 100 mg	IIB, III, and IV	EVT
Yeo, 2018[32]	Cohort	PAD	195	Clopidogrel; or DAPT ASA + clopidogrel. If naïve LD clopidogrel 300-600 mg, LD ASA 300 mg	II-IV	Operation; EVT

ASA: aspirin; CAD: cardiovascular disease; DAPT: dual antiplatelet therapy; EVT: endovascular treatment; LD: loading dose; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention; TEA: thromboendarterectomy.

composite endpoint (death, major stroke, major amputation, target vessel revascularisation, and bypass) was a PRU >234, with a sensitivity of 92.1% and a specificity of 82.4%.[29]

Thirteen studies described HTPR to aspirin[12,14,16,17,19,21,22,25,28,32,35,37,39] Borowski et al. described changes in HTPR to aspirin pre- and post-percutaneous transluminal angioplasty (PTA), with the PFA-200 assay.[16] This study showed 37.8% HTPR pre-PTA and 46.7% resistance post-PTA. Changes in HTPR to aspirin were further explored by Saunders et al.[28] When adopting LTA, 18.8% of patients had HTPR to aspirin at baseline, with 13.8% of those showing a response at 6-month follow-up. Similarly, the PFA-100 technique identified 38% of patients with HTPR for aspirin at baseline, with only 9.9% of those patients remaining resistant at follow-up. Personalised aspirin dosage as a solution to platelet resistance was described by Khan et al.[14] Using LTA 14% (n = 9) of patients were identified as having HTPR to 81 mg of aspirin, with six patients showing an adequate response to 162 mg of aspirin, and three patients to 325 mg aspirin.

Dual HTPR to clopidogrel and aspirin was mentioned in six studies.[12,17,19,22,26,35] Dual resistance to anti-platelet therapy ranges from 4.1 to 20.9%. Gupta et al. explored the difference in dual resistance in CAD patients

Table 2. Description of the HTPR testing methods and outcomes of included studies in the present systematic review.

Study	HTPR test	Definition HTPR	Timing test	HTPR clopidogrel	HTPR ASA	HTPR clopidogrel + ASA	Results
Akinosoglou, 2018[11]	VerifyNow	PRU >208	Presentation; 28 days postrecovery	77% of septic patients; 29% of patients in recovery	-	-	-
Borowski, 2020[16]	PFA-200	-	Pre- and post-PTA	Post-PTA 73.7%	Pre-PTA 37.8%; post-PTA 46.7%	-	-
Busch, 2021[17]	VASP LTA	VASP: clopidogrel PRI >50% LTA: ASA MoA >20%	1-day post-PTA	36%	11%	11%	-
Cassar, 2006[18]	Whole blood flow cytometry	-	Baseline; 12 h after clopidogrel loading dose, day 30	9.8% at 12 h & 30 days	-	-	-
Clavijo, 2018[33]	Verify Now VASP	VerifyNow: PRU >208 VASP clopidogrel: PRI >50%	Before and 6 h after last clopidogrel/ ticagrelor dose	Verify Now: 36% before clopidogrel; 30% after clopidogrel; VASP 44% before clopidogrel; 42% after clopidogrel	-	-	-
Clavijo, 2018[35]	Verify Now VASP	VerifyNow: PRU >208 VASP clopidogrel: PRI >50%	After minimum 1-week DAPT	Verify Now: 33% VASP: 46%	Verify Now: 25%	HTPR to ASA or clopidogrel 35%; HTPR to both 8%	-
Dhillon, 2020[34]	Verify Now VASP	VerifyNow: PRU >208 VASP: -	Before (baseline) and 6 h after last clopidogrel (steady state); after 2 weeks ticagrelor	VerifyNow: Baseline: 36%; steadystate: 30%	-	-	-
Grifoni, 2018[19]	LTA	ASA: LTA AA >20% Clopidogrel: LTA ADP >70%	Within 24 h of PTA	32%	45%	20.9%	-
Guo, 2014[20]	TEG	Clopidogrel: ADP >70%	After >5 days DAPT	22%	-	-	-
Gupta, 2017[12]	VerifyNow	PRU >208; ARU >550; dual resistance PRU >208 + ARU >550	Clopidogrel: 600 mg > 6 h before testing; 300 mg > 12 h before testing; 75 mg > 5 days before testing. ASA: 300 mg > 6 h before PCI, 324 mg chewed/ 250 mg IV >30 min before PCI	45.4%	6.9%	4.1%	Subjects with PAD more likely to have dual resistance (4.1% with PAD versus 2.4% without) (p = .002)

(continued)

Table 2. (continued)

Study	HTPR test	Definition HTPR	Timing test	HTPR clopidogrel	HTPR ASA	HTPR clopidogrel + ASA	Results
Hartinger, 2018[21]	LTA Impedance aggregometry If discrepancy: PFA-200 VerifyNow	LTA: ARA >20%; EPI >44% Impedance aggregometry: <65% aggregation	>6 days ASA therapy; within 30 days after revascularisation	-	50%	-	-
Hernandez-Suarez, 2018[36]	VerifyNow	PRU >230	>7 consecutive days	39%	-	-	-
Hernandez-Suarez, 2017[13]	VerifyNow	PRU >230	>7 consecutive days	35%	-	-	-
Karnabatidis, 2014[22]	VerifyNow	ARU >550 PRU >234	>5 days continuous therapy	50.8%	20.7%	12.5%	
Khan, 2020[14]	LTA	MoA >20%	-		14%		67% of HTPR patients sensitive to 162 mg ASA 3 patients sensitive to 325 mg ASA
Lee, 2019[23]	VerifyNow	-	-	-	-	-	45% carriers of CYP2C19 polymorphism
Leunissen, 2016[24]	VerifyNow VASP PACT	VerifyNow: PRU <235, PRU <208, >40% inhibition VASP: PRI >50% PACT: % inhibition pre- + post-intervention	1–5 days after loading dose	VerifyNow: PRU >235: 43.3%; PRU >208: 60.0%; 40% inhibition: 83.3% PACT: 53.8%	-	-	VASP: PRI varied widely IQR 53.2-84.5
Linnemann, 2009[25]	LTA PFT-100	LTA: MoA >78% PFT-100: <192s	Baseline after 14 consecutive days ASA. FU 17 months	-	Baseline: LTA: 4.1% PFT-100: 12.2% FU: 7% change in responsiveness	-	-
Linnemann, 2010[26]	PFA-100 LTA	PFA-100: 87 s LTA: ADP 2 μM >42.9%; ADP 5 μM > 72.1%	Baseline; 3 weeks after intervention with >14 days clopidogrel	Group 1: PFA-100: 27.3% LTA: 27.3% with 2 μM; 18.2% with 5 μM Group 2: 11.1% on dual therapy	-	-	-
Linnemann, 2010[38]	LTA	LTA: ADP 2 μM > 42.9%	Baseline: >14 consecutive days, FU 17.5 months	Baseline: 35.2% FU: 17.6%	-	-	-
Madsen, 2011[39]	PFA-100 LTA Multiplate	PFA-100: <167 s LTA: AA >20%, ADP 5 > 50%, ADP 20 > 70%. Multiplate: AUCAA> 300, AUCADP >468	Baseline, 14 days, FU 3 months	LTA: ADP5 23.3% ADP 20 0% Multiplate: AUCADP >468 23.0%	PFA-100: 17% baseline + FU LTA: 4.9% baseline, 8.1% FU, none showed HTPR at both visits Multiplate: 6.1% baseline, 5.1% FU	-	-

(continued)

Table 2. (continued)

Study	HTPR test	Definition HTPR	Timing test	HTPR clopidogrel	HTPR ASA	HTPR clopidogrel + ASA	Results
Mazur, 2013[15]	LTA VASP	LTA: ADP 5 > 50%, ADP 20 > 59% VASP: PRI >50%	-	-	-	-	Platelet aggregation to ADP 5 was higher in PAD + stent thrombosis than in PAD (p = .0003) and CAD (p = .002)
Pasala, 2016[37]	VerifyNow	ARU >550	>4 weeks ASA. Blood samples on day of enrollment	-	25.8%	-	-
Pastromas, 2013[27]	VerifyNow	PRU >235	>3 months of clopidogrel intake, after the procedure	53.9%	-	-	-
Saunders, 2011[28]	PFA-100 LTA	LTA: AA >30%, ADP 10 > 70% PFA-100: <164 s	Within 2 h of sample correlation at baseline + FU 6 months	-	LTA: 18.8% baseline, 13.8% of those showed response to ASA at 6-month FU PFA-100: 38.0% baseline, of which 9.9% remained HTPR at FU	-	-
Spiliopoulos, 2013[29]	VerifyNow	-	>1 month DAPT; During admission, always before the procedure	-	-	-	ROC analysis: PRU ≥234 optimal cut-off. Sensitivity 92.1%, Specificity 84.2%
Spiliopoulos, 2014[30]	VerifyNow	PRU >234	>5 days clopidogrel 75 mg; Before procedure	51.9%	-	-	-
Tepe, 2012[31]	Multiplate	-	Directly before intervention	After LD of clopidogrel 300 mg 30%	-	-	-
Yeo, 2018[32]	VerifyNow	ARU >550 PRU >235	If naïve to clopidogrel test performed 5–7 h after LD	49%	17%	-	-

ARU: aspirin reaction units; ASA: aspirin; CAD: cardiovascular disease; FU: follow-up; HTPR: high on-treatment platelet reactivity; LD: loading dose; LTA: light transmittance aggregometry; PACT: platelet activation assay; PAD: peripheral arterial disease; PFA: platelet function analyser; PFT: platelet function test; PTA: percutaneous transluminal angioplasty; PRU: platelet reaction units; TEG: thromboelastography; VASP: vasodilator-stimulated protein phosphorylation assay.

with and without PAD, and found patients with PAD more likely to have dual resistance (4.1% with PAD vs 2.4% without) (relative risk: 1.73; 95% CI = 1.21–2.47).[12]

Analysis of the correlation between various platelet resistance testing methods is described in seven studies.[21,24–26,28,33,39] Of the articles included in this review, not one assessed the correlation between all platelet resistance techniques. Leunissen et al. describe no significant correlation between VASP and VerifyNow, while Clavijo et al. only showed a moderate correlation (r = 0.6, p = .001) for the same aforementioned techniques.[24,33]

Genetic testing

Genetic testing of CYP2C19 loss of function (LOF) alleles and a correlation to HTPR for clopidogrel was performed in four studies.[20,23,24,36] Carriers of a CYP2C19 LOF allele were shown to have a relative reduction of 23.5% in platelet inhibition, compared to patients with a wild type allele (p = .022).[20] Three studies noted no significant difference in the prevalence of HTPR between patients with and without a CYP2C19 LOF allele.[20,24,36] Lee et al. describe a significant association between all-cause mortality at 1-year follow-up and gene polymorphism number.[23] Guo et al. show that CYP2C19 LOF alleles affect the risk for developing ischemic events (adjusted HR = 2.688; 95% CI = 1.366–5.288).[20]

Clinical outcomes

Twelve studies assessed clinical outcomes.[12,15,17,19,20,23,25,27,29,31,32,37] Five studies showed an increased risk of limb-related events in the presence of HTPR.[12,15,17,27,32]

MALE. Yeo et al. described significantly higher rates of target vessel revascularisation within 12 months in patients with HTPR for clopidogrel (20 vs 6%, p = .02), while Pastromas et al. identified HTPR for clopidogrel to be the only independent predictor for a decrease in target limb revascularisation-free survival (HR = 0.536; 95% CI = 0.31–0.90).[27,32] Contrarily, two studies found no significant association between HTPR for clopidogrel or aspirin and limb-related events, with a follow-up of 6 and 23 months [Grifoni aspirin HR = 1.2 (0.6–2.5); clopidogrel HR = 0.7 (0.3–1.7); and Pasala aspirin HR = 0.94 (0.43–2.11)][19,37]

MACE. In a multivariate analysis performed by Pasala et al., aspirin resistance was an independent predictor of long-term adverse cardiovascular events (HR = 3.73; 95% CI = 1.43–9.81), with Gupta et al. demonstrating similar results for clopidogrel and aspirin through a 2-year follow-up (HR = 1.27; CI = 1.03–1.57).[12,37] Mortality rates in patients with HTPR for clopidogrel or aspirin are significantly higher than in non-resistant patients, with Grifoni et al. describing resistance to aspirin or clopidogrel (HR = 3.75; 95% CI = 1.20–11.66, and HR = 4.78; 95% CI = 1.57–14.52, respectively), and dual antiplatelet resistance as a strong independent risk factor for mortality (HR = 6.0; 95% CI = 2.3–15.9).[12,19]

Discussion

Antiplatelet therapy is a pivotal element in the management of PAD for the prevention of both limb-related and cardiovascular events. The ESVS advises clopidogrel as the antiplatelet therapy of choice, specifically for its' superior effect on cardiovascular event reduction in comparison to aspirin.[1] The references supporting this recommendation are limited, with evidence largely retracted from the 1996 CAPRIE study.[40] Furthermore, while a large body of research focussing on platelet resistance in CAD and stroke patients shows an increased risk of major adverse cardiac events (MACE) in HTPR patients, evidence in the field of PAD is limited.[5,41,42] Summarising present and gathering new evidence of this relationship is of great importance as intermittent claudication is often the first, and relatively benign, presentation of cardiovascular disease. Furthermore, PAD patients require lengthy antiplatelet treatment and deserve optimal medical treatment in order to prevent adverse cardiovascular and limb events. With the growing elderly population, prevention of major cardiovascular disease should have priority from a patient's, physician's, and societal point of view.

The aim of this systematic review was to summarise measurement methods of HTPR and their correlation to clinical outcomes in PAD patients. Measurement methods of HTPR varied and cut-off values were calculated differently or at least different in outcome. This resulted in a wide range of HTPR for clopidogrel treatment from 9.8 to 77%, and during aspirin treatment from 4.1 to 50% of PAD patients. Independent of these varying numbers, HTPR to clopidogrel was found to negatively influence target limb revascularisation-free survival[27] and HTPR to aspirin and clopidogrel were significant predictors of death in patients undergoing peripheral vascular interventions.[19] A substudy of the SMART-CHOICE trial further validates the findings of this systematic review.[43] HTPR was found to be significantly associated with an increased risk of MACE in clopidogrel-treated patients, regardless of maintenance with aspirin.

From a methodologic point of view, differences between study outcomes (such as cut-off HTPR values) may be due to different timing of testing. Treatment with antiplatelet therapy and invasive treatments such as PTA's have also shown to influence HTPR measurements. Data extracted from interventional cardiology literature could be of assistance in guiding the choice of optimal cut-off values and timing of testing. A study by Brar et al. describes a cut-off VerifyNow PRU of 230 for predicting MACE following PCI, an intervention with similarities to endovascular treatment of PAD.[44] Similarly, a post-hoc analysis of the GRAVITAS trial found that no further post-PCI MACE events occurred below a threshold PRU value of 208.[45] A myriad of clinical contributors such as diabetes mellitus; chronic kidney disease; advanced age; increased BMI and a medical history of atherothrombotic events, are also known to impact HTPR.[46] PAD patient characteristics can be of importance in the clinician's decision to adapt the antiplatelet regimen based on HTPR results. The smoker's paradox (Figure 1(b)), the positive effect of smoking on platelet reactivity in clopidogrel-treated patients, is one example of an influential factor in antiplatelet strategies.[47]

Genetic polymorphisms are another factor warranting consideration in the identification of HTPR patients. Recent studies have primarily focused on CYP2C19, as demonstrated in this review, with LOF alleles as the primary contributor to HTPR. CYP2C19 is of central importance due to its involvement in both steps of the metabolic pathway of clopidogrel, namely clopidogrel bisulfate to 2-oxo-clopidogrel to R130964.[48] While genetic testing focussing on CYP2C19 LOF alleles provides promising grounds for exploring HTPR, it should be noted that this is one of many CYP isozymes. Furthermore, numerous variations on the theme of loss-of-function exist given that a total of 14 CYP2C19 alleles have been identified (CYP 2C19*1A, *2A, *3, *4, *5A, *6, *7, *8, *9, *10, *12, *13, *14, and *17).

To be clinically applicable, testing for antiplatelet sensitivity should ideally be performed before treatment initiation while alternatives for standard treatment regimens should be available. Initial genetic testing for CYP2C19 is one possibility and cost-effectiveness has been shown in other fields.[49,50] However, the studies included in this review show that genetic testing results in conflicting data on the CYP2C19 polymorphisms in PAD patients and occurrence of future clinical events.[20,23,24,36] Yet, in other fields of cardiovascular disease CYP2C19 polymorphisms are in fact associated with adverse clinical events.[7,51,52] This suggests that genetic testing might be advantageous in reducing limb-related or cardiovascular morbidity with implementation in conjunction with alternative strategies improving sensitivity.[53] Alternative approaches include a loading dose with clopidogrel pre-procedure and testing with VerifyNow, an inexpensive, whole blood assay with no sample preparation and rapid turnaround.[32] Another approach is combined genetic- and HTPR testing. In the event of HTPR and LOF alleles, a tailored clopidogrel dose can be applied, or a switch can be made to prasugrel or ticagrelor.[54]

Future studies need to identify alternative antiplatelet therapy strategies based on the resistance results in PAD patients, exploring personalised dosage schemes in intermediate metabolisers, or a switch to alternatives such as ticagrelor or direct oral anticoagulants in poor metabolisers. While a growing body of intervention cardiology research supports the switch to P2Y12 inhibitors prasugrel and ticagrelor, evidence in the field of PAD is limited and conflicting.[55–58] Vorapaxar, a thrombin receptor antagonist, is a further alternative warranting consideration. A recent analysis of patients with PAD and CAD demonstrated a significant reduction in MACE, while a reduction in MALE in PAD patients with a history of revascularisation is also described.[59] Another important aspect to consider in tailored treatment is the assessment of bleeding risk in increased or alternative antiplatelet strategies. A recent study focussing on acute coronary syndrome demonstrated comparative safety and improved efficacy of P2Y12 inhibitors prasugrel and ticagrelor compared to clopidogrel when adopting a guided (i.e., platelet function or genetic testing) rather than standard dosage scheme.[60] Lastly, dual antiplatelet resistance is an infrequently mentioned yet pivotal aspect of HTPR research. The identification and tailored treatment of dual antiplatelet resistance, specifically surrounding endovascular of surgical interventions, could lead to improved postoperative outcomes.[19,61]

Based on results from this review, in the case of HTPR, clopidogrel could be substituted while aspirin dose could be increased. The diagnosis and treatment algorithm should however be adapted based on the population characteristics, including genetic and nongenetic contributors. For example, in geographical areas with very low occurrence of CYP2C19 polymorphisms, only HTPR testing can be considered. Recent data provides region-specific CYP2C19 data for the European region and can aid clinical implementation of genetic screening.[62] Contrarily, in multicultural areas genetic testing of a relevant sample size could aid adapting this algorithm. Currently, there is insufficient evidence to indicate differences in HTPR in geographical areas.

Limitations and quality of evidence

Regarding the strength of evidence supporting the results of this systematic review, there are several limitations. First, a diagnostic standard reference test for detecting high on-treatment platelet resistance has not yet been established, nor an agreed-on cut-off value for the selected platelet resistance assays, factors that impact the quality of the data presented. A pooled meta-analysis could not be performed due to the heterogenicity in study design and outcome measures. A meta-analysis has recently been performed on a subgroup of patients receiving clopidogrel following endovascular intervention.[63] Second, selection bias must be considered. Only one randomised control study was identified.[31] Not all studies included focused specifically on PAD patients, and thus these PAD patients were grouped without PAD classification, or mixed with CAD patients. A large number of articles focused on patients undergoing revascularisation procedures, a potential source of selection bias for measuring platelet resistance due to possible correlation with limb-related events. Third, very few articles provide a detailed overview of the specifics surrounding antiplatelet therapy, such as dual platelet therapy dosage or combination specifics; and the relationship between an event and specific antiplatelet therapy at that time point. Regarding antiplatelet therapy dosage, no standard combinations were used by the studies included. Lastly, despite independent review of the included articles, bias in the selection articles and extraction of data cannot be completely ruled out.

Conclusion

High on-treatment platelet resistance is probably moderate-to-highly prevalent in patients with PAD. Studies on this subject carry data with limited evidence. Adaption of antiplatelet therapy may have the potential to reduce morbidity and mortality by lengthening target limb revascularisation-free survival, and lowering cardiovascular morbidity or mortality. The small number of studies and lack of randomised control trials reporting on this topic highlights the deficit of knowledge on this subject. Further research is needed in this specific patient population, investigating platelet resistance assays, genetic testing, cut-off values and timing of testing, as well as clinical correlation. Advances in this field could allow for evidence-based implementations in clinical practice and may increase the quality of life in this fragile population and improve the societal impact of cardiovascular disease.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Research Fund Haaglanden Medical Centre and Bronovo Research Fund.

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Supplemental Material

Supplemental material for this article is available online.

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