ARTYKUŁ ORYGINALNY / ORIGINAL ARTICLE

Predictors of successful acetylsalicylic acid resistance suppression after percutaneous coronary revascularisation

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Abstract

Background and aim: There is no established management of resistance to acetylsalicylic acid (ASA) in patients with coronary artery disease (CAD). We hypothesised that simply doubling the usual daily dose of ASA could be effective in overcoming ASA resistance.

Methods: Our study comprised 40 subjects with CAD (male 67.5%, mean age 60.5 ± 8.8 years, mean body mass index 26.9 ± 2.7 kg/m² and median aspirin reaction unit [ARU] value obtained with a VerifyNow Aspirin Test 612 [573–634]) with resistance to 75 mg/daily ASA defined as ARU ≥ 550. According to the overcoming of resistance or lack thereof in a repeated test after four weeks of 150 mg daily ASA treatment, we defined two subsets: subjects who regained ASA sensitivity, and those who did not.

Results: Successful overcoming of ASA resistance was observed in 62.5% of patients. Multivariate analysis regression confirmed that two variables independently determined successful ASA resistance suppression: male gender (OR 6.88; 95% CI 1.29–36.75; p = 0.024), and ARU for 75 mg daily (OR 0.97 per unit at 75 mg; 95% CI 0.94–0.99; p = 0.039). ROC analysis indicated that the threshold value at which ARU at 75 mg ASA treatment was predictive of successful ASA resistance overcoming was ≤ 608 ARU. Using a simple point score (one point for male gender and one for initial ARU ≤ 608), we found that ASA resistance was overcome in 8%, 36% and 56% of patients, when zero, any single, or two predictors were present.

Conclusions: ASA resistance overcoming by dose doubling can be achieved more often in males and in subjects with lower ARU value at ASA 75 mg.

Key words: resistance to acetylsalicylic acid, coronary artery disease, rapid platelet function assay, VerifyNow Aspirin Test, antiplatelet therapy

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of mortality in urbanised countries. Acetylsalicylic acid (ASA) irreversibly inhibits cyclooxygenase (COX) by acetylation of serine 529 in the catalytic centre of COX-1 and serine 516 in human COX-2. As a consequence, it inhibits the transformation of arachidonic acid (AA) into prostaglandin H₂ and the synthesis of thromboxane A₂.

A suboptimal response to ASA may be defined and measured by laboratory tests such as adenosine diphosphate (ADP) and AA-induced aggregometry, rapid automated methods, including rapid platelet function assay, such as VerifyNow or PFA-100, and measurements of plasma and urine concentrations of thromboxane B₂ metabolites. ASA resistance, defined as preserved aggregation capability due to insufficient blockade of platelet COX-1 in patients using chronic preventive doses of the drug, has been recently recognised as a common and potentially significant clinical problem.

The estimated prevalence of resistance to ASA depends on the laboratory method, but may affect as much as half of the subjects who are treated with this drug. However, reported resistance rates vary markedly, which may partly...
depend on the inherent limitations of some assays to detect ASA-mediated biological effects, or to underlying platelet reactivity variability independent of ASA-mediated cyclooxygenase-1 inhibition [1].

ASA resistance has adverse clinical consequences [2]. In the population of 15,603 subjects of the CHARISMA trial, there was a significant interaction between bleeding and potency of antiplatelet therapy for all-cause (p = 0.002), cardiovascular (p = 0.02), and cancer mortality (p = 0.03) [3]. In a study of 120 diabetic patients treated with primary percutaneous coronary intervention (PCI) due to ST elevation myocardial infarction (STEMI), increased platelet activity was related to a higher rate of restenosis and recurrent acute coronary syndrome (ACS) during a six month follow-up [4]. Similarly, in a study on 83 STEMI patients treated with primary PCI, excessive platelet activation during reperfusion despite dual antiplatelet therapy was associated with greater microvascular impairment [5]. Also, Foussas et al. [6] reported that ASA resistance detected using a PFA-100 analyser was the most potent predictor of one year cardiovascular mortality in patients with non-ST segment elevation ACS (HR = 2.8; 95% CI 1.7–4.6; p < 0.001).

The principal aims of this study were: (1) to evaluate the prevalence of suboptimal response to treatment with ASA in a population of Polish CAD patients treated in a tertiary centre with PCI based on rapid platelet function assay (VerifyNow Aspirin Test); and (2) to assess whether doubling of initial daily ASA dose from 75 to 150 mg would be effective in restoring reactivity to ASA and to identify clinical predictors of a positive response.

METHODS
Screened cohort

Over a period of two years, a group of 299 patients was screened for ASA resistance. The inclusion criterion was the presence of CAD (at least one coronary stenosis > 70% or left main coronary stenosis > 50%) in recent coronary angiography including the subjects who had undergone coronary angioplasty (elective or due to ACS) justifying chronic use of ASA 75 mg/day. ASA resistance was defined as an aspirin reaction unit (ARU) value ≥ 550 in a VerifyNow Aspirin Test, whereas ASA sensitivity was defined as ARU < 550. Successful restoration of ASA reactivity (i.e. overcoming ASA resistance) was defined as a change in ARU from an initial value ≥ 550 to less than 550. Patients with contraindications to ASA, treated with non-steroidal anti-inflammatory drugs and vitamin K antagonists, with neoplastic diseases, thrombocytopenia (platelet count < 50 × 10^3/µL), chronic inflammatory diseases including: autoimmune, coeliac, inflammatory bowel, sarcoidosis, vasculitis, asthma and rheumatoid arthritis or with an estimated life-span below six months were excluded from the study. Standard treatment according to the current guidelines of the European Society of Cardiology included 75 mg daily ASA dose (enteric coated tablets) taken for the median time of 70 ± 33 days before screening [11]. All patients received standardised pharmacological treatment typical for CAD including beta-blockers, statins, angiotensin converting enzyme inhibitors or angiotensin receptor blockers. Diabetic patients were treated with metformin.

Informed, written consent was obtained from all subjects and the Bioethics Committee of the Medical University of Lodz approved this research project.

Target study group (resistant patients)

A VerifyNow Aspirin Test was initially performed in the population of 299 subjects; those who had an ARU value ≥ 550 were included in the intervention group composed of 40 CAD patients with ASA resistance. The initial test was performed at baseline during chronic (at least four weeks) administration of 75 mg ASA daily, and the test was repeated four weeks after the initiation of the investigated intervention — treatment with ASA 150 mg daily, to redefine the resistance status.

Platelet reactivity testing

The study protocol included two measurements of platelet reactivity in all subjects using a VerifyNow Aspirin Test (Accumetrics Inc.) which is a qualitative test of platelet function due to aspirin ingestion in citrated whole blood applicable in the point-of-care or laboratory setting. Whole blood samples were collected 2–4 h after the ingestion of a morning ASA dose from a peripheral vein using a 21 gauge or larger needle in a partial fill 3.2% citrate vacuum collection tube.

The VerifyNow Aspirin Test utilises AA as the agonist to quantify the antiplatelet effect of ASA specifically along this pathway using light transmittance to detect aggregation of platelets in whole blood samples. The therapeutic range of ARU values in patients on ASA treatment varies from 350 to 549 [12]. This assay is not designed for use in patients with underlying congenital platelet abnormalities, with non-aspirin induced acquired platelet abnormalities, or receiving non-aspirin antiplatelet agents.

Study treatment

To test the possibility of overcoming ASA resistance, all participants were switched to daily dose 150 mg (the same pharmaceutical form) for the following four weeks. Depending on the ASA resistance or lack thereof in the repeated test (using
the same definition of ASA resistance), two groups were distinguished: group I — patients in whom sensitivity to ASA was restored (ASA-150 sensitive); and group II — patients who remained resistant (ASA-150 resistant). Clinical and demographic data of both groups was then analysed to define the predictors of successful restoration of ASA resistance by higher daily dose.

**Statistical analysis**
Continuous variables were expressed as mean (± standard deviations) for normally distributed data or median (with interquartile range) values. Categorical variables were expressed as counts and percentages. The D’Agostino-Pearson test was used to determine normal distribution. Student’s T-test was used for normally distributed continuous variables, otherwise Mann-Whitney’s test was applied to assess the differences between the ASA 150 mg sensitive and resistant groups. Differences in the distribution of categorical variables were tested using Fisher’s exact test. The predictors of a satisfactory response to increased ASA 150 mg daily dose were defined using univariate logistic regression and used to build a multivariate regression model based on variables tested in Table 1 containing independent predictors of successful ASA resistance overcoming. Receiver operating characteristic (ROC) curve analysis indicated the threshold value of ARU at 75 mg ASA daily providing optimal prediction of sufficient response to be 15 mg ASA dose. Statistical analysis was performed with a Medcalc 12.2.1.0 (MedCalc Software, Belgium) package.

**RESULTS**
Forty (13.4%) patients were identified as resistant to ASA within the screened population of 299 participants (mean age 60.5 ± 10.5 years, mean body mass index [BMI]...
27.1 ± 3.8 kg/m², median ARU 472 [249–665], male gender 65%, arterial hypertension 58.5%, diabetes mellitus 26% and atrial fibrillation (AF) 12%. No significant differences in the ARU values were present at baseline between men and women: 593 (552–663) vs. 613 ± 30, p = 0.32, respectively.

The final subset of 40 patients with confirmed ASA resistance had a mean age of 60.5 ± 8.8 years, mean BMI 26.9 ± 2.7 kg/m², median ARU 612 (573–634) and included 27 (67.5%) males. Ten out of 40 patients had recent (i.e. within the previous month) coronary angioplasty due to ACS (5/10) or elective indication (5/10), and the remaining 30 of the 40 were tested after an elective PCI earlier in their history. Ten subjects after recent angioplasty were additionally treated with clopidogrel 75 mg/day. Patients with AF did not receive oral anticoagulation and were treated with parenteral low molecular weight heparin over the study period. Clinical characteristics of the study group are presented in Table 2.

Successful overcoming of resistance to ASA using a 150 mg dose was confirmed in 62.5% (n = 25) of patients. Individual changes in ARU values from at baseline to after four weeks of 150 mg daily ASA treatment are presented in Figure 1. An analysis of factors related to the overcoming of ASA 75 mg resistance is shown in Table 1. There were differences between the ASA-150 sensitive and resistant groups regarding the prevalence of male gender (p = 0.006), ARU value during 75 mg daily aspirin treatment (p = 0.01), and white blood cell count (6.8 × 10³/µL ± 1.8 × 10³/µL vs. 8.4 × 10³/µL ± 2.7 × 10³/µL; p = 0.039). A trend towards lower platelets count was noted in the ASA-150 sensitive group (227.5 × 10³/µL ± 53.3 × 10³/µL vs. 264 × 10³/µL ± 60.5 × 10³/µL; p = 0.053). Qualitative clinical parameters such as time from PCI to first ARU measurement, type of index event: (ACS vs. unstable angina) or concomitant medications (clopidogrel, heparin) had no statistically significant impact on the prevalence of ASA resistance or the chance of successfully overcoming the resistance phenomenon.

Based on univariate predictors of overcoming ASA resistance (male gender, lower values of ARU for 75 mg ASA and white blood cell count), multivariate logistic regression revealed that only two variables (p = 0.0011 for model) independently predicted overcoming of ASA resistance: male gender (OR...
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Table 3. Predictors of successful overcoming of ASA resistance status in univariate and multivariate logistic regression

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>7.87</td>
<td>1.78–34.83</td>
<td>0.004</td>
</tr>
<tr>
<td>ARU at 75 mg ASA</td>
<td>0.97*</td>
<td>0.95–0.99</td>
<td>0.003</td>
</tr>
<tr>
<td>White blood cell count [× 10³/µL]</td>
<td>0.72</td>
<td>0.51–1.01</td>
<td>0.353</td>
</tr>
<tr>
<td>Multivariate analysis</td>
<td></td>
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</tr>
<tr>
<td>Male gender</td>
<td>6.88</td>
<td>1.29–36.75</td>
<td>0.024</td>
</tr>
<tr>
<td>ARU at 75 mg ASA</td>
<td>0.97*</td>
<td>0.94–0.99</td>
<td>0.039</td>
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*per unit; ARU — aspirin reaction unit; ASA — acetylsalicylic acid; ARU at 75 mg ASA — ARU at 75 mg ASA daily obtained in VerifyNow Aspirin Test

DISCUSSION

The most important finding of our study is that it is possible to overcome the resistance to 75 mg aspirin daily dose by doubling the standard dosage of 75 mg, which is usually recommended for the secondary prevention of ischaemic heart disease. Such management is simple, cheap and according to our findings can be especially promising in male patients with moderately elevated ARU values at a standard ASA dose.

ASA dose increase: clinical aspects

The prevalence of ASA resistance in our study was slightly more than 13%, which was less than in previously published data [8, 13, 14]. One of the possible mechanisms of ASA resistance is suboptimal dosage of this drug. According to current guidelines, the preventive dose of ASA, which is the most frequent recommendation, differs from 75 to 160 mg, and maximally up to 325 mg daily. A maintenance dose of 75 to 100 mg ASA daily is believed to have the same efficacy as higher doses, and carries a lower risk of gastrointestinal intolerance which may require drug discontinuation in up to 1% of the population [15]. Bringing the patient to a higher dosage and a higher level of platelet inhibition might increase the risk of bleeding. We did not specifically address this issue in our short-term study. But based on a meta-analysis of 24 randomised controlled trials covering almost 66,000 participants which evaluated the incidence of gastrointestinal (GI) haemorrhage associated with long-term aspirin therapy (75–1,500 mg daily), no evidence exists that such a dose change significantly affects the incidence of GI haemorrhage [16]. Thus, we considered the tested regimen of increased enteric-coated ASA dose to be a safe treatment modification with no expected impact on bleeding risk.

The response to antithrombotic drugs is subject to various factors, dependent on the specific medications, potent-related factors and drug interactions [17]. Lee et al. [8] confirmed that a daily ASA dose ≤ 100 mg is related to a 2-fold increase in ASA resistance rate (VerifyNow Aspirin Test) in patients with stable CAD (95% CI 1.12–4.44; p = 0.022) [8]. Malý et al. [18] studied this phenomenon in 424 subjects with stable ischaemic heart disease using two different tests: platelet aggregation induced by propyl gallate (CPG) and assessment of platelet function by PFA-100. Among the patients studied by CPG, ASA resistance rate decreased from 12.1% (100 mg ASA daily) to 7.6% (after increasing the dose of ASA to 200 mg) and in only 20% of patients was the daily ASA dose of less than 100 mg sufficient to inhibit platelet function. Using the PFA-100 test, the authors estimated ASA resistance to occur in 15.2% of treated patients although the study protocol did not allow the detection of differences between low and high doses. Data from this study indicates that increasing the ASA dose from 100 to 200 mg per day almost halved the risk of resistance. This is consistent with the findings from our research, in which the success in overcoming ASA resistance was 62.5%. It is very important that both doses of ASA (75 mg and 150 mg daily) used in our study are in the recommended range for patients with ischaemic heart disease.

Gurbel et al. [19] demonstrated that the assessment of ASA resistance may be assay-dependent. Assays that use AA to stimulate platelet aggregation: LTA, TEG and VerifyNow Aspirin, result in resistance estimates that are lower than methods that use stimuli other than AA. However, this study of 125 outpatients with CAD corroborates our results, which indicate that a higher ASA dose reduces the prevalence of ASA resistant patients.
Helgason et al. [20] studied the effect of ASA treatment (from 325 to 1,300 mg/daily) on platelet aggregation in 306 patients using it for ischaemic stroke prevention after two weeks and thereafter at approximately six-month intervals. At initial test, 78 subjects had partial inhibition of platelet. During the follow-up, 119 of those who had complete inhibition, and 52 who had partial inhibition, had undergone repeat testing which revealed that 39 of the 119 (32.7%) with complete inhibition at baseline converted from complete to partial inhibition without a change in aspirin dosage. Importantly, 35 patients of the 52 with partial platelet inhibition at initial measurement achieved complete inhibition either by aspirin dosage escalation (in 325 mg/d. increments) or fluctuation of response at the same dosage. Despite these findings, our study protocol assumed a short four-week follow-up to eliminate the impact of spontaneous fluctuations of platelet aggregation.

Non-compliance is another potential reason for ASA resistance and a predictor of poor outcome. Cotter et al. [21] confirmed that in a cohort of MI survivors for whom aspirin treatment was recommended, adverse events and readmissions were more common in the non-adherent group compared to an adherent group or a potentially biologically resistant group (42% vs. 6% vs. 11% and 67% vs. 11% vs. 11%, respectively). Similarly, Postula et al. [22] stated that low patient compliance (OR 0.8; 95% CI 0.20–0.35; p = 0.001) was a major factor related to non-response to ASA measured by PFA 100 test in 92 patients with stable CAD who were taking 75–150 mg of ASA daily. Available data suggests that this problem may affect up to 40% of patients treated with aspirin in secondary prevention [23]. In our study, the compliance was assessed using forms completed by respondents and was high, with 100% of patients maintaining a 150 mg dose over four weeks. Compliance assessment evaluated based on forms filled out by patients is a potential limitation. However, it can be considered acceptable for this specific group and the short duration of the follow-up, although the short duration of our study and a higher motivation level due to recent PCI might contribute to a 100% self-reported compliance rate.

Gender differences and ASA resistance
In our study, we identified a higher prevalence of male gender in the ASA 150 sensitive group compared to the ASA resistant group (84% vs. 40%, p = 0.006). Gender may play a role in response to antiplatelet drugs. In a population of 800 patients with stable CAD, gender-related differences in the concentration of inflammatory and haemostasis markers were found [24]. Women had a higher platelet count and a higher rate of platelet activation (measured by thromboglobulin excretion), which may explain this pro-aggregation tendency.

Gum et al. [25] indicated that with a standard 75 mg dosage, ASA resistance in CAD patients appears to be more prevalent in women than men. Similarly, other studies have shown that female gender was associated with a from 2- to 4-fold increased rate of ASA resistance. Dorsch et al. [26] confirmed that among patients with CAD and MI, female gender was associated with a 4-fold increased rate of ASA resistance [2, 27].

Gender-related differences in overcoming ASA resistance using dose escalation observed in our study are consistent with existing data. Becker et al. [28] confirmed that platelets in women were significantly more reactive at baseline; however after aspirin therapy, their percentage aggregation to AA decreased more than in men (p < 0.001). In multivariate analysis, female gender significantly predicted aggregation to 2 µM and 10 µM of ADP (p = 0.02 and p < 0.001, respectively) and collagen at 5 µg/mL (p < 0.001) independent of risk factors, age, race, menopausal status, and hormone therapy. Women experienced the same or greater decreases in platelet reactivity after ASA therapy, retaining modestly higher platelet reactivity compared to men. Becker et al. [28] demonstrated that healthy women treated with 81 mg ASA daily had a better platelet response than men.

Limitations of the study
Our study included only a small population of patients with CAD defined as resistant to ASA by a clinically approved point-of-care test. This decreases the statistical power of our findings including multivariate regression analysis and means that it would be necessary to find confirmatory data from a larger group. Our population was unselected, encompassing the clinical spectrum of CAD and thus heterogeneous but reflective of the clinical reality of the population treated in a tertiary cardiac centre. Our research was planned as exploratory and thus was not focused on ‘hard’ clinical endpoints but rather on surrogates defined as overcoming of aspirin resistance.

Another limitation of our study is the unknown fraction of spontaneous responders, who become ASA sensitive on a stable dose. Due to the small number of participants who met the definition of ASA resistance, we decided not to create a control group with ongoing low dose of ASA and thus could not assess how many patients changed their platelet aggregation status after four weeks of maintained 75 mg/day ASA treatment.

Another limitation of our study is the use of a single method to assess ASA resistance considering that this phenomenon is assay-dependent. However, VerifyNow Aspirin is a point-of-care, clinically accepted test, likely to be increasingly used in routine clinical management and, notably, it is the first and only CLIA-waived test (Clinical Laboratory Improvement Amendments) to help physicians assess the antiplatelet effect of ASA [12].

CONCLUSIONS
The prevalence of ASA resistance in a cohort of CAD patients treated in a tertiary care cardiac centre using a standard ASA
dose of 75 mg/day was 13.4%. A simple strategy of dose increase from 75 mg to 150 mg/day allowed for the overcom-
ing of resistance in > 60% patients. An appropriate response to 150 mg ASA was more likely in men and in subjects with lower ARU values on 75 mg ASA. These findings carry a po-
tential clinical significance, but require confirmation in larger scale studies.

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Conflict of interest: none declared

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Czynniki predykcyjne skutecznego przełamania oporności na kwas acetylosalicylowy u pacjentów po zabiegach przezskórnej rewaskularyzacji wieńcowej

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Streszczenie

Wstęp i cel: Nie ma ustalonego postępowania w przypadku oporności na kwas acetylosalicylowy (ASA) u pacjentów z chorobą wieńcową. Autorzy pracy założyli, że proste podwojenie dziennej dawki ASA może być efektywnym postępowaniem w przełamywaniu oporności na ASA.

Metody: Badaną grupę stanowiło 40 pacjentów z chorobą wieńcową [mężczyźni 67.5%, średni wiek 60.5 ± 8.8 roku, średni wskaźnik masy ciała 26,9 ± 2,7 kg/m² i mediana ARU (aspirin reaction unit) 612 (573–634)] i opornością na ASA w dawce 75 mg/d. zdefiniowaną jako wartość ARU ≥ 550 uzyskaną w teście VerifyNow Aspirin Test. W zależności od przełamania zjawiska oporności na ASA lub jego braku po 4 tygodniach leczenia ASA w dawce 150 mg/d. wyodrębniono dwie grupy: pacjentów, u których udało się uzyskać ponownie wrażliwość na ASA i osoby, które pozostały oporne.

Wyniki: Skuteczne przełamanie oporności na ASA obserwowano u 62,5% badanych. W analizie regresji wieloczynnikowej wykazano, że dwie zmienne w sposób niezależny determinują skuteczne przełamanie oporności na ASA: płeć męska (OR 6,88; 95% CI 1,29–36,75; p = 0,024) i ARU dla 75 mg ASA dziennie (OR 0,97 na jednostkę przy 75 mg ASA; 95% CI 0,94–0,99; p = 0,039). Analiza krzywej ROC pokazała, że wartość punktu odcięcia ARU dla leczenia 75 mg ASA wskazująca na skuteczne przełamanie oporności na ASA wynosiła £ 608 ARU. Budując prosty system klasyfikacji, w którym przyznawano jeden punkt dla płci męskiej i jeden dla wyjściowej wartości ARU £ 608, wykazano, że skuteczne przełamanie oporności na ASA wystąpiło u 8%, 36% i 56% pacjentów, gdy odpowiednio żaden, jeden lub dwa z wyżej wymienionych czynników były obecne.

Wnioski: Podwojenie dawki leku jest skutecznym sposobem przełamania oporności na ASA i może być częściej osiągnięte u mężczyzn oraz osób z niższymi wartościami ARU w trakcie leczenia ASA w dawce 75 mg/d.

Słowa kluczowe: oporność na kwas acetylosalicylowy, choroba wieńcowa, szybki test funkcji płytek, VerifyNow Aspirin Test, terapia przeciwpłytkowa

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