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High On-Treatment Platelet Reactivity in Peripheral Arterial Disease: A Pilot Study to Find the Optimal Test and Cut Off Values

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WHAT THIS PAPER ADDS

Cardiology studies have shown inter-individual variety in platelet reactivity with clopidogrel treatment. This pilot trial investigated the proportion of peripheral arterial disease (PAD) patients with high clopidogrel platelet reactivity (HCPR), according to different platelet reactivity tests and a single DNA test. It was found that HCPR is also largely present in PAD patients. Furthermore, it was shown that platelet reactivity testing within 24 hours of the loading dose is not reliable and that the accurate timing should be determined. Further investigation is required to draw conclusions about the optimal test and optimal timing of testing.

Objective: Restenosis and stent thrombosis after endovascular intervention in patients with peripheral arterial disease (PAD) can potentially be tackled by more intensive antiplatelet therapy, such as dual antiplatelet therapy (DAPT) consisting of aspirin and $P2Y_{12}$ inhibitor. Despite clopidogrel treatment, some patients still display high platelet reactivity (HCPR). Tailored antiplatelet therapy, based on platelet reactivity testing, might overcome HCPR. However, more data are warranted regarding the proportion of patients with HCPR in the PAD population, different platelet reactivity tests, their correlation, and the optimal timing for these tests as a stepping stone for a future trial investigating the potential benefit of tailored antiplatelet therapy in PAD patients.

Methods: Thirty patients on DAPT after percutaneous transluminal angioplasty underwent platelet reactivity testing by VerifyNow, vasodilator-stimulated phosphoprotein (VASP) and platelet activation assay, and CYP2C19-polymorphism testing.

Results: The proportion of patients with HCPR measured by VerifyNow varied between 43.3% and 83.3%, depending on the cut off values used. Testing within 24 hours of initiation of DAPT gave a higher proportion of HCPR than testing after more than 24 hours. According to DNA testing, 14.8% were CYP2C19*2 homozygote, 22.2% heterozygote, and 63% CYP2C19*2 negative. VASP assay revealed 24% HCPR. The highest HCPR rate was found with a VerifyNow cut off of less than 40% inhibition, whereas the lowest HCPR rate was found with the VASP assay. There was a low correlation between the tests.

Conclusion: HCPR is present in PAD patients and research on HCPR is needed in this population; timing of tests is relevant and standardisation of tests is needed. The optimal conditions for platelet function testing should be determined.

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INTRODUCTION

Restenosis and stent thrombosis remain the main challenges after endovascular treatment of peripheral arterial disease (PAD). The 1 and 5 year patency rates of PTA alone are 71% and 49%. With additional stent placement, the patency rates increase to 74% and 65% after 1 and 3 years.[1–6]

Platelets have a significant role in restenosis and stent thrombosis, hence antiplatelet therapy (APT) is given to prevent these complications. Numerous publications from

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the Antithrombotic Trialists' Collaboration have concluded that use of aspirin in patients with cardiovascular disease will result in a 25% odds reduction in subsequent cardiovascular events (CVE).[7] The prescription of dual antiplatelet therapy (DAPT), consisting of aspirin and $P2Y_{12}$ inhibitor, for patients undergoing endovascular treatment has increased, although evidence is lacking for DAPT after peripheral endovascular procedures (unpublished data).[1]

Clopidogrel, a $P2Y_{12}$ inhibitor, is often the first choice APT added to aspirin, although previous trials have shown that 40% of patients show high platelet reactivity, despite additional clopidogrel treatment.[8] The few trials reporting on high on-clopidogrel platelet reactivity (HCPR) in PAD patients indicate an even higher incidence.[9–11] In some patients, this phenomenon can be caused by a mutation in the genes coding for cytochrome P450 2C19 (CYP2C19) activity, a liver enzyme that converts the clopidogrel prodrug into its active metabolite.[12] Other independently related factors to HCPR are non-compliance, diabetes mellitus, renal failure, and non-smoking.[13–16]

The existence of HCPR has led to the concept of tailored APT, the idea that simply testing platelet reactivity in response to APT and adjusting the regimen based on the results, will lead to improved clinical outcomes.[17] Most research on this concept is performed in cardiac patients undergoing percutaneous coronary intervention. To date, clinical trials on tailored APT have shown diverging results. Two large clinical trials (GRAVITAS,[18] $n = 2214$ and ARCTIC,[19] $n = 2240$) showed no difference in primary outcome (composite of cardiovascular death, non-fatal acute myocardial infarction, and ST elevation) or bleeding complications after tailored APT compared with standard therapy. However, a few smaller studies have shown a beneficial effect of tailored APT compared with standard therapy.[20–22] No studies have been performed regarding tailored APT for PAD patients.

Currently, there is a need for randomised trials investigating the benefit of (tailored) APT in PAD patients after endovascular treatment. To perform such trials, more knowledge is needed concerning the proportion of patients with HCPR in this population, the optimal timing, test, and cut off values to identify HCPR.

Therefore, the aim of this pilot study was to determine the proportion of patients with HCPR in the PAD population and to evaluate different platelet reactivity tests, their correlation, and optimal timing for these tests.

MATERIALS AND METHODS

Study design

The study was a prospective, observational pilot study with 30 patients. As no previous trial results were available regarding PAD patients with HCPR, an adequate power calculation was not possible. The study was conducted with approval of the local ethics committee and in accordance with the declaration of Helsinki. All patients gave written informed consent prior to the procedure.

Patient selection

Patients were included if they were adults scheduled to undergo an endovascular revascularisation (PTA, percutaneous transluminal angioplasty) of the superficial femoral artery or popliteal artery and were on aspirin treatment prior to the intervention.

Exclusion criteria were treatment with heparin, oral anticoagulants, or $P2Y_{12}$ inhibitors at the time of the PTA, as these patients would not be suitable to receive DAPT post intervention.

Procedures

Participating patients underwent regular PTA with or without additional stenting. Procedures were performed by either the interventional radiologist or vascular surgeon, and aspirin was continued during endovascular treatment. Blood was drawn from the arterial access sheath directly prior to the angioplasty for platelet reactivity testing. Prescription of DAPT after intervention was left to the treating physician's discretion. Patients with an indication for DAPT received a loading dose (LD) of 300 mg clopidogrel on the same day as the procedure, with platelet reactivity tests performed between 1 and 5 days after the LD. In addition to the platelet reactivity tests, a CYP2C19 polymorphism DNA test was performed using the Spartan RX CYP2C19 DNA testing system (Spartan Bioscience Inc, Ottawa, Canada), to determine the presence of CYP2C19*2 loss of function alleles.

Platelet reactivity measurements

VerifyNow. Platelet reactivity was assessed using the VerifyNow $P2Y_{12}$ assay (Accumetrics, San Diego, CA, USA), which is a cartridge based optical detection system using whole blood. Blood was collected in a Greiner Bio-One 3.2% citrate Vacuette tube. Although the VerifyNow is a widely used point of care test, different cut off values are used in clinical and research settings. Some researchers advocate the use of $P2Y_{12}$ reaction units (PRU), whereas others use the percentage of platelet inhibition as measurement of sufficient response to $P2Y_{12}$ inhibitors. In the current literature the most commonly used cut off value is post PRU <235,[23] although the manual of the VerifyNow advises a cut off value of <208 PRU.[24] A third commonly used cut off value is $>40\%$ inhibition.[22,25] The present study compared these three cut offs to evaluate the differences in (non)-responders to clopidogrel. At the other end of the spectrum, low platelet reactivity caused by clopidogrel treatment increases the risk of bleeding. Low platelet reactivity is defined as PRU <95 by the VerifyNow manual.

VASP assay. Vasodilator-stimulated phosphoprotein (VASP) is an intracellular platelet protein that is not phosphorylated when the $P2Y_{12}$ receptors are active. Persistent VASP phosphorylation, as measured with flow cytometry, correlates with $P2Y_{12}$ receptor inhibition, reflecting the effect of antiplatelet therapy. Blood was collected in a 0.105 M trisodium citrate tube. Flow cytometry analysis of VASP phosphorylation was performed using a commercial kit (PLT