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On-Treatment Platelet Reactivity is a Predictor of Adverse Events in Peripheral Artery Disease Patients Undergoing Percutaneous Angioplasty

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WHAT THIS PAPER ADDS

On-treatment platelet reactivity, the so called antiplatelet resistance, has been associated with cardiovascular events in patients with coronary artery disease undergoing PCI. In the present study of PAD patients, the entity of platelet inhibition on dual antiplatelet therapy is associated not only with thrombotic, but also with bleeding complications, suggesting that a "therapeutic window" exists, within which the predicted risk of ischaemic and bleeding complications is the lowest. Future studies are needed to evaluate the potential utility of assessing platelet function, in the clinical setting of PAD, to ensure the patient is given the best tailored antiplatelet therapy.

Objectives: Few data are available on the association between a different entity of platelet inhibition on antiplatelet treatment and clinical outcomes in patients with peripheral artery disease (PAD). The aim of this study was to evaluate the degree of on-treatment platelet reactivity, and its association with ischaemic and haemorrhagic adverse events at follow up in PAD patients undergoing percutaneous transluminal angioplasty (PTA).

Methods: In this observational, prospective, single centre study, 177 consecutive patients with PAD undergoing PTA were enrolled, and treated with dual antiplatelet therapy with aspirin and a P2Y12 inhibitor. Platelet function was assessed on blood samples obtained within 24 h from PTA by light transmission aggregometry (LTA) using arachidonic acid (AA) and adenosine diphosphate (ADP) as agonists of platelet aggregation. High on-treatment platelet reactivity (HPR) was defined by LTA $\geq$ 20% if induced by AA, and LTA $\geq$ 70% if induced by ADP. Follow up was performed to record outcomes (death, major amputation, target vessel re-intervention, acute myocardial infarction and/or myocardial revascularisation, stroke/TIA, and bleeding).

Results: HPR by AA and HPR by ADP were found in 45% and 32% of patients, respectively. During follow up (median duration 23 months) 23 deaths (13%) were recorded; 27 patients (17.5%) underwent target limb revascularisation (TLR), two (1.3%) amputation, and six (3.9%) myocardial revascularisation. Twenty-four patients (15.6%) experienced minor bleeding. On multivariable analysis, HPR by AA and HPR by ADP were independent predictors of death [HR 3.8 (1.2–11.7), $p = .023$ and HR 4.8 (1.6–14.5), $p = .006$, respectively]. The median value of LTA by ADP was significantly lower in patients with bleeding complications than in those without [26.5% (22–39.2) vs. 62% (44.5–74), $p < .001$). LTA by ADP $\leq$ 41% was independently associated with bleeding HR 14.6 (2.6–24.0), $p = .001$] on multivariable analysis.

Conclusions: In this study a high prevalence of on-clopidogrel and aspirin high platelet reactivity was found, which was significantly associated with the risk of death. Conversely, a low on-clopidogrel platelet reactivity was associated with a higher risk of bleeding. These results document that the entity of platelet inhibition is associated with both thrombotic and bleeding complications in PAD patients.

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INTRODUCTION

Peripheral artery disease (PAD) is most commonly caused by atherosclerosis, and is characterised by an increased risk of myocardial infarction, stroke, and cardiovascular death.[1] Given the markedly elevated cardiovascular risk among patients with PAD, antiplatelet therapy would be expected to be of great benefit. However, there is little evidence that antiplatelet therapy can alter the natural history of PAD, both in asymptomatic and symptomatic patients, and which antiplatelet agent could be the best option is not well defined. Although dual antiplatelet therapy (DAPT) is generally recommended after peripheral vascular intervention, this is largely based on extrapolation from coronary percutaneous intervention data.[2] In this setting a different entity of on-treatment platelet function inhibition is associated with different clinical outcomes. Several studies have demonstrated that high on-treatment platelet reactivity (HPR), so called antiplatelet resistance, is associated with an increased risk of ischaemic complications (especially stent thrombosis),[3–5] and there is a growing body of evidence that, on the contrary, low on-treatment platelet reactivity (LPR) could be associated with bleeding risk.[6] High on-aspirin platelet reactivity measured by light transmission aggregometry (LTA) induced by arachidonic acid (HPR by AA) and high on-clopidogrel platelet reactivity measured by LTA induced by adenosine diphosphate (ADP) (HPR by ADP) have been used to defined the variable responses to antiplatelet therapy in major clinical studies. Concerning the role of HPR in PAD, few data are available in the literature. HPR on-clopidogrel has been associated with an increased risk of adverse events (cardiovascular death, major amputation, and re-intervention) during follow up in patients with PAD undergoing percutaneous transluminal angioplasty (PTA).[7–9] The role of so called aspirin resistance in PAD is not completely defined; indeed, available studies are not homogenous in terms of clinical presentation of the disease, time of blood sampling, and methods used for the determination of HPR on-aspirin therapy.[10–17]

The aim of this study was to evaluate the degree of on-treatment platelet reactivity, and the association between the entity of platelet inhibition and ischaemic and haemorrhagic adverse events at follow up, in patients with PAD undergoing PTA with or without stenting.

MATERIALS AND METHODS

Study population

The study enrolled 177 PAD patients (118 males, median age 75 years) undergoing primary and elective PTA, with or without stenting, who were referred to the University Hospital of Florence, Italy from January 2012 to December 2013. Inclusion criteria were ankle brachial index (ABI) <0.9 or >1.3 and age >18 years. Patients aged <18 years and/or unable to sign the informed consent were excluded. A platelet count $<100 \times 10^9$/L, a haemoglobin <9 g/dL, and a haematocrit $<$ 28% were additional exclusion criteria. The study was approved by the local ethics committee. All patients gave written informed consent.

Percutaneous transluminal angioplasty and antiplatelet management

All interventions were performed according to current standards, and the use and type of stent implanted was at the discretion of the operator. All patients, unless they were already on antiplatelet therapy for a previous coronary revascularisation, underwent a P2Y12 inhibitor loading dose. Patients were discharged on DAPT with a P2Y12 inhibitor and aspirin. Clopidogrel was the first choice P2Y12 inhibitor (according to the guidelines) unless patients were on treatment with prasugrel or ticagrelor for a recent acute coronary syndrome. Aspirin, 100—325 mg once daily, was recommended for an indefinite period, and a P2Y12 inhibitor for at least 6 months.

Platelet function assessment

Platelet function was assessed by light transmission aggregometry (LTA) (APACT4, Helena Laboratories, Milan, Italy), performed on platelet rich plasma, using AA and ADP as agonists of platelet aggregation. Blood samples anticoagulated with 0.109 M sodium citrate (ratio 9:1) were obtained within 24 h of PTA. Platelet rich plasma, obtained by centrifuging whole blood for 10 min at 200 g, was stimulated with 1 mM AA and 10 μM ADP. According to literature data, HPR was defined by LTA $\geq$ 20% if induced by AA, and LTA $\geq$70% if induced by ADP.[18]

Follow up

Follow up was performed to record the occurrence of ischaemic and bleeding events. All patients had scheduled examinations at 1, 6, and 12 months and then annually thereafter. Adherence to antiplatelet treatment was assessed during scheduled or unscheduled examinations. All other possible information derived from hospital readmission or by the referring physician, relatives, or municipality live registries was collected.

Outcomes

The study's outcomes were death, major amputation, target vessel re-intervention (TVR), acute myocardial infarction and/or myocardial revascularisation, stroke/TIA, and bleeding, classified as major and non-major according to the TIMI classification.[19]

Statistical analysis

Statistical analysis was performed using the software package SPSS 20 (SPSS Inc., Chicago, Il, USA). Discrete data were summarised as frequencies, and continuous data were expressed as means and standard deviations or medians and interquartile ranges (IQRs), as appropriate. The χ^2 test was used for comparison of categorical variables, and the unpaired two tailed Student *t* test or Mann—Whitney rank sum test were used to test differences among continuous variables. The ability of platelet aggregation values by ADP and AA to predict outcomes was examined by receiver operating characteristics (ROC) curves. ROC curves were

constructed by plotting the sensitivity against the corresponding false positive rate which equals 1 specificity. Survival curves were generated using the Kaplan–Meier method, and the difference between groups was assessed by log-rank test. A multivariable Cox proportional hazard model adjusted for clinical and laboratory variables (age, sex, BMI $>$ 30 kg/m^2, dyslipidaemia, diabetes, smoking habits, EF $<$ 45%, renal failure, and Leriche-Fontaine classification) was performed to evaluate the independent contribution of platelet hyper- or hypo-reactivity to the outcomes. A landmark analysis was computed by the Kaplan–Meier method for mortality using a starting point of 6 months after the index procedure. All tests were two sided, and a p value $<$ .05 was considered significant.

Table 1. Baseline characteristics of the study population.

	All patients (n = 177)
Age, years, median (IQR)	75 (68–81)
BMI, kg/m^2, median (IQR)	25.2 (22.8–27.6)
BMI $\geq$ 30 kg/m^2, n (%)	25 (14.1)
Smokers, n (%)	35 (19.9)
Hypertension, n (%)	151 (85.3)
Dyslipidaemia, n (%)	163 (92.3)
Diabetes, n (%)	57 (32.3)
Renal failure, n (%)	21 (11.8)
Ejection fraction $<$ 45%, n (%)	36 (20.2)
Leriche-Fontaine Class, n (%)	
I	2 (1.3)
IIa	14 (7.9)
IIb	103 (57.9)
III	7 (3.9)
IV	51 (28.9)

IQR = interquartile range; BMI = body mass index.

RESULTS

Baseline demographic and clinical characteristics of the study population are reported in Table 1. Regarding the severity of PAD, most patients were class IIb according to the Leriche-Fontaine classification. In about 90% of patients PTA was followed by stent implantation. No PTA failure was recorded in PAD patients. All patients were discharged on DAPT with aspirin and a P2Y12 inhibitor (130 with clopidogrel, 39 with prasugrel, and 8 with ticagrelor).

HPR by AA was found in 45% of patients, and showed a non-significant association with older age and a higher prevalence of renal failure (Table 2). HPR by ADP was found in 32% of patients, and was significantly associated with older age (Table 2).

The median follow up was 23 (13–27) months. During follow up 23 deaths (13%) were recorded. Among survivors, 27 patients (17.5%) underwent TVR, two (1.3%) underwent amputation, and six (3.9%) myocardial infarction and/or myocardial revascularisation; no patients experienced stroke or TIA. Twenty-four patients (15.6%) experienced a bleeding complication, which was minor in all cases (16 epistaxis, 3 mouth bleedings, 2 haematuria, 1 gastrointestinal bleeding, 2 limb bruisings). Patients who died were significantly older than survivors, and had a significantly higher prevalence of renal failure and left ventricular systolic dysfunction (Table 3). The median value of LTA by both AA and ADP was significantly higher in patients who died than in survivors [43% (16–75) vs. 18% (14–75), p = .005 for LTA by AA; 75% (56–83) vs. 56 (38–83)%, p = .001 for LTA by ADP, respectively]. Patients who died had a significantly higher prevalence of HPR by AA and HPR by ADP than survivors (73.9% vs. 26.1%, p = .002; 65.2% vs. 34.8%,

Table 2. High on-treatment platelet reactivity by AA and ADP.

	No HPR by AA (n = 98)	HPR by AA (n = 79)	p	No HPR by ADP (n = 121)	HPR by ADP (n = 56)	p
Males/females, n (%)	65/33 (66.3/33.7)	53/26 (67.1/32.9)	.999	82/39 (67.8/32.2)	36/20 (64.3/35.7)	.732
Age, years, median (IQR)	73.5 (67.0–79.0)	77.0 (70.0–83.0)	.058	73 (67–78)	79 (70.5–84.0)	.001
BMI, kg/m^2, median (IQR)	25.5 (23–28)	25 (22.0–27.6)	.419	25.2 (22.4–27.3)	25.7 (23.1–29.3)	.338
BMI $\geq$ 30 kg/m^2, n (%)	16 (16.3)	9 (11.4)	.237	17 (14.0)	8 (14.3)	.567
Smokers, n (%)	19 (19.4)	16 (20.2)	.954	27 (22.3)	8 (14.3)	.204
Hypertension, n (%)	86 (87.8)	65 (82.3)	.306	106 (87.6)	45 (80.4)	.448
Dyslipidaemia, n (%)	92 (93.9)	71 (89.9)	.241	113 (93.4)	50 (89.3)	.255
Diabetes, n (%)	32 (32.6)	25 (31.6)	.887	39 (32.2)	18 (32.1)	.990
Renal failure, n (%)	8 (8.2)	13 (16.4)	.090	14 (11.6)	7 (12.5)	.859
Ejection fraction $<$45%, n (%)	17 (17.3)	19 (24.1)	.271	23 (19.0)	13 (23.2)	.518

HPR = high on-treatment platelet reactivity; AA = arachidonic acid; ADP = adenosine diphosphate; IQR = interquartile range; BMI = body mass index.

Table 3. Univariable and multivariable analysis for death.

	Univariable HR (95% CI)	*p*	Multivariable HR (95% CI)	*p*
Females vs. males	0.67 (0.25—1.81)	.432	0.45 (0.16—1.28)	.134
Age	1.10 (1.03—1.16)	.002	1.10 (1.02—1.18)	.016
BMI $\geq$ 30 kg/m^2	0.54 (0.12—2.47)	.429	3.85 (0.74—20.12)	.110
Smoking	0.16 (0.02—1.23)	.079	0.33 (0.04—2.80)	.308
Hypertension	0.32 (0.12—0.88)	.028	0.19 (0.06—0.61)	.004
Dyslipidaemia	0.33 (0.09—1.16)	.083	1.30 (0.31—5.38)	.721
Diabetes	1.14 (0.45—2.88)	.777	1.13 (0.39—3.29)	.817
Renal failure	3.27 (1.12—9.56)	.030	2.39 (0.79—7.22)	.123
Ejection fraction < 45%	3.79 (1.50—9.56)	.005	2.78 (1.07—7.18)	.035
Fontaine classification (I+IIa+IIb vs III+IV)	4.90 (1.99—12.04)	.001	3.16 (1.05—9.87)	.049
HPR by AA	4.20 (1.57—11.26)	.004	3.51 (1.24—9.94)	.018
HPR by ADP	5.17 (2.04—13.09)	.001	4.62 (1.63—13.10)	.004

HR = hazard ratio; BMI = body mass index; HPR = high platelet reactivity; AA = arachidonic acid; ADP = adenosine diphosphate.

$p < .001$, respectively). No significant associations were found between TVR, MI, and HPR [TVR: HR 1.2 (0.6—2.5) $p = .666$ for HPR by AA and HR 0.7 (0.3—1.7); $p = .434$ for HPR by ADP; MI: HR 1.6 (0.3—8.1) $p = .551$ for HPR by AA and HR 1.2 (0.2—6.3) $p = .875$ for HPR by ADP].

Dual antiplatelet resistance was detected in 37 patients (20.9%). The incidence of mortality was 35.1% (13/37) in patients with HPR by ADP and AA and 7.1% (10/140) in patients who did not have dual antiplatelet resistance ($p < .001$).

Kaplan—Meier analysis showed a significantly higher risk of death in patients with HPR by AA and HPR by ADP than in those without (Fig. 1). On multivariable analysis HPR by AA and HPR by ADP remained independent predictors of death [HR 3.75 (1.20—11.66), $p = .023$ and HR 4.78 (1.57—14.52), $p = .006$, respectively] (Table 3). The univariable and multivariable Cox regression analysis demonstrated that dual antiplatelet resistance is a strong independent risk factor for death [HR 5.6 (2.4—12.8), $p < .001$; HR 6.0 (2.3—15.9), $p < .001$, respectively]. The landmark analysis using the prespecified starting point of 6 months showed that the differences in mortality between patients with and without HPR by ADP emerged both in the short-term follow up as well as from 6 months to long term, whereas HPR by AA was significantly associated only with long-term mortality (Fig. 1). No significant association was observed between TLR and HPR by AA or HPR by ADP. Bleeding complications were significantly associated with younger age (Table 4). The median value of LTA by ADP was found to be significantly lower in patients who experienced bleeding complications than in those who did not [26.5% (22.0—39.2) vs. 62.0% (44.5—74.0), $p < .001$). On ROC curve analysis the cutoff of platelet aggregation induced by ADP with the best sensitivity and specificity for increased risk of bleeding was 41%. Using this value as the cutoff of low on-treatment platelet reactivity, a significant association was found with bleeding (Fig. 2). On multivariable analysis LTA by ADP lower than 41% remained independently associated with bleeding [HR 14.6 (2.6—24.0), $p = .001$] (Table 4).

DISCUSSION

In PAD patients enrolled in this study it was found that: (i) HPR by ADP and HPR by AA are predictors of death; and (ii) low on-treatment platelet reactivity by ADP is a predictor of bleeding complications. To the authors' knowledge, this is the first study which concurrently assessed the role of platelet hyper- or hypo-reactivity in predicting mortality or bleeding events during a 2 year follow up in PAD patients who underwent PTA on DAPT.

This study showed a high prevalence of HPR by ADP and AA in the acute phase of the disease, and the assessment of HPR by ADP and AA after PTA was able to identify those patients who died during follow up. These findings are consistent with those obtained in the clinical setting of acute coronary syndromes, in which the presence of ADP and/or AA induced HPR was associated with a significantly increased risk of ischaemic events, and cardiac death.[3—5]

Few studies are available on the role of HPR, and in particular on the so called clopidogrel resistance, in the occurrence of adverse events in PAD patients on DAPT. Pastromas and co-workers demonstrated that, in patients with PAD treated with clopidogrel and aspirin for 6 months, the post-PTA evaluation of HPR by ADP (assessed by the point-of-care test VerifyNow) provided prognostic information on the occurrence of target limb re-intervention.[8] In the PRECLOP study, the assessment of HPR before PTA, by the same point-of-care test, provided the optimal PRU cut off value to identify PAD patients at high risk of developing the combined endpoint of death, TVR, and amputation during one year follow up.[9] Consistent with these previous observations, PAD patient carriers of at least one CYP2C19 loss-of-function allele had a reduced pharmacodynamic response to clopidogrel (measured as platelet aggregation induced by ADP), and those with both HPR and a CYP2C19 loss-of-function allele had a significantly higher risk of ischaemic events.[20] Recently, two studies have demonstrated an association between aspirin resistance detected by VerifyNow and clopidogrel resistance detected by VerifyNow and VASP and thrombotic complication in a significantly lower number of PAD patients.[17,20,21]

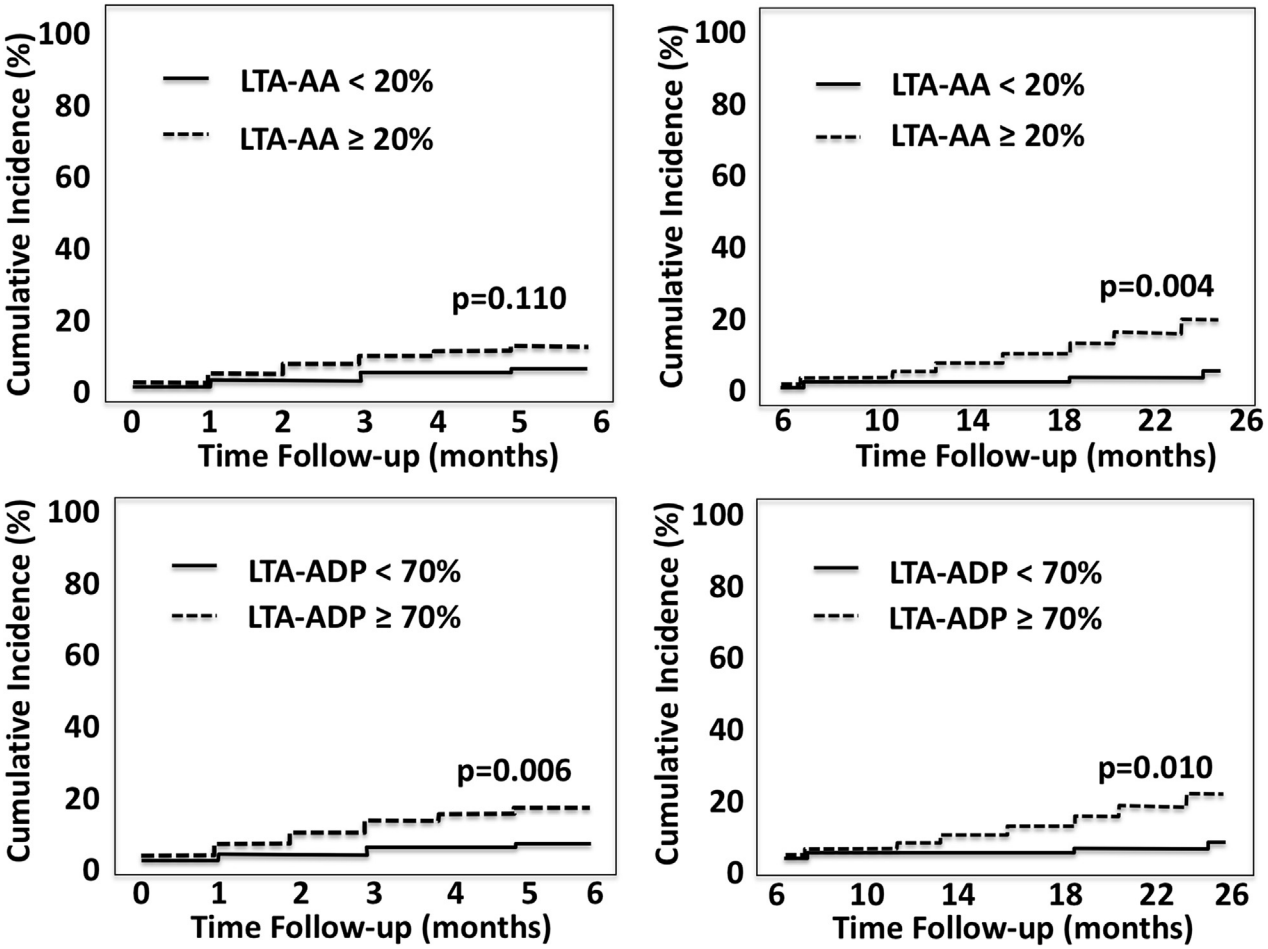

Figure 1. Kaplan—Meier landmark analysis survival curves for death in patients with and without HPR by AA and ADP using the prespecified starting point of 6 months. HPR = high platelet reactivity; AA = arachidonic acid; ADP = adenosine diphosphate.

The present results demonstrate that in PAD patients, not only HPR by ADP, but also HPR by AA were found to be independent predictors of death in PAD patients treated by PTA.

At variance, three previous studies on PAD patients treated by PTA failed to demonstrate a significant association between HPR by AA and adverse events during follow up.[10,15,22] Although the clinical setting of these studies was similar to that of the present study, namely symptomatic PAD patients treated by PTA, the timing of HPR evaluation was different. In particular, in 109 symptomatic PAD patients, the intra-individual variance of HPR assessed by AA induced LTA before PTA did not correlate with restenosis, reocclusion, death, stroke, or myocardial infarction at one year follow up. In the present study, HPR was evaluated 24 h after a P2Y12 loading dose, unless patients were already on antiplatelet therapy for a previous coronary revascularisation, whereas in the above mentioned studies platelet function was assessed before PTA.[10,15,22] In the clinical setting of acute coronary syndromes, only platelet function assessment after percutaneous coronary intervention was significantly associated with the occurrence of cardiovascular events. It is likely that platelet reactivity detected in the acute phase of the disease (both coronary and peripheral artery) reflects the presence of an "aggressive" blood, which may play a key role in making the patient a "vulnerable" one. A complex network of pro-inflammatory and anti-inflammatory cytokines,[23] and an increased platelet turnover with a higher number of reticulated platelets may be the determinants of HPR in the acute phase of the disease. Indeed, reticulated platelets, which are the youngest platelets released into the circulation from the bone marrow, rich in mRNA content and particularly hyper-reactive, have been associated with adverse outcomes in ACS patients.[24]

The different methods used to determine HPR should also be taken into account to explain the different results obtained. In the present study, HPR was assessed by LTA on platelet rich plasma, which is considered the gold standard for platelet function assessment, and was used by the present and other groups to define the HPR by AA cutoff value of 20%, which was that associated with adverse cardiovascular outcomes in ACS patients.[18] Moreover, the definition of the best cutoff value for identifying PAD at higher risk of clinical outcomes is a crucial point too. In PAD patients treated with aspirin, the whole platelet aggregation induced by AA was completely inhibited in all patients, whereas only 40% of patients showed the expected effect

Table 4. Univariable and multivariable analysis for bleeding.

	Univariable HR (95% CI)	*p*	Multivariable HR (95% CI)	*p*
Males/Females	0.80 (0.31—2.05)	.642	1.33 (0.49—3.56)	.574
Age	0.95 (0.92—0.99)	.019	0.95 (0.91—0.99)	.022
BMI $\geq$ 30 kg/m^2	0.51 (0.11—2.34)	.389	1.68 (0.36—7.94)	.513
Smoking	1.43 (0.52—3.91)	.491	1.27 (0.46—3.52)	.652
Hypertension	1.24 (0.34—4.49)	.745	1.45 (0.40—5.21)	.567
Dyslipidaemia	2.14 (0.27—17.11)	.475	1.51 (0.15—14.88)	.725
Diabetes	1.06 (0.43—2.65)	.899	0.67 (0.26—1.74)	.410
Renal failure	1.07 (0.29—3.96)	.918	1.76 (0.47—6.60)	.405
Ejection fraction $<$ 45%	1.76 (0.67—4.64)	.252	2.44 (0.93—6.39)	.070
Fontaine classification (I+IIa+IIb vs III+IV)	4.90 (1.99—12.04)	.001	1.23 (0.40—3.42)	.698
LTA-ADP $<$ 41%	14.96 (5.18—43.21)	$<$.001	9.28 (3.21—26.87)	.001

HR = hazard ratio; BMI = body mass index; LTA-ADP = light transmission aggregometry by adenosine diphosphate.

of aspirin on whole platelet aggregation induced by ADP or collagen.[10] Interestingly, only patients with HPR by ADP and collagen, but not by AA were at high risk of re-occlusion following peripheral angioplasty. In a prospective study which enrolled 98 PAD patients treated by PTA and followed for 12 months, the point-of-care assay PFA-100 was used, and the authors defined as non-responders to antiplatelet therapy those patients who had epinephrine closure time $<$ 170 s or ADP closure time $<$ 120 s.[22] There was no evidence of a greater incidence of complications in aspirin non-responders, whereas patients with clopidogrel resistance experienced a higher incidence of restenosis or re-occlusion after peripheral PTA compared with clopidogrel responders. However, the small number of aspirin non-responders observed in this study (5 patients) reduces the significance of these data. Furthermore, the choice of PFA-100 as a method to investigate clopidogrel and aspirin resistance, and the cut off values used for HPR definition are questionable. Consistent with the present results, a recent paper demonstrated that aspirin resistance by means of the VerifyNow Aspirin Assay was highly prevalent (25.8%) and was an independent predictor of death, myocardial infarction, or ischaemic stroke in symptomatic PAD patients.[25]

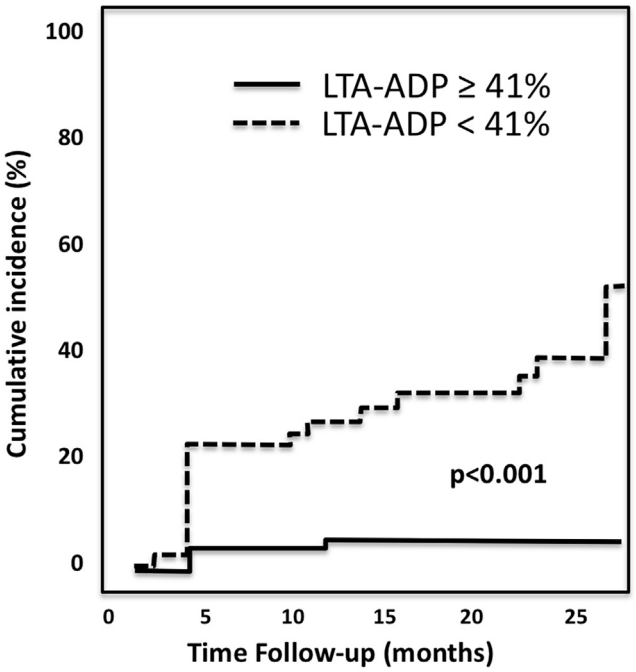

Figure 2. Kaplan—Meier survival curves for bleeding in relation to LTA-ADP. LTA-ADP = light transmission aggregometry by adenosine diphosphate.

Consistent with the results obtained in the clinical setting of acute coronary syndromes, in PAD patients in the present study the dual antiplatelet resistance (HPR by ADP and AA) is a strong and independent predictor of death, evidencing that the different pathways involved in platelet reactivity (i.e. cyclooxygenase-1 and P2Y12) should both be adequately inhibited by antiplatelet treatment to reduce mortality in these patients.

The present study has shown for the first time in the clinical setting of PAD that patients with bleeding complications had significantly lower values of LTA by ADP than those without; moreover, a LTA by ADP value $<$ 41% was identified as cut off independently associated with bleeding events during follow up. A possible link between low on-treatment platelet reactivity and bleeding has also been reported in ACS patients undergoing percutaneous coronary intervention.[6] The finding of excessive inhibition of platelet function as a risk factor for bleeding suggests the hypothesis that a "therapeutic window", that is an optimal range of P2Y12 inhibition exists, within which the predicted risk of ischaemic and bleeding complications is the lowest.

The present results strengthen and extend to PAD patients the evidence that impaired and reduced inhibition of platelet function by clopidogrel and aspirin in the acute phase of the disease is associated with worse subsequent clinical outcomes, underlining the importance of optimal platelet inhibition.

It must be recognised that, even if DAPT has mainly replaced mono-antiplatelet therapy and it is recommended after arterial endovascular revascularisation, recommendations vary. A recent meta-analysis of randomised controlled trials comparing DAPT with mono-antiplatelet therapy after peripheral arterial revascularisation documented no clear benefit of DAPT over mono-antiplatelet therapy.[26]

The strengths of this study were the duration of the follow up (about 23 months), which is longer than in the other published studies, and the evaluation of both platelet hyper- and hypo-reactivity in PAD patients.

Limitations

The study suffers from some limitations. First, the lack of further blood sampling to determine if platelet hyper- or hypo-reactivity detected in the acute phase of the disease is maintained over time, and second, whether changes in platelet response during follow up could be associated with clinical outcomes.

Although patients included in this study were high risk patients, a low rate of myocardial infarction and cerebrovascular events was documented during follow up. Therefore it was not possible to definitively exclude the association between the above mentioned outcomes and platelet hyper reactivity in PAD patients.

CONCLUSIONS AND FUTURE DIRECTIONS

In this study a high prevalence of HPR by AA and HPR by ADP was found, which were both significantly associated with risk of death; low on-treatment platelet reactivity by ADP was significantly associated with bleeding. These results document that the entity of platelet inhibition is associated with both thrombotic and bleeding complications in PAD patients. Future studies are needed to evaluate the potential utility of assessing platelet function, in the clinical setting of PAD, to ensure the patient gets the best tailored antiplatelet therapy.

CONFLICT OF INTEREST

None.

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REFERENCES

1 van Haelst STW, Koopman C, den Ruijter HM, Moll FL, Visseren FL, Vaartjes I, et al. Cardiovascular and all-cause mortality in patients with intermittent claudication and critical limb ischaemia. *Br J Surg* 2018;**105**:252—61.
2 Singh P, Harper Y, Oliphant CS, Morsy M, Skelton M, Askari R, et al. Peripheral interventions and antiplatelet therapy: role in current practice. *World J Cardiol* 2017;**9**:583—93.
3 Sofi F, Marcucci R, Gori AM, Abbate R, Gensini GF. Residual platelet reactivity on aspirin therapy and recurrent cardiovascular events-a meta-analysis. *Int J Cardiol* 2008;**128**:166—71.
4 Wisman PP, Roest M, Asselbergs FW, de Groot PG, Moll FL, van der Graaf Y, et al. Platelet-reactivity tests identify patients at risk of secondary cardiovascular events: a systematic review and meta-analysis. *J Thromb Haemost* 2014;**12**:736—47.
5 Parodi G, Marcucci R, Valenti R, Gori AM, Migliorini A, Giusti B, et al. High residual platelet reactivity after clopidogrel loading and long-term cardiovascular events among patients with acute coronary syndromes undergoing PCI. *JAMA* 2011;**306**:1215—23.
6 Parodi G, Bellandi B, Venditti F, Carrabba N, Valenti R, Migliorini A, et al. Residual platelet reactivity, bleedings, and adherence to treatment in patients having coronary stent implantation treated with prasugrel. *Am J Cardiol* 2012;**109**:214—8.
7 Spiliopoulos S, Pastromas G. Current status of high on-treatment platelet reactivity in patients with coronary or peripheral arterial disease: mechanisms, evaluation and clinical implications. *World J Cardiol* 2015;**7**:912—21.
8 Pastromas G, Spiliopoulos S, Katsanos K, Diamantopoulos A, Kitrou P, Karnabatidis D, et al. Clopidogrel responsiveness in patients undergoing peripheral angioplasty. *Cardiovasc Intervent Radiol* 2013;**36**:1493—9.
9 Spiliopoulos S, Pastromas G, Katsanos K, Kitrou P, Karnabatidis D, Siablis D. Platelet responsiveness to clopidogrel treatment after peripheral endovascular procedures: the PRECLOP study: clinical impact and optimal cutoff value of on-treatment high platelet reactivity. *J Am Coll Cardiol* 2013;**61**:2428—34.
10 Mueller MR, Salat A, Stangl P, Murabito M, Pulaki S, Boehm D, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemost* 1997;**78**:1003—7.
11 Linnemann B, Prochnow S, Mani H, Schwonberg J, Lindhoff-Last E. Variability of non-response to aspirin in patients with peripheral arterial occlusive disease during long-term follow-up. *Ann Hematol* 2009;**88**:979—88.
12 Linnemann B, Schwonberg J, Toennes SW, Mani H, Lindhoff-Last E. Variability of residual platelet function despite clopidogrel treatment in patients with peripheral arterial occlusive disease. *Atherosclerosis* 2010;**209**:504—9.
13 Madsen EH, Gehr NR, Johannesen NL, Schmidt EB, Kristensen SR. Platelet response to aspirin and clopidogrel in patients with peripheral atherosclerosis. *Platelets* 2011;**22**:537—46.
14 Saunders J, Nambi V, Kimball KT, Virani SS, Morrisett JD, Lumsden AB, et al, ELIMIT Investigators. Variability and persistence of aspirin response in lower extremity peripheral arterial disease patients. *J Vasc Surg* 2011;**53**:668—75.
15 van der Loo B, Braun J, Koppensteiner R. On-treatment function testing of platelets and long-term outcome of patients with peripheral arterial disease undergoing transluminal angioplasty. *Eur J Vasc Endovasc Surg* 2011;**42**:809—16.
16 Guirgis M, Thompson P, Jansen S. Review of aspirin and clopidogrel resistance in peripheral arterial disease. *J Vasc Surg* 2017;**66**:1576—86.
17 Yeo KK, Armstrong EJ, López JE, Chen DC, Westin GG, Li CS, et al. Aspirin and clopidogrel high on-treatment platelet reactivity and genetic predictors in peripheral arterial disease. *Catheter Cardiovasc Interv* 2018;**91**:1308—17.
18 Gori AM, Marcucci R, Paniccia R, Giusti B, Fedi S, Antonucci E, et al. Thrombotic events in high risk patients are predicted by evaluating different pathways of platelet function. *Thromb Haemost* 2008;**100**:1136—45.
19 Bovill EG, Terrin ML, Stump DC, Berke AD, Frederick M, Collen D, et al. Hemorrhagic events during therapy with recombinant tissue-type plasminogen activator, heparin, and aspirin for acute myocardial infarction: results of the Thrombolysis in Myocardial Infarction (TIMI), phase II trial. *Ann Intern Med* 1991;**115**:256—65.
20 Guo B, Tan Q, Guo D, Shi Z, Zhang C, Guo W. Patients carrying CYP2C19 loss of function alleles have a reduced response to

clopidogrel therapy and a greater risk of in-stent restenosis after endovascular treatment of lower extremity peripheral arterial disease. *J Vasc Surg* 2014;**60**:993—1001.

21 Leunissen TC, Peeters Weem SM, Urbanus RT, den Ruijter HM, Moll FL, Asselbergs FW, et al. High on-treatment platelet reactivity in peripheral arterial disease: a Pilot study to find the optimal test and cut off values. *Eur J Vasc Endovasc Surg* 2016;**52**:198—204.

22 Ziegler S, Maca T, Alt E, Speiser W, Schneider B, Minar E. Monitoring of antiplatelet therapy with the PFA-100 in peripheral angioplasty patients. *Platelets* 2002;**13**:493—7.

23 Gori AM, Cesari F, Marcucci R, Giusti B, Paniccia R, Antonucci E, et al. The balance between pro- and anti-inflammatory cytokines is associated with platelet aggregability in acute coronary syndrome patients. *Atherosclerosis* 2009;**202**:255—62.

24 Cesari F, Marcucci R, Gori AM, Caporale R, Fanelli A, Casola G, et al. Reticulated platelets predict cardiovascular death in acute coronary syndrome patients. Insights from the AMI-Florence 2 Study. *Thromb Haemost* 2013;**109**:846—53.

25 Pasala T, Hoo JS, Lockhart MK, Waheed R, Sengodan P, Alexander J, et al. Aspirin resistance predicts adverse cardiovascular events in patients with symptomatic peripheral artery disease. *Tex Heart Inst J* 2016;**43**:482—7.

26 Peeters Weem SM, van Haelst ST, den Ruijter HM, Moll FL, de Borst GJ. Lack of evidence for dual antiplatelet therapy after endovascular arterial procedures: a meta-analysis. *Eur J Vasc Endovasc Surg* 2016;**52**:253—62.