

Variability of non-response to aspirin in patients with peripheral arterial occlusive disease during long-term follow-up

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Abstract Non-responsiveness to aspirin as detected by laboratory tests may identify patients at high risk for future vascular events. The aim of this prospective study was to evaluate whether non-responsiveness to aspirin is stable over time. Ninety-eight patients with stable peripheral arterial occlusive disease (PAOD) treated with 100 mg/d aspirin were followed over a median timeframe of 17 months. Platelet function tests were performed initially and at follow-up using arachidonic acid-induced light transmittance aggregometry (LTA) in native platelet-rich plasma with the Behring Coagulation Timer® and by measuring the collagen-epinephrine closure time (CT) on a Platelet Function Analyzer (PFA-100®). When determining platelet function using LTA, four patients (4.1%) had residual platelet function (i.e., MaxAggr $\geq 78\%$) despite aspirin treatment, whereas, according to the PFA-100® results, 12 patients (12.2%) were identified as non-responders (i.e., CT < 192 s). Fifty-seven patients who were still under treatment with 100 mg/d aspirin at the time of follow-up provided a second blood sample. Further platelet function tests with the PFA-100® system identified a persistent non-responsiveness to aspirin over time in three patients (5.3%) whereas four (7.0%) and 15 (26.3%) patients had changes in response status when platelet function was assessed by LTA and on the PFA-100®, respectively. We conclude that true non-responsiveness to aspirin is a rare phenomenon in stable PAOD patients.

Furthermore, we conclude that in a number of patients, aspirin non-responsiveness is not stable over time.

Keywords Platelet reactivity · Aspirin · Peripheral arterial occlusive disease · Aggregometry · PFA-100®

Introduction

Atherosclerosis is the underlying cause of arterial occlusive disease, and thrombosis is known to be the main cause of acute ischemic events. Peripheral arterial occlusive disease (PAOD), ischemic heart disease (IHD), and cerebral artery disease are all manifestations of atherosclerosis. Thus, it is not surprising that these conditions coexist in the majority of patients. Peripheral arterial occlusive disease patients generally have widespread arterial disease and, in addition, are at increased risk of myocardial infarction, stroke, and cardiovascular death. Ischemic heart disease causes 40–60% of deaths among PAOD patients, while cerebral artery disease accounts for 10–20% of deaths [1].

Platelets play a central role in arterial thrombosis, which develops on the surface of a ruptured atheromatous plaque or as a consequence of an arterial endothelial lesion. Therefore, anti-platelet therapy has become a cornerstone of therapy not only in secondary prevention of cardiovascular and cerebrovascular disease, but also in PAOD patients [1, 2]. The Antithrombotic Trialists' Collaboration meta-analysis found that, among 9,214 patients with PAOD in 42 trials, aspirin (acetylsalicylic acid; ASA) at doses of 80 to 325 mg/d lowered the risk of serious vascular events by 23% [3]. Patients with intermittent claudication, those with peripheral bypass or endarterectomy, and those with peripheral angioplasty all benefited to a similar degree. The Physicians' Health Study, a primary prevention study,

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found that 325 mg ASA taken every other day decreased the need for surgical peripheral arterial reconstructive surgery. However, no difference was observed between the ASA and the placebo group with respect to the development of intermittent claudication [4].

Acetylsalicylic acid acts as an inhibitor of platelet aggregation. ASA irreversibly inhibits cyclooxygenase-1 by acetylating a serine residue at position 529, thereby preventing the conversion of arachidonic acid to the unstable prostaglandin intermediate PGH₂, which is then converted to thromboxane TxA₂, a potent vasoconstrictor and platelet agonist. Regular administration of low-dose ASA (e.g., 100 mg/d) usually results in >95% inhibition of thromboxane generation. Therefore, therapeutic monitoring has been considered unnecessary. However, data exist that suggest that patients on ASA who experience a cardiovascular event have a worse prognosis than patients not taking ASA [5]. This led to the suggestion that ASA might be ineffective in certain patients. Other investigators demonstrated that the anti-platelet properties of ASA can vary between individuals, and that recurrent events in some patients may be explained by residual platelet function due to “ASA resistance” or “non-responsiveness” [6, 7].

Light transmittance aggregometry (LTA) is a widespread routine test for the assessment of platelet function and its pharmacologic inhibition [8]. However, LTA is time-consuming, not yet standardized, and its use is mostly limited to specialized laboratories [9]. Therefore, interest has increased in whole blood point-of-care methods (e.g., PFA-100®), which offer the possibility of rapid and reliable identification of patients who do not respond to ASA, and which may be used at the patient's bedside on a daily basis.

Few studies have investigated the efficacy of ASA therapy in PAOD patients. The objective of our study was to determine the prevalence of non-responsiveness to ASA in patients with clinically stable PAOD, as indicated by LTA and by means of the PFA-100® Platelet Function Analyzer. In addition, we evaluated whether non-responsiveness to ASA was persistent during long-term follow-up.

Material and methods

Study population

This study comprised 98 consecutive PAOD patients (66 males, 32 females) seen in our outpatient department and treated with 100 mg/d ASA as the only antithrombotic drug. Patient ages ranged from 44–90 years (median 67.7 years). PAOD was defined as an ankle brachial index ≤ 0.9 , as measured by the Doppler ultrasound technique. The disease was required to be in a stable

condition, i.e., without any clinical deterioration within the last 3 months. ASA therapy was performed over a period of at least 14 days. Patients with additional medication known to influence platelet function (e.g., non-steroidal anti-inflammatory drugs, NSAIDs) were excluded, as well as patients with known coagulation disorders, liver cirrhosis, alcohol abuse, end-stage renal failure, malignant disease, or other concomitant diseases with a life expectancy lower than 1 year. A platelet count $<100 \times 10^9/l$, a hemoglobin <9 g/dl, and a haematocrit $<28\%$ were additional exclusion criteria. This study was approved by the local Ethics Committee, and all patients provided written, informed consent to participate. Using a standardized questionnaire, clinical data detailing vascular and concomitant disease, cardiovascular risk factors, and co-medication were recorded. In the case of a preceding acute cardiovascular event (e.g., acute coronary syndrome), patients were assessed at least 4 weeks after the cardiovascular event. Compliance with ASA therapy was determined by interview both at study commencement and at follow-up. All patients confirmed that they had taken ASA regularly as directed over the previous 14 days. The last ASA dose was administered 1 to 24 h before blood sampling.

Follow-up

Of the 98 patients included in this study, 88 were invited for a second blood sampling after a median time of 17 months (range=10–37). Ten patients (10.2%) could not be followed-up. Of these, four patients had died and six patients were lost to follow-up due to a change of residence. Of those who were contacted, 16 (16.3%) refused to attend our outpatient department for a second blood sampling because they were too ill or otherwise no longer willing to participate. Seventy two patients (73.5%) were able and willing to provide a second blood sample. Of those, a few patients were no longer taking ASA ($n=6$) or their dosage had been increased ($n=3$). Of the remaining 63 cases (64.3%), seven patients had been placed on a combination therapy with clopidogrel (75 mg/d). Changes of antithrombotic medication had been indicated mainly due to a major cardiovascular event or the need for a surgical or catheter-based interventional revascularization procedure. One patient had refused any antithrombotic treatment due to becoming asymptomatic during the follow-up period. These patients were excluded from repetitive assessment of platelet function. Thus, 57 patients (58.2%), who were receiving 100 mg/d ASA monotherapeutically at the time of follow-up and who had not experienced any acute cardiovascular event during the last 4 weeks, were eligible to provide a second sample. As described above, platelet function tests at follow-up required a platelet count $\geq 100 \times$

$10^9/l$, a hemoglobin concentration ≥ 9 g/dl, and a hematocrit $\geq 28\%$.

Blood sampling

Blood was drawn by clean venipuncture from an antecubital vein using a 21-gauge butterfly cannula system (Multifly®-Set, 21 G \times 1.5 TW, 0.8 \times 19 mm, Sarstedt, Nümbrecht, Germany). EDTA and citrate (0.129 M (3.8%) and 0.106 M (3.2%) trisodium citrate)-supplemented blood was collected using plastic syringes (Monovette®, Sarstedt, Nümbrecht, Germany). Platelet count was measured on the Sysmex® KX-21 (Roche Diagnostics, Basel, Switzerland), an automatic multi-parameter blood cell counter. Platelet counts between 100 and 500/nl were necessary for subsequent platelet function testing. Platelet-rich plasma (PRP) was obtained by centrifuging 0.106 M (3.2%) citrated whole blood at room temperature at 140 $\times g$ for 5 minutes. To produce platelet-poor plasma (PPP, platelet count <10/nl), citrated whole blood was centrifuged more vigorously at 1500 $\times g$ for 15 min. The time interval between blood sampling and testing was at least one hour and did not exceed 3 h. All platelet function tests were performed in duplicate.

Light transmittance aggregometry (LTA)

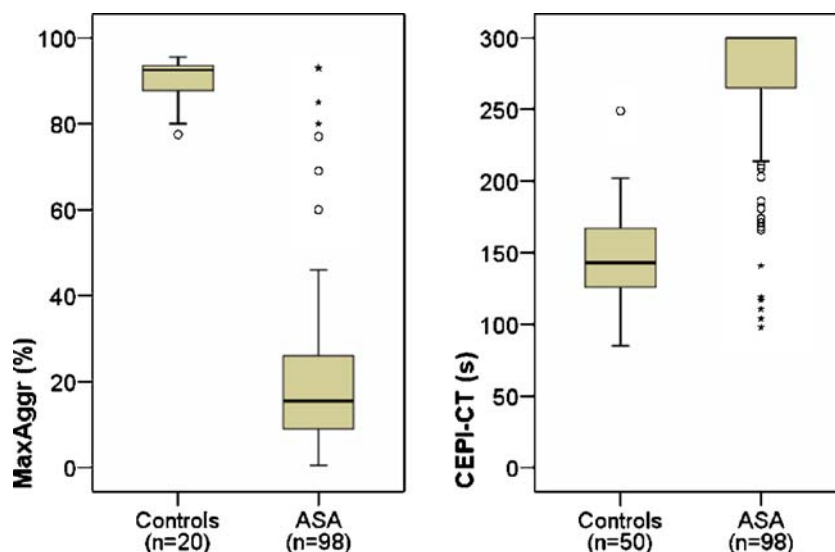
Classical LTA was performed on the Behring Coagulation Timer® (BCT®; Dade Behring, Düdingen, Switzerland). The BCT® is a fully automatic machine used for both routine and special coagulation testing. The BCT® detects platelet aggregate formation in PRP by monitoring changes in light transmission (monochromatic light; wavelength 620 nm) at 37°C. Platelet aggregation agonists (15 μ l reagent) were

automatically introduced to PRP (135 μ l plasma) by stirring at a velocity of 600 rpm. In this study, arachidonic acid (ARA; Moelab, Hilden, Germany), at a final concentration of 0.5 mg/mL, was used to stimulate aggregation. The extent of induced aggregation was defined by the slope of the aggregation curve obtained from the change in light transmission over time. The maximum aggregation response (MaxAggr), which is seen approximately 90 s after addition of the agonist, was recorded. Light transmission was measured in PRP at the beginning and at the time of MaxAggr and was compared to that in PPP. Maximum aggregation was calculated from the formula

$$\text{MaxAggr}[\%] = 100\% \times \frac{(\text{start aggr PRP} - \text{max. aggr PRP})}{(\text{start aggr PRP} - \text{aggr PPP})}$$

In accordance with recommendations given at the 53rd Annual Scientific and Standardization Committee Meeting of the International Society of Thrombosis and Haemostasis (ISTH), Geneva 2007, the 5th–95th percentile of maximum aggregation measured in duplicate in a group of healthy volunteers ($n=20$) was considered as the reference range (i.e., 78% to 96%). In a previous investigation, we also determined the within-day precision of LTA by drawing blood samples from five healthy volunteers, performing platelet function testing on five consecutive days, and calculating the coefficient of variation (CV). The CV using 0.5 mg/ml ARA as the agonist in non-adjusted PRP was <3% [9]. We demonstrated that MaxAggr using ARA at a concentration of 0.5 mg/mL in non-adjusted PRP differentiates well between ASA responsive patients and healthy subjects (Fig. 1). Non-responsiveness to ASA was defined as the maximum aggregation values within the reference range (i.e., $\geq 78\%$)

Fig. 1 Maximum aggregation values on the BCT System and CEPI-CT values on the PFA-100® system comparing healthy subjects and PAOD patients receiving ASA medication (100 mg/day) at baseline



despite ASA medication. An example aggregation recording of one patient who was responsive to ASA and another who was classified as a non-responder is presented in Fig. 2.

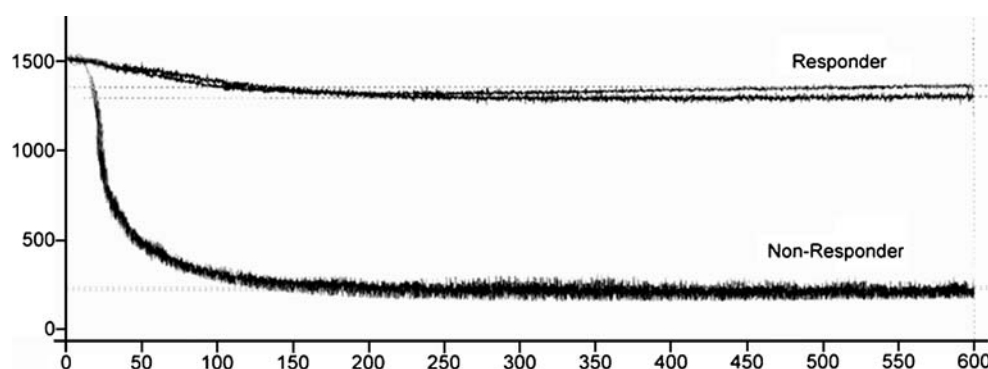
Platelet Function Analyzer PFA®-100

The PFA-100® (Dade Behring, Marburg, Germany) is a point-of-care method for assessing high shear stress-dependent platelet function by simulating the complex process of primary hemostasis *in vitro*. The device aspirates 800 µl of a whole blood sample that is anti-coagulated with 0.129 M (3.8%) trisodium citrate at high shear rates (5,000–6,000 s⁻¹) through a 147-µm diameter aperture in a capillary membrane coated with 2 µg equine type I collagen and 10 µg epinephrine bitartrate (CEPI-cartridge). The membrane triggers platelet adhesion, activation, and aggregate formation, leading to occlusion of the central aperture and cessation of blood flow. The time required for complete occlusion of the aperture and interruption of blood flow is defined as the closure time (CT). The maximum allowable value for CT is 300 s, and values greater than 300 s are reported as non-closure. The reference range of <192 s was previously determined by our group from the 95th percentile of measurements in a group of 50 healthy volunteers. Fig. 1 illustrates the distribution of the CT values in ASA-treated patients in comparison to the group of healthy volunteers. A CT within the reference range was considered to indicate non-responsiveness to ASA.

Assessment of vascular events and revascularization procedures

All vascular events and revascularization procedures (i.e., peripheral arterial catheter intervention or vascular surgery) that occurred during the observation period were assessed by interview. Reported events were only considered if they occurred under treatment with 100 mg/d ASA and if they were confirmed by medical reports from general practitioners or admitting hospitals. The composite endpoint of a cardiovascular event was defined as the composite of death from cardiovascular cause, myocardial infarction, acute coronary syndrome (ACS), stroke, amputation, or gangrene.

Fig. 2 Example aggregation curves of LTA with ARA 0.5 mg/ml on the BCT® System (duplicate measurement) for a responder and a non-responder to ASA. The graph shows the change in light transmission (extinction mE) over time (seconds)



Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 15.0, Chicago, IL, USA). Besides descriptive statistics with frequencies, mean and standard deviation, median and range, we performed the chi-squared and Fisher's exact tests using cross-tabulations. Cohen's kappa was calculated as a measure of the agreement between LTA and PFA-100®. The criterion for statistical significance was a *p* value of less than 0.05. Results are also presented as box plots with the bare length indicating the interquartile range (25th–75th percentile). Outliers are defined as values differing 1.5–3.0 bare lengths, whereas extreme values are those differing >3.0 box lengths from the upper or lower edge of the box. In the figures, outliers are illustrated as circles and extreme values as stars.

Results

Ninety-eight PAOD patients (66 males, 32 females) aged 44–90 years (median=67.7 years) who were taking 100 mg/d ASA as an antithrombotic therapy for at least 14 days were enrolled in this study. The prevalence of cardiovascular risk factors and comorbidities are presented in Table 1.

Prevalence of non-responsiveness to ASA

With the use of the aforementioned cut-off values, the percentages of patients in the total cohort (*n*=98) with non-responsiveness to ASA were 4.1% (*n*=4) when performing LTA with 0.5 mg/ml ARA, and 12.2% (*n*=12) when measuring the CEPI-CT on the PFA-100®. The agreement between the two tests was low ($\kappa=0.14$, *p*=ns). Only two patients did not respond to either method.

Variability of responsiveness to ASA during long-term follow-up

When repeating LTA in 57 patients at follow-up after a median period of 17 months, we identified 53 patients

Table 1 Baseline characteristics of the study cohort ($N=98$): prevalence of vascular risk factors and comorbidities

	<i>N</i> (%)
Vascular risk factor	
Smoking	31 (31.6%)
Arterial hypertension	84 (85.7%)
Diabetes mellitus	45 (45.9%)
Hyperlipidemia	76 (77.6%)
Obesity ($BMI \geq 30 \text{ kg/m}^2$)	16 (18.0%)
Chronic renal failure	36 (36.7%)
Peripheral arterial occlusive disease (PAOD)	98 (100.0%)
Prior amputation	4 (4.1%)
Prior vascular surgery	11 (11.2%)
Prior foot ulcer/gangrene	11 (11.2%)
Prior catheter intervention (PTA/Stenting)	47 (48.0%)
Ischemic heart disease (IHD)	47 (48.0%)
Prior myocardial infarction	22 (22.4%)
Prior aorto-coronary bypass surgery	17 (17.3%)
Prior percutaneous coronary intervention (PCI)	26 (26.5%)
Cerebrovascular disease (CVD)	23 (23.5%)
Prior cerebral infarction	9 (9.2%)
Carotid artery stenosis/occlusion	16 (16.3%)
Prior carotid artery revascularisation (stent-PTA or surgery)	4 (4.1%)

(96.5%) who were responders in both examinations, while four (7.0%) showed a change in responsiveness (i.e., two initial non-responders became responders and two responders became non-responders over time; Fig. 3). No patient showed non-responsiveness in LTA at both visits. When repeating tests using the PFA-100®, 39 patients (68.4%) presented with a prolonged CT in both examinations and 15 (26.3%) showed a change in responsiveness to ASA (Fig. 3). The remaining three patients (5.3%)

presented with a normal CT despite ASA medication at both visits.

The scatter plots in Fig. 4 show the absolute values of MaxAggr on the BCT® and the CTs on the PFA-100®. The diagrams show that a change in responsiveness, which was observed when comparing test results at baseline and at follow-up, was not solely due to small changes in values at or near the cut-off in the majority of cases.

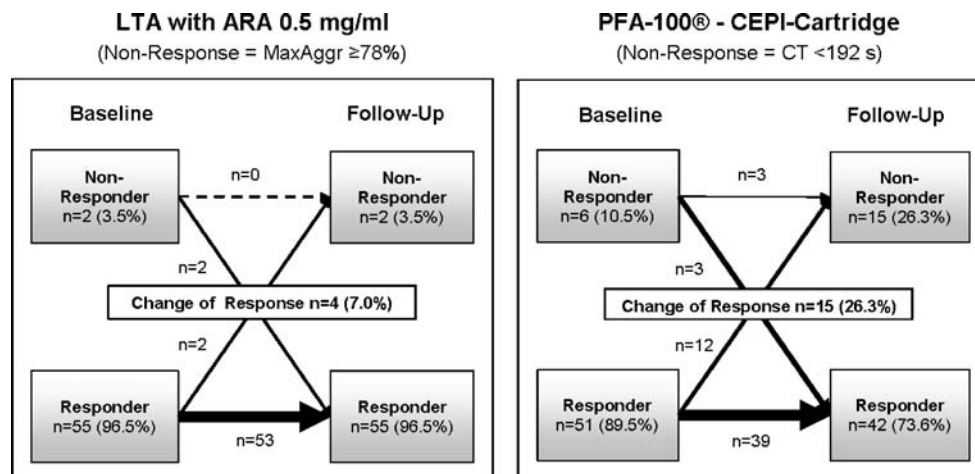
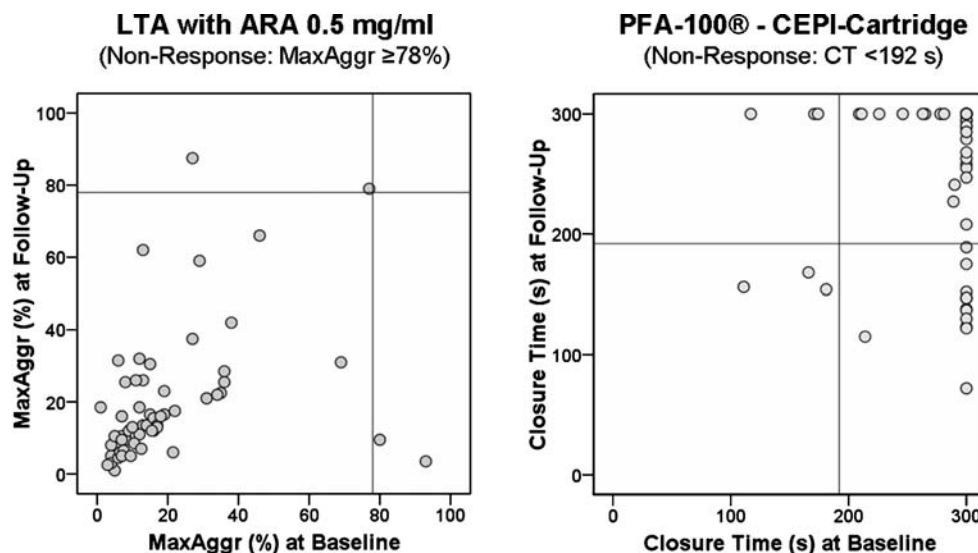
Fig. 3 Responsiveness to aspirin at baseline and at follow-up as determined by LTA and on the PFA-100® system

Fig. 4 Absolute aggregation values of LTA and the PFA-100® CEPI-cartridge at baseline and at follow-up. The inserted lines represent the cut-off values used to differentiate between responders and non-responders



Comparison of LTA and PFA-100® test results in intermittent non-responders to ASA

Test results from the subgroup of patients who exhibited a change in ASA responsiveness as determined either by LTA or on the PFA-100® system at follow-up ($n=18$) are summarized in Table 2. Only two patients (Patient No. 1 and No. 4) showed consistent test results in LTA and on the PFA-100® in one of both measurements. Of the three persistent non-

responders on the PFA-100® system, one showed a persistently good inhibition of platelet function when measured by LTA, whereas the other two were identified as intermittent responders by LTA during long-term follow-up. The remaining 15 patients with residual platelet function in one test were shown to have inhibited platelet function in the other test system and vice versa. Thus, no patient showed consistent results in the two test systems when the results of both blood samples were considered.

Table 2 Comparison of aggregation results determined by LTA with 0.5 mg/ml ARA and by using the CEPI-cartridge on the PFA-100® in patients with intermittent non-responsiveness to ASA ($n=18$) either at baseline or at follow-up (R responder, NR non-responder)

Patient no.	1st blood sample			2nd blood sample		
	LTA	PFA-100®	NR in both test systems	LTA	PFA-100®	NR in both test systems
1	NR	NR	Yes	R	NR	No
2	NR	R	No	R	R	No
3	R	NR	No	NR	R	No
4	R	NR	No	NR	NR	Yes
5	R	NR	No	R	NR	No
6	R	NR	No	R	R	No
7	R	NR	No	R	R	No
8	R	R	No	R	NR	No
9	R	R	No	R	NR	No
10	R	R	No	R	NR	No
11	R	R	No	R	NR	No
12	R	R	No	R	NR	No
13	R	R	No	R	NR	No
14	R	R	No	R	NR	No
15	R	R	No	R	NR	No
16	R	R	No	R	NR	No
17	R	R	No	R	NR	No
18	R	R	No	R	NR	No

Clinical outcome according to ASA responsiveness

Data on clinical outcomes after a median time of 17 months (range 10–37 months) were available from all patients whose platelet function was assessed twice ($n=57$). During this period, four patients (7.0%) had experienced a cardiovascular event (ACS $n=2$, stroke $n=2$), and 19 patients (33.3%) had required a peripheral arterial revascularization procedure because of clinical deterioration of PAOD. Regarding patients with a persistent non-response or a change of responsiveness to ASA during follow-up, we did not observe any severe cardiovascular event in this subgroup. The number of peripheral arterial revascularization procedures was increased among PAOD patients with persistent or intermittent ASA non-response when compared to patients with stable responsiveness, but due to small patient numbers, this was not definitely conclusive (Table 3).

Drop-out patients

In a notable number of patients, a second blood sample could not be obtained. Among those were four patients who had died and another 16 in whom antithrombotic medication had been changed, mainly due to an acute cardiovascular event (i.e., acute coronary syndrome and/or PCI ($n=7$), cerebral infarction ($n=1$)) or the need for a peripheral arterial revascularization (i.e., surgical or catheter interventional procedure ($n=6$)) during follow-up. When compared to patients who were seen for follow-up, we did not observe any difference in the prevalence of ASA non-responsiveness in the group of drop-out patients. There were 4.9% (2/41) and 14.6% (six out of 41) ASA non-responders in this group when tested by LTA and on the PFA-100®, respectively. However, in the group of patients who provided a second blood sample at the time of follow-up, the corresponding values were 3.5% (two out of 57) and 10.5% (six out of 57; $p=ns$). The four patients who had died were all responders to ASA when tested by LTA, and only one of them exhibited a

normal CT with the CEPI-cartridge when tested on the PFA-100® system.

Discussion

Our study investigated platelet function by means of ARA-induced LTA and with the CEPI-cartridge on the PFA-100® in 98 subjects with PAOD treated with 100 mg/d ASA as the only antithrombotic drug. Our results demonstrate that persistent platelet reactivity, despite ASA treatment, is very uncommon. LTA and the PFA-100® system obviously identified different patients with residual platelet function despite ASA therapy. Moreover, non-responsiveness to ASA was not stable over time. During long-term follow-up, a notable number of patients switched between responsiveness and non-responsiveness.

The prevalence of non-responsiveness, defined as aggregation values within the reference range despite administration of ASA medication, was 4.1% by LTA and 12.2% by the PFA-100® when determined at baseline. These results correspond to those of Gum et al. [10], who investigated stable cardiovascular patients in a former study. Using LTA and the PFA-100®, non-responsiveness was present in 5.5% (17/326) and 9.5% (31/326) of patients, respectively. In the literature, the prevalence of ASA non-responsiveness is reported to vary from 0–57%, depending on the method used to assess platelet function, the definition of non-responsiveness, and the population studied. According to a recent meta-analysis, non-responsiveness is less prevalent when assessed by LTA using arachidonic acid as the agonist (i.e., 6%) relative to point-of-care methods such as the PFA-100® (i.e., 26%) [11].

The fact that LTA and the PFA-100® system identify different patients as ASA non-responders has been attributed to the fact that the two methods measure different aspects of platelet function. ARA-induced LTA is a specific test for ASA inhibition of thromboxane A₂ synthesis, whereas the PFA-100® system is considered non-specific

Table 3 Cardiovascular events and peripheral arterial revascularization procedures in patients with persistent or intermittent non-responsiveness to ASA relative to those with stable inhibition of platelet function

	Persistent non-response ($n=0$)	LTA with ARA 0.5mg/ml			PFA-100® with CEPI-cartridge			
		Intermittent non-response ($n=4$)	Stable responsiveness ($n=53$)	p value	Persistent non-response ($n=3$)	Intermittent non-response ($n=15$)	Stable responsiveness ($n=39$)	p value
Peripheral arterial revascularisation	–	2 (50.0%)	17 (32.1%)	0.575	2 (66.7%)	6 (40.0%)	11 (28.2%)	0.328
PTA/stenting	–	1 (25.0%)	12 (22.6%)	–	1 (33.3%)	3 (20.0%)	9 (23.1%)	–
Bypass surgery or TEA	–	1 (25.0%)	5 (9.4%)	–	1 (33.3%)	3 (20.0%)	2 (5.1%)	–

and may be influenced by several additional factors. Shear stress in LTA is low, while the PFA-100® is a high shear stress test of platelet function. Therefore, closure time, as determined on the PFA-100®, is strongly influenced by the amount of von Willebrand factor (vWF) [12]. Increased plasma vWF levels and a higher vWF-ristocetin cofactor activity have been related to shorter CT values on the PFA-100®, and these patients may be considered poor responders to ASA [13, 14]. In our study, the agreement between the LTA and PFA-100® test results was poor, as only two patients were identified as non-responders using both methods (2.0%). This discrepancy has been previously described by other investigators [10, 15].

An important finding from our study is that non-responsiveness to ASA is not stable over time. During the observation period, four of 57 patients (7.0%) switched between responsiveness and non-responsiveness when tested by LTA, whereas on the PFA-100® system, 15 patients (26.3%) changed the status of their response. No patient showed persistent non-responsiveness to ASA when platelet function was assessed by LTA, and only three patients had a persistent normal CT (i.e., <192 s) on the PFA-100® when platelet function tests were repeated after a median time of 17 months. However, two of these patients simultaneously showed good inhibition of platelet aggregation as assessed by LTA, and this would seem to rule out true resistance to ASA. LTA test results in the other patient indicated a change of ASA responsiveness over time. Based on repeated platelet function testing, persistent resistance to ASA seems to be extremely rare in stable PAOD patients; this has been reported before for both healthy subjects and IHD patients [16, 17].

Our results also demonstrate that the PFA-100® CEPI-cartridge is not very sensitive to ASA therapy. The rate of non-responders and those who change response status over time according to the PFA-100® results is much higher than the LTA data would suggest. In a recently published investigation, duplicate measurements of CT with the CEPI-cartridge were performed in ASA-treated patients. The results revealed an inconsistent classification as responder or non-responder in about 29% of patients [18].

A change in response to ASA over time has also been observed by other investigators. Andersen et al. repeated CT measurements with the CEPI-cartridge on the PFA-100® after a period of 4 months in IHD patients treated with 160 mg/d ASA. They reported that 10% (four of 40) of patients changed from being a responder to a non-responder and vice versa [19]. In an earlier study, Helgason et al. observed changes in the response to ASA in about every third patient receiving 325 mg/d ASA for secondary prevention after stroke, when repeating ARA-induced LTA after 6 months [20]. Just recently, Harrison et al. published data that also indicated a poor agreement of test results

from LTA, the PFA-100®, and the VerifyNow® Aspirin system over a 1-year period [21].

Several factors might explain the changes in responsiveness. First, methodological inconsistency should be considered. However, in a previous investigation, we determined the within-day precision of LTA performed on the BCT® system to be very good (CV <3%) [9]. With respect to the PFA-100® system, CVs of 9–13% were reported [12]. Additionally, non-compliance with ASA therapy, inadequate dose response, or interactions with other drugs are also possible [22, 23].

In a study by Schwartz et al., non-compliance was considered the main reason for “resistance” to ASA [22]. In their study, 17/190 patients were identified as non-responders to ASA when assessing platelet function using LTA. However, after confirmed observations of ASA compliance and after a further LTA test session, only one patient who admitted having taken an NSAR within the last 12 h presented with a persistent non-inhibition of platelet function. Corresponding results were found by other investigators, and non-compliance was related to a poor clinical outcome in those cases as well [17, 24]. In our study, compliance was assessed by patient self-reporting, making it impossible to rule out non-compliance with the directed medication regimen. All patients stated that they had taken ASA regularly for the past 14 days. Importantly, no patient was identified as a persistent non-responder by both test systems over time. Thus, it seems unlikely that changes of responsiveness in our study could be attributed to non-compliance alone. However, two measurements revealed a consistent non-response with both test systems thus indicating that non-compliance may explain a change of responsiveness at least in some cases.

The status of platelets in some patients might change over time, for instance, in relation to changing disease activity and related differences in platelet activation pathways. Increased platelet reactivity and diminished platelet inhibition by ASA have been described in patients with acute coronary syndromes and after coronary artery bypass and carotid surgery (LTA) [25–27]. Because of this, we performed platelet function tests at least 4 weeks after a preceding acute ischemic event.

Thus, it ultimately remains unclear why certain patients switch between responsiveness and non-responsiveness over time. A fluctuation in the antithrombotic effect of ASA may put patients at risk of developing thrombotic vascular events when platelet aggregation is not completely inhibited. Unfortunately, the total number of cases in our study is too small to answer the question of whether adverse patient outcomes can be predicted through detection of persistent or intermittent residual platelet function by means of LTA or on the PFA-100® system. In recent years, several studies have investigated if laboratory-

assessed non-responsiveness to ASA is predictive of future ischemic vascular events, primarily in the case of patients with IHD or cerebrovascular disease [28–30]. The authors confirmed that residual platelet function in ASA-treated patients was associated with an unfavorable outcome. In the majority of studies published so far, platelet function tests were performed at study entry and were not repeated during follow-up.

Some limitations of the present study require consideration. First, tests to detect non-responsiveness to antithrombotic therapy should ideally be performed before starting ASA treatment and under steady-state conditions. However, this is not realistic in clinical practice. The majority of our patients were on ASA medication long before study entry, and it would have been unethical to stop anti-platelet therapy to obtain aggregated measurements without drug interference, as this would have placed patients at higher risk for vascular thrombotic events. Secondly, a major drawback of our study is that we did not measure serum thromboxane B2 (TxB2), which reflects the degree of enzymatic inhibition of platelet COX-1 [31]. However, TxB2 levels are not specific because thromboxane is also generated by monocytes, endothelial cells, and platelets through the action of COX-2 [32]. In our study the anti-platelet effects of ASA were characterized by measuring ARA-induced platelet aggregation, which reflects thromboxane-dependent platelet function. Additionally, we assessed the CT using the CEPI-cartridge on the PFA-100®, a widely used whole blood point-of-care method. To date, there has been no agreement on how to define non-responsiveness to anti-platelet therapy. We classified a patient as being non-responsive to ASA if aggregation test results of ARA-induced LTA and the PFA 100® CEPI-cartridge were within the reference range determined as the 5th–95th percentile in a group of healthy subjects [9]. This approach is in accordance with the recommendations given at the 53rd Annual Scientific and Standardization Committee Meeting of the ISTH, Geneva 2007.

Third, platelet function tests were repeated in only 57/98 patients (58%) after a median of 17 months. The drop-out rate was high, but this can be largely explained by an increased dose, change of antithrombotic medication or serious comorbidity that prevented the patient from returning to our facility for follow-up. A selection bias can be ruled out insofar as non-responsiveness to ASA was no more prevalent among the drop-out patients that we were able to monitor only once at baseline.

Despite these limitations, our data confirms that true non-responsiveness to ASA is a rare phenomenon in stable PAOD patients treated with 100 mg/day ASA. Due to the inconsistency in test results obtained with the two different functional methods and because of the fluctuations in responsiveness to ASA over time, repeated assessments of

platelet function may be helpful to identify patients with consistent or intermittent non-responsiveness to ASA. Additional work is required to standardize and validate laboratory tests of the anti-platelet properties of ASA, as well as to answer the question of whether routine screening for ASA non-responsiveness might provide any benefit to PAOD patients.

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