

Evaluation of aspirin resistance and the presence of unstable carotid plaque in patients undergoing carotid endarterectomy

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Abstract

Background: Antiplatelet therapy using low dose acetylsalicylic acid (ASA) is routinely recommended in patients undergoing surgical or endovascular treatment of carotid artery disease. Despite this treatment, a significant proportion of postoperative complications may result from inadequate response to antiplatelet therapy which may contribute to the development of unstable plaques.

Aim: Assessment of correlation between ASA resistance and the presence of unstable carotid plaque in patients after carotid endarterectomy.

Methods: We studied 66 patients (27 women and 39 men) aged 41–80 years who were operated for carotid stenosis. All patients received ASA (75 mg once daily) in the pre- and perioperative period. Measurements of platelet reactivity were performed using the PFA-100 platelet analyser at the second day after the operation. Plaque stability was evaluated using a computer analysis system based on the greyscale median method. The patients were divided into two groups, showing good response to ASA or ASA-resistant.

Results: ASA resistance was identified in 19 (32%) patients. In multivariate logistic regression analysis, unstable plaque showed a borderline correlation with ASA resistance ($p = 0.051$).

Conclusions: 1. Prevalence of increased platelet reactivity despite ASA treatment (ASA resistance) in patients treated surgically for carotid artery disease is high, suggesting a possibility of ineffective antiplatelet therapy. 2. A modest correlation between abnormal platelet response during treatment with ASA and the presence of unstable plaques suggests that these two phenomena may coexist but we were unable to show a clear association between them.

Key words: acetylsalicylic acid resistance, carotid endarterectomy

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INTRODUCTION

Stroke is third most common cause of mortality in the developed countries. Ischaemic strokes comprise 75–80% of all strokes, and 20% of them are due to extracranial carotid artery disease. In two thirds of patients with symptoms of cerebral insufficiency, these lesions may be removed surgically, as in most cases stenosis or obstruction is due to atherosclerotic plaques.

According to the National Stroke Association (NSA) consensus recommendations, also endorsed by the American Heart Association (AHA), internal carotid artery endarterectomy is recommended in patients with symptomatic > 70% stenosis [1]. Effectiveness of this method for primary stroke prevention was shown in the European Carotid Surgery Trial (ECST) [2] and North American Symptomatic Carotid Endarterectomy Trial (NASCET) [3] studies.

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Unstable coronary plaques are not considered a recommendation for carotid endarterectomy. Despite popular belief that stroke risk increases with the severity of coronary artery stenosis, the importance of carotid plaque morphology remains unclear [4, 5]. Ischaemic stroke may result from a decrease in cerebral perfusion distally to a site of critical stenosis (or occlusion) [6]. Multiple evidence suggest an important role of factors complicating unstable atherosclerotic plaques, including rupture, ulceration, thrombosis, or central nervous system (CNS) microembolisation [7].

Although platelet activation plays an important role in the pathophysiology of thrombosis in patients operated due to vascular diseases, factors affecting its variability in the postoperative period have not been clearly identified. Antiplatelet drugs are among major therapies to prevent thrombosis following vascular surgery, as also reflected in the current Trans Atlantic Inter-Society Consensus (TASC II) guidelines. However, the guidelines do not include any recommendations regarding the management of cases in which no expected platelet response to antiplatelet drugs including acetylsalicylic acid (ASA) is seen, and thus this issue remains unsolved.

The aim of the study was to evaluate the rate of concomitant presence of unresponsiveness to ASA and unstable carotid plaques in patients after carotid endarterectomy.

METHODS

Study group

Patients received ASA (75 mg/day) for at least 10 days before the surgery and indefinitely following the surgery. In patients receiving clopidogrel preoperatively, this drug was stopped at least 10 days before the surgery due to high risk of bleeding and bridge therapy with low-molecular-weight heparin was initiated. ASA treatment was started in these patients 2 h after the surgery (day 0).

Before the surgery, patients underwent carotid ultrasonography to evaluate the extent of atherosclerotic lesions and the degree of vessel stenosis. Images of atherosclerotic lesions were subjected to computer analysis using the grey-scale median (GSM) method to identify stable and unstable plaques, followed by carotid endarterectomy. All operations were performed in the Department of Vascular Surgery and Angiology at the Centre of Postgraduate Medical Education, Warsaw, Poland. Platelet reactivity during ASA treatment was evaluated using the PFA-100 analyser at the second day after the surgery.

The study was performed in 2010–2011 in the Department of Vascular Surgery and Angiology, Centre of Postgraduate Medical Education, and the 1st Department of Cardiology, Medical University of Warsaw, both in Warsaw, Poland.

The study was approved by the bioethics committee at the Centre of Postgraduate Medical Education, Warsaw, Poland.

Ultrasonographic analysis

All examinations were performed using the Aloka ProSound F75 system. Atherosclerotic plaques were imaged using a linear 7.5 MHz probe in longitudinal and transverse views. Images were stored digitally on an USB flash memory and then transferred to CD-ROM discs using a personal computer. Images were stored in the TIFF format and subjected to further computer analysis.

Evaluation of platelet response to ASA

After obtaining written patient consent for participation in the study, blood samples were collected to test tubes containing 3.2% sodium citrate. All collections of the biological material during the study were performed by the same person. Within 30 min to 2 h after blood sampling, platelet reactivity as expressed by clotting time (CT) was measured using the PFA-100 platelet analyser with collagen/epinephrine (CEPI) test cassettes.

The reference CT range in healthy subjects not receiving ASA was defined as 98–185 s. Patients with on-treatment CT values below 98 s were considered responders, and those with CT values in the range of 98–185 s during treatment with ASA were considered showing abnormal response to the drug.

GSM analysis

To analyse ultrasound images using the Adobe Photoshop software, the GSM method described by Nicolaides [8] was used.

Multiple studies showed a positive correlation between GSM values and the presence of unstable plaques in carotid arteries, forming the basis for a recommendation to use this method. Our study was primarily based on the findings of the multicentre ICAROS study [9], the first one to use GSM values for stroke prevention during carotid artery stenting. GSM score values > 25 were considered the criterion of plaque instability also in our study, and patients with GSM values > 25 were included in the unstable plaque group.

Statistical analysis

Variables in the study group were described using standard descriptive statistics including mean values and standard deviation (SD), medians and quartiles, and contingency tables. Variables were compared using the χ^2 test or the Student *t* test, with a correction for unequal variances if applicable.

Using stepwise elimination, we selected variables that were significant at the alpha level of 0.05, but with retention of the variables with $p < 0.1$ in the model. The predictive value of the models was evaluated using receiver operating characteristic curves, based on the area under the curve values which range from 0 to 1, with higher values indicating higher predictive values. *P* values < 0.05 were considered statistically significant. All tests were 2-sided. Calculations were performed using the Stata 7.0 software.

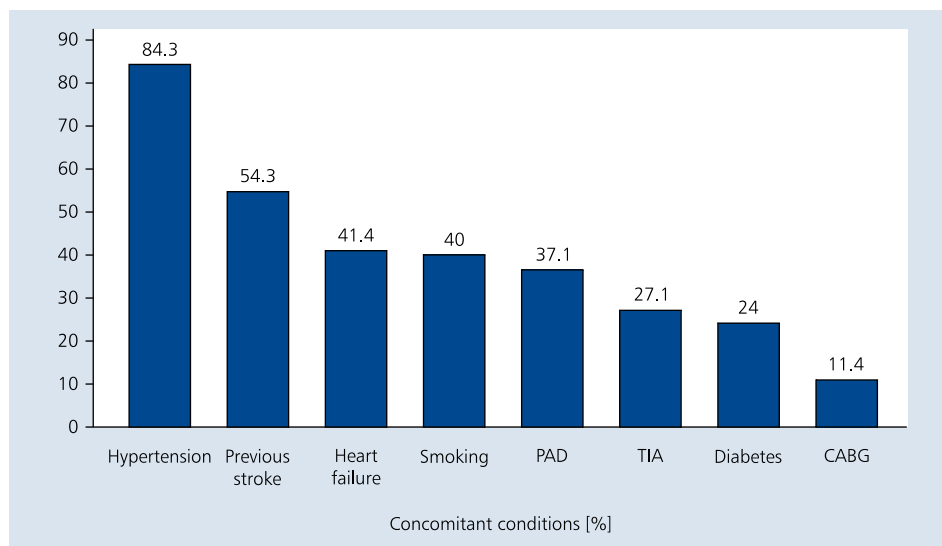


Figure 1. Concomitant conditions in patients with carotid artery disease; PAD — peripheral arterial disease; TIA — transient ischaemic attack; CABG — coronary artery bypass grafting

RESULTS

Baseline patient characteristics

We studied 66 patients referred for carotid endarterectomy due to haemodynamically significant, symptomatic ($n = 59$, 89%) or asymptomatic ($n = 8$, 11%) internal carotid artery stenosis. We operated 27 women and 39 men aged 41–80 years.

The largest age subset were patients aged 71–80 years. This group included 29 (45%) patients, followed by 23 (34%) patients aged 61–70 years, 10 (16%) patients aged 51–60 years, and 4 patients aged 41–50 years.

During preoperative evaluation, 31 (54.3%) symptomatic patients were found to have a history of stroke, 16 (27.1%) patients had a transient ischaemic attack (TIA), and other symptoms related to coronary atherosclerosis (dizziness, amaurosis fugax) were present in 11 (18.6%) patients.

Asymptomatic carotid artery stenosis ($> 70\%$) was found in 8 patients during routine diagnostic testing before other scheduled procedures such as surgery for Leriche's syndrome, abdominal aortic aneurysm, and coronary artery bypass grafting. In all these patients, carotid endarterectomy was performed prior to these planned procedures.

Concomitant conditions in patients with carotid artery disease are summarised in Figure 1.

Carotid endarterectomy was performed in 66 patients, including left-sided endarterectomy in 45 (64.7%) patients, and right-sided endarterectomy in 21 (35.3%) patients. Plaque ulceration was present in the left side in 15 (23.4%) patients and on the right side in 7 (10.8%) patients.

Twenty-seven (37.7%) patients were operated using a temporary shunt, and 39 (59.1%) patients were operated without the use of a temporary shunt.

Table 1. Collagen/epinephrine clotting time values at visit 2

Test	Overall				
	N	Mean	SD	Min	Max
PFA 100 v2 [s]	66	241.6	68.6	87	300

Characteristics of the response to ASA

In the study group, 45 (68.2%) patients showed the expected response to ASA, and 19 (31.8%) patients did not show an adequate response to ASA.

Table 1 shows the mean (with SD), minimum, and maximum CEPI CT values by PFA-100 testing.

Characteristics of atherosclerotic plaques using the GSM method

In the study group, 25 (38%) patients were found to have stable plaques by the GSM method ($GSM > 25$), and 41 (62%) patients had unstable plaques ($GSM < 25$).

Among patients with unstable plaques, increased platelet reactivity was found in 25 (59.5%) patients, and normal platelet reactivity in 16 (40.5%) patients.

In the study group, we performed logistic regression analysis to evaluate the relation between the presence of unstable plaques and an increased likelihood of increased platelet reactivity during treatment with ASA.

Our findings indicate that unstable plaques showed a borderline correlation with no response to ASA ($p = 0.051$, Table 2).

DISCUSSION

Our findings indicate a possible coexistence of unstable plaques and resistance to ASA.

Table 2. Odds ratio for plaque instability in relation to aspirin resistance

Parameter	Odds ratio	Lower confidence limit	Upper confidence limit	P
Plaque instability	1.088	0.219	7.223	0.051

This interesting coincidence raises a question whether these two abnormalities just coexist, or rather the lack of significant correlation resulted from the fact that our patient sample was too small.

The phenomenon of no expected platelet response to ASA in patients with cardiovascular disease is a subject of intensive research. In the current literature, the term ASA resistance is replaced with “inappropriate response to ASA” or similar terms, reflecting the fact that a complete lack of response to the antiplatelet effect of a drug is actually a marginal issue, while the truly significant aspects are complex problems related to platelet response that result from heterogeneity and individual variation of patients treated with ASA. This is also reflected by complex temporal dynamics of platelet reactivity during treatment. In biochemical terms, inappropriate response to ASA is manifested by inadequate platelet inhibition seen in laboratory tests [10, 11].

Numerous hypotheses have been put forward to explain the lack of expected platelet response to ASA. One hypothesis is related to turbulent flow caused by atherosclerotic plaques, leading to increased shear stress and platelet activation. The site of carotid endarterectomy also shows a predisposition to thrombosis, with subsequent embolisation and CNS ischaemia.

Based on simple, rapid bedside tests, it is possible to determine a biochemical response to ASA therapy and its changes in the perioperative period. However, most studies evaluated ASA therapy in patients with coronary artery disease, while ASA is also used to prevent thrombosis in patients with carotid artery disease. In light of multiple studies evaluating resistance to the antiaggregatory effect of ASA in the treatment of cardiac conditions, it seems interesting to evaluate this issue also in patients after carotid endarterectomy.

Depending on the patient population and the method used, the prevalence of inappropriate platelet reactivity has been estimated at 5–40% [12]. In our study, the proportion of patients showing increased platelet reactivity during treatment with ASA was 31.8%, which is consistent with the reported literature data. A systematic review of inappropriate response to the antiplatelet effect of ASA included 22 studies which used the PFA-100 platelet analyser to evaluate platelet reactivity in patients receiving ASA. Overall, the prevalence of

inappropriate platelet response to ASA as assessed using the PFA-100 platelet analyser was 37.0% [13]. In another recent metaanalysis, the proportion of patients showing inappropriate response to ASA, evaluated using various methods to test the response to ASA, was 31% [14]. These proportions were different in other studies in patients with stable coronary artery disease. Of note, the observed differences may result from two major reasons discussed in the literature, namely the criteria of resistance to ASA (based on CEPI CT using the PFA-100 analyser) and the used dose of ASA.

One problem when evaluating risk in patients referred for carotid artery surgery is the fact that the indications for surgery were based on studies that were more than 10 years old [15]. It should be noted that in these studies, the main criterion for surgery was the degree of vessel stenosis evaluated radiographically. These indications do not take into account plaque instability resulting from processes that take place in the plaque itself, while histopathological studies indicate a number of factors predisposing to microembolisation. The most important of these include the presence of a large lipid core adjacent to the vessel lumen [16], rupture of the fibrous cap, bleeding into the plaque, and the presence of an ulceration as a cause of thrombogenicity. Based on numerous recent reports indicating a major role of unstable carotid plaques in the prediction of cerebral microembolisation and stroke risk, recognition of this problem may be expected to increase and probably it will also become an indication for surgery. In our study, an increased risk of inappropriate platelet reactivity was associated with a trend for more frequent occurrence of unstable plaques, which may clinically lead to intracerebral microembolism. This may be explained by the fact that most microemboli are small thrombi detached from unstable plaque due to lacking protective antiplatelet effect of ASA.

A transient intraoperative increase in platelet reactivity was reported by Ackerstaff et al. [17] in their study on patients operated due to carotid artery stenosis. These authors evaluated transient (lasting up to several hours) increase in platelet aggregation after the surgery despite routine administration of ASA combined with intraoperative unfractionated heparin bolus during carotid endarterectomy. These findings confirm data from other reports suggesting that the antiplatelet effect of ASA in patients operated due to carotid artery disease may be transiently abolished by intraoperative heparin treatment [18]. Other studies reported that this transiently abolished antiplatelet effect of ASA in the postoperative period was associated with an increased expression of P-selectin, a receptor protein present in the platelet membrane [19], although Ackerstaff et al. [17] did not observe any significant increase in the activity of P-selectin despite the fact that a significant reduction of the antiplatelet effect of ASA was seen after the surgery. This may be explained by a low number of patients included into their study (n = 27), or increased platelet aggregation due to

other factors. One of these may be the glycoprotein IIb/IIIa receptor present on the platelet membrane, which increases platelet activity against fibrinogen [20].

Limitations of the study

As we used only one of the many available methods to evaluate platelet reactivity, we are aware of limitations of the present study, particularly in light of heterogeneous results of studies on platelet hyperreactivity during ASA treatment that were reported in the literature, resulting from a vast array of tests to evaluate platelet function. We are also aware of the fact that the method used to evaluate platelet reactivity in the present study is currently not widely recommended for research applications. The recommended approaches include testing for platelet reactivity using arachidonic acid or by indirect measurements of thromboxane A2 metabolites in serum or urine. Our choice was based on advantages of the selected device, which is easy to use and allows rapid testing at the bedside. In addition, the PFA-100 platelet analyser was approved by the Food and Drug Administration as one of the devices to evaluate platelet dysfunction.

Measurement of platelet reactivity on the second day after carotid thrombendarterectomy may also seem a limitation of our study. We believe, however, that our approach to the evaluation platelet function after the surgery is justified and may shed new light on the effectiveness of the prevention of thromboembolic complications in this patient group. In our opinion, carotid endarterectomy itself has no significant effect on long-term platelet reactivity, despite intraoperative administration of a small dose of unfractionated heparin. The action of the latter (including the antiplatelet effect) is transient and in contrast to ASA, it does not lead to complete platelet inactivation, and the drug half-life is only 30–150 min.

CONCLUSIONS

1. The prevalence of increased platelet reactivity despite treatment with ASA in patients treated surgically for carotid artery disease is high, indicating a possibility of ineffective antiplatelet therapy.
2. A modest correlation between abnormal platelet response during treatment with ASA and the presence of unstable plaques suggests that these two phenomena may coexist but we were unable to show a clear association between them.

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Conflict of interest: none declared

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Korelacja zjawiska nieprawidłowej reakcji na przeciwplatekowe działanie kwasu acetylosalicylowego i niestabilnej blaszki miażdżycowej u chorych po endarterektomii tętnic szyjnych

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Streszczenie

Wstęp: Terapia przeciwplatekowa małymi dawkami kwasu acetylosalicylowego (ASA) zalecana jest rutynowo u pacjentów leczonych operacyjnie i endowaskularnie z powodu miażdżycowego zwężenia tętnic szyjnych. Mimo tego postępowania znaczny odsetek powikłań pooperacyjnych może być spowodowany brakiem oczekiwanej reakcji na leki przeciwplatekowe mogącej być jedną z przyczyn powstania tzw. niestabilnej blaszki miażdżycowej.

Cel: Celem pracy była ocena współwystępowania nieprawidłowej reakcji płytek krwi na działanie ASA i niestabilnej blaszki miażdżycowej.

Metody: Do badania zakwalifikowano 66 pacjentów operowanych z powodu istotnego zwężenia tętnicy szyjnej wewnętrznej: 27 kobiet i 39 mężczyzn, w wieku 41–80 lat. Pacjenci w okresie okołoperacyjnym przyjmowali ASA w dawce 75 mg/d. Pomiar reaktywności płytek wykonano przy użyciu analizatora funkcji płytek krwi PFA-100 w 2. dobie po operacji. Pacjenci zostali podzieleni na grupę prawidłowo reagujących na ASA i na grupę pacjentów, których płytki krwi mimo stosowania ASA nadal charakteryzowały się zwiększoną reaktywnością. Obie grupy analizowano pod względem stabilności blaszki miażdżycowej. Ocenę stabilności blaszki miażdżycowej wykonano, wykorzystując analizę mediany GSM.

Wyniki: Nieprawidłową reakcję na ASA stwierdzono u 19 (32%) pacjentów. W modelu wieloczynnikowej analizy regresji logistycznej wykazano, że niestabilność blaszki miażdżycowej korelowała na granicy istotności statystycznej z brakiem odpowiedzi na ASA ($p = 0,051$).

Wnioski: 1. Częstość zjawiska zwiększonej reaktywności płytek krwi mimo przyjmowania ASA u pacjentów leczonych operacyjnie z powodu miażdżycowego zwężenia tętnic szyjnych jest wysoka, co wskazuje na prawdopodobny brak skutecznej terapii przeciwplatekowej tym lekiem. 2. Zaznaczona w niniejszej pracy niewielka korelacja między nieprawidłową reakcją płytek krwi po podaniu ASA a niestabilnością blaszki miażdżycowej pozwala przypuszczać, że oba zjawiska mogą ze sobą współistnieć. Jednak w niniejszej pracy, poza tendencją w kierunku współwystępowania obu zjawisk, nie wykazano definitywnie powyższej zależności.

Słowa kluczowe: endarterektomia tętnic szyjnych, aspirynooporność

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