Review Article

Clinical implications of aspirin resistance

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Summary

Aspirin reduces major atherothrombotic events across a wide spectrum of patients with atherosclerotic disease. The occurrence of ischemic events despite of aspirin treatment is a failure of therapy, often denoted 'clinical aspirin resistance'. This is distinguished from laboratory assays showing an insufficient inhibition of platelet function, which indicate 'laboratory aspirin resistance'. Laboratory aspirin resistance has been reported in up to 60% of patients after stroke or peripheral arterial disease, up to 70% in stable coronary heart disease and even up to 80% in acute myocardial infarction. However, this data must be interpreted carefully because of small sample sizes and potential confounding factors such as compliance, co-morbidities and large differences between the laboratory methods used for detection. During the past years, evidence has accumulated that laboratory aspirin resistance is associated with an increased incidence of major atherothrombotic events, with an up to 13-fold increased risk of events in patients with cardiovascular disease. Thus, an individualized antiplatelet therapy will have to consider the possibility of aspirin resistance, and the identification of aspirin non-responders may improve antiplatelet therapy in future. Whether an increased dose of aspirin or another antiplatelet drug (e.g. clopidogrel) instead or in addition to aspirin should be given is unclear. Prospective trials are underway which address this issue. This review gives an overview on the various clinical studies that have investigated the prevalence and clinical importance of laboratory aspirin resistance. Moreover, therapeutic options, as well as future perspectives are discussed.

Keywords

Aspirin resistance, atherosclerosis, platelet function, prevalence, clinical outcome

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Introduction

Since the time of its introduction, aspirin has become a cornerstone in the secondary prevention of cardiovascular events. Aspirin inhibits platelet thromboxane (TX) A_2 release by acetylation of cyclooxygenase (COX)-1 (1–5). TXA_2 potently activates platelets. Because platelets lack the synthetic machinery to generate relevant amounts of new COX-1, aspirin-induced inhibition lasts for the lifetime of the platelet.

Beyond the molecular mechanism of action, the treatment benefit of aspirin is established for the prevention of cardio- and cerebrovascular events, management of acute coronary syndromes and as an adjunct to percutaneous and surgical revascularization. The Antithombotic Trialists' Collaboration documented that aspirin reduces non-fatal myocardial infarction (MI) by one third, non-fatal stroke by one quarter, and vascular mortality by one sixth in patients with vascular disease (6).

However, not all individuals equally respond to antiplatelet therapy and thrombembolic events may occur in presence of antiplatelet therapy with aspirin. This has been called "aspirin resistance" or "non-response". A generally accepted definition based on valid diagnostic criteria has not been established.

In general, "resistance" means that a drug is unable to hit its pharmacological target, either due to the inability to reach it or as a consequence of an altered target. Accordingly, "aspirin resistance" can be defined as the inability of aspirin to inhibit COX-1-dependent TXA_2 production, and consequently TXA_2-dependent platelet functions (7). Since laboratory assays are necessary to identify this situation, the term "*laboratory resistance*" may be used as well.

On the other hand, the phenomenon that patients experience atherothrombotic events while on antiplatelet therapy has been designated "*clinical resistance*", even though "treatment failure" seems more appropriate. The problem of treatment failure is that we are unable to determine whether a patient who experienced an atherothrombotic event might have undergone a more serious or even lethal event without aspirin. Moreover, within the in-vivo or in-vitro assays available today, the ranges of "normal" and "re-

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sistant" are defined differently or not defined at all. Altogether, this leads to divergent data concerning the prevalence of aspirin resistance and divergent readouts of aspirin´s clinical impact.

Aspirin resistance has received much attention in medical journals and public media. This review focuses on clinical aspects of aspirin resistance. A short overview is also given regarding supposed mechanisms and diagnostic methods. The prevalence of aspirin resistance in different patient groups and the impact on clinical outcome are discussed in more detail. Finally, possible therapeutic approaches and future perspectives are presented.

Supposed mechanisms of aspirin resistance

The mechanisms of aspirin resistance are uncertain. Pharmacokinetic and -dynamic mechanisms are likely to play a role, including genetic, biological and clinical factors. The few known and many hypothetical mechanisms have been discussed in detail by excellent recent reviews (7–10). Above all, non-compliance may be a "mechanism" of aspirin resistance. Many studies on aspirin effects did not assess it. Non-compliance in aspirin trials may be in the range of 10% (11), but is likely higher in clinical practice. The galenic preparation may also be important: a comparison of enteric-coated and plain aspirin demonstrated the former to be less effective (12).

Probably the best established molecular mechanism of laboratory aspirin resistance is the blockade of aspirin´s target, platelet COX-1, by analgesic, antipyretic and antiphlogistic drugs, such as salicylic acid, ibuprofen, naproxen and pyrazoles (13–16). This may also be true for other organic compounds, such as gallic acid (17). COX-2-inhibitors (coxibs) and paracetamol are unlikely to interact with aspirin.

An increased sympathetic activity attenuates the antiplatelet effect of aspirin in healthy volunteers and patients with previous MI (18, 19). Following surgery or haemorrhage, an increased platelet turnover can result in a higher fraction of platelets capable to form TXA_2 in spite of aspirin treatment (20).

Alternative pathways for TXA_2 synthesis and the identification of prostaglandin-like compounds (isoprostanes) might also explain a blunted antiplatelet effect of aspirin. For example, the COX-2 isoenzyme may (besides COX-1) occur in platelets and, since it is less sensitive to aspirin, contribute to aspirin resistance (21, 22).

Platelet agonists other than thromboxane may also be important. An increased sensitivity to collagen (23) and to ADP (24) have been suggested to cause aspirin-insensitive platelet activation, since both stimuli are only partly TXA_2-independent.

Several gene variants have been identified, which potentially contribute to heterogeneous responses to aspirin. These include enzymes involved in arachidonic acid metabolism (including COX-1), platelet glycoprotein and collagen receptors, as well as proteins involved in blood coagulation (25). However, the importance of genetic mechanisms for laboratory resistance or treatment failure is uncertain.

Diagnostic methods

Only specific tests that measure the pharmacological effect of aspirin will clarify whether platelet hyperreactivity is due to an insufficient pharmacological drug effect or caused by other reasons (7). Since the definition of aspirin resistance should be limited to situations where its failure to hit the target has been documented with appropriate laboratory tests, the problem is that the biologically important platelet agonists in a particular patient are not known. Hence, laboratory assays of aspirin resistance are surrogate measures. Many tests evaluate platelet function and do not distinguish aspirin effects from other platelet disorders. Moreover, no standardized definition or laboratory test is presently available to quantify aspirin resistance in relation to normal ranges of the diagnostic methods. Nevertheless, numerous tests are available. These are summarized in Table 1 together with their advantages and limitations.

A simple method for study of natural haemostasis is the bleeding time, determined by a standard skin incision and measurement of the time until bleeding stops. The diagnostic value is very limited since it is non-specific and poorly standardized.

Probably the best evaluated method is light transmission aggregometry (LTA), which measures the change in light transmission of a platelet suspension during aggregation *in vitro* (turbidimetry according to Born). Because aspirin blocks platelet TXA_2 synthesis, LTA using arachidonc acid as agonist (about 1 mM) is an excellent assay to detect platelet inhibition by aspirin. LTA induced by low collagen concentrations (≤1 µg/ml) is also relatively dependent on TXA_2. In contrast, *ADP-induced LTA* does not necessarily involve the arachidonic acid pathway and is quite insensitive to aspirin inhibition. The effect of epinephrine in LTA is variable.

Using the same agonists, impedance aggregometry measures the change in electrical impedance between platinum-electrodes. This method of aggregation allows investigating the antiplatelet effect of aspirin in whole blood, obviating the need to prepare a platelet suspension (26, 27). Again, arachidonic acid is the optimal and most specific agonist to determine the effect of aspirin.

To reduce the laboratory requirements, several "point-of-care" assays have been developed, such as the platelet function analyzer (PFA-100) and the rapid platelet function assay (RPFA). These use whole blood and can be adapted for outpatient evaluation since analysis times are short (minutes).

The PFA-100 simulates an artificial vessel with a thrombogenic membrane coated with collagen and either epinephrine or ADP. A constant negative pressure aspirates anticoagulated blood through this vessel (a capillary mimicking the resistance of a small artery) with high shear force. A platelet plug forms and gradually occludes the aperture until flow stops. The time taken to interrupt blood flow (closure time) is recorded. The RPFA (Ultegra VerifyNow Aspirin) is a simple and rapid bedside test detecting the agglutination of fibrinogen-coated beads in response to arachidonic acid by an increase of light transmission. If aspirin produced an antiplatelet effect, the beads do not agglutinate and light transmission is unchanged.

The RPFA moderately correlated with the PFA-100 (epinephrine/collagen cartridge) with a correlation coefficient of 0.73 (28), but the prevalence of aspirin resistance in patients with coronary disease substantially differed between LTA (arachidonic acid) and PFA-100 (29). Though "point-of-care" assays are attractive for clinical practice, they are not always prospec-

Table 1: Overview on the methods currently used for the evaluation of laboratory aspirin resistance.

Test	Method / principle	Preferential use / validity	Advantages	Limitations
		In-vivo test		
Bleeding time	Measurement of the time necessary for bleeding to stop after standardized skin incision.	Influenced by several variables, including platelet count, red blood cells, vessel wall, skin trophism.	– easy and rapid to use – widely available	– invasive – operator-dependent – little sensitivity – higly variable
		Aggregometry		
Light transmission aggregation (LTA)	Analysis of light transmission after stimulation of platelets with arachidonic acid, collagen, ADP or other agonists. (– anticoagulated platelet-rich plasma)	Using the TXA_2-specific agonist arachidonic acid, this technique has been successful to study the antiplatelet effect of aspirin.	– widely available – well-established in literature	– laboratory-onsuming – operator-dependent – difficult to tandardize – limited sensitivity
Impedance aggregation ("whole blood aggregometry")	Measurement of electrical impedance between two electrodes in whole blood after stimulation with arachidonic acid, collagen, ADP or other agonists (– anticoagulated whole blood)	Less technically demanding and time- consuming than LTA. Few studies have been performed in patients on aspirin treatment.	– fast and relatively simple – allows for blood cell-platelet interactions	– may be operator-ependent – limited sensitivity
		Point-of-care assays		
Platelet function analyzer (PFA-100)	Assessment of aggregation under high shear. Whole blood is aspirated through an aperture coated with collagen and either epinephrine or ADP. (– whole blood)	Sensitive to many variables (platelet count, hematocrit, von-Willebrand-factor, others), but relatively poor correlation with serum TXB_2.	– simple – rapid – semi-automated – numerous studies	– dependence on latelet-independent actors – high cost – limited sensitivity
Rapid platelet function assay (Ultegra RPFA-ASA, VerifyNow Aspirin)	Analysis of light transmission in a test cartridge containing fibrinogen-coated beads. (– whole blood)	Probable cross-sensitivity to multiple variables (platelet count, red blood cells, platelet reactivity to collagen, von-Willebrand-factor).	– simple – rapid – semi-automated	– expensive – limited specificity – limited sensitivity – controversial orelation to ggregometry
		Thromboxane		
TXB_2	Radioimmunoassay or ELISA. TXA_2 is rapidly converted into the stable metabolite TXB_2. (– serum)	Reflects platelet capacity to form TXA_2. Most specific test to measure the antiplatelet effect of aspirin. Can be determined in conjunction with in vitro aggregation induced by various stimuli, including arachidonic acid.	– highly specific: directly depends on aspirin-induced inhibition of platelet COX-1	– time and laboratory onsuming – expensive
11-dehydro-TXB_2 (AspirinWorks and others)	Measurement using radioimmunoassay or ELISA. Systemic TXB_2 undergoes hepatic transformation into 11-dehydro-TXB_2. (Native TXB_2 in urine comes from renal tissues and does not represent platelet activation). (– urine)	This metabolite indicates TXA_2 synthesis *in vivo*. A fraction of urinary 11-dehydro-TXB_2 (about 20%) is probably not platelet-derived.	– represents platelet activity in vivo – time-integrated mesurement (hours) – non-invasive	– not entirely latelet-specific – expensive – requires correction or renal function creatinine)
		Miscellaneous techniques		
Flow cytometry	Automated laser detection of platelet activation markers (e.g. P-selectin, CD63, changes in GP IIb/IIIa complex conformation) using antibodies. (– anticoagulated whole blood)	Expensive equipment not available in many laboratories. Represents a highly specific assay for measuring the antiplatelet effect of aspirin in small volumes of whole blood. Unable to detect aggregation.	? high specificity ? high sensitivity ? high reproducibility	– expensive – experienced perator required – few studies
Thrombelastography	Analysis of clot strength from formation to lysis after stimulation with arachidonic acid or ADP. (– whole blood)	Influenced by coagulation factors, natural inhibitors of coagulation and fibrinolysis. Needs to be validated in a clinical setting.	? simple ? rapid ? semi-automated	– poor correlation to ggregometry – few studies

tively validated in patients with atherosclerotic disease and with respect to the incidence of thrombembolic events in the patients identified as aspirin resistant.

Another laboratory test for diagnosing aspirin resistance is flow cytometry, which measures activation-dependent changes in platelet surface markers (e.g. P-selectin) after stimulation by various agonists, including arachidonic acid. This method can be performed with whole blood (30), but requires some experience and an expensive equipment.

Since the antiplatelet effect of aspirin is caused by the inhibition of COX-1, TXA_2 is a particularly suitable parameter to assess the antiplatelet effect of aspirin. TXA_2 rapidly (within minutes) hydrolyzes to TXB_2, for which laboratory test kits (immunoassays) are commercially available. It has been suggested that aggregometry using arachidonic acid tends to overestimate the incidence of aspirin resistance, while TXB_2 is the most specific parameter to measure the pharmacological effect of aspirin (7). Still, TXB_2 measurements require platelets to be stimulated *in vitro*. This may be done by addition of platelet agonists, but is equally well achieved by preparation of serum at 37°C. Hepatic metabolites of TXB_2 (11-dehydro-TXB_2) may be determined in urine as a measure of TXA_2 formation *in vivo*, but a proportion of 11-dehydro-TXB_2 probably comes from other TXA_2 forming cells than platelets, which limits the specificity for aspirin resistance.

Taken together, optical and impedance aggregometry using arachidonic acid as stimulus, as well as the determination of TXB_2, are appropriate methods to determine the antiplatelet effect of aspirin. Other agonists also depend on aspirin-insensitive activation pathways, providing limited and less specific information about aspirin's antiplatelet effect. This may explain why systematic comparisons of different laboratory methods showed only a weak or no correlation among each other (7).

Prevalence of aspirin resistance and impact on clinical outcome

A discussion of aspirin resistance in clinical practice requires two questions to be addressed: first, how common is laboratory aspirin resistance and second, does it matter clinically? Table 2 gives an overview about the studies which provided data on the clinical impact on aspirin resistance in defined patient groups. An extended version of the table with additional study information, including trials without clinical outcome data, is available online at www.thrombosis-online.com.

Taking into account the unsettled use of the term "aspirin resistance" and the diverse diagnostic methods, it is not surpprising that the available prevalence data are inconsistent. For example, the prevalence estimates for laboratory aspirin resistance varies in coronary artery disease (CAD) from 5.2–69, 22.5–83.3 and 20–74% in patients with stable CAD, acute coronary syndrome (ACS), and early after coronary artery bypass grafting (CABG), respectively. Patients with cerebrovascular disease and previous stroke were aspirin-resistant in 5–60%. Moreover, 9–65% of patients with peripheral arterial disease were aspirin resistant.

Hovens et al. found wide differences in the prevalence of aspirin resistance in a meta-analysis of patients taking aspirin for secondary prevention and calculated a mean prevalence of 22.4%, 26.0% and 27.3% in patients with CAD, stroke and miscellaneous diseases, respectively (31). As expected, the analytical method was an important determinant of aspirin resistance. The prevalence was lower in studies using arachidonic acid-induced LTA (15.4%) compared with the PFA-100 method (28.1%), reflecting that the PFA-100 probably also detects TXA_2- and aspirin-independent platelet activation.

Nevertheless, there is increasing evidence that laboratory aspirin resistance is clinically important. Several follow up studies have explored the relationship among aspirin therapy, laboratory data on platelet reactivity and the risk of vascular events and these have been integrated in several large meta-analyses (32, 33), some specifically addressing the PFA-100 method (34, 35).

Krasopoulos et al. reviewed 20 studies which related laboratory aspirin resistance to cardiovascular outcome (32). Among 2,930 patients, 810 (28%) were classified as laboratory aspirin resistant. Overall, cardiovascular events occured in 39% of the patients in the aspirin-resistant cohort and 16% of the aspirin-sensitive patients (odds ratio [OR] 3.85, $p<0.001$). The OR for increased mortality in laboratory aspirin-resistant patients was even higher (OR 5.99, $p<0.003$).

Another meta analysis by Snoep et al. pooled 12 studies, comprising 1,813 patients (33). The mean prevalence of laboratory aspirin resistance was 27%. When studies with cardiovascular outcomes were pooled, an OR of 4.4 was calculated for laboratory aspirin resistance. The OR of myonecrosis after percutaneous coronary intervention (PCI) was 3.1. The pooled OR of major cardiovascular events in all included studies was 3.8. All were statisitcally significant. However, no differences were found among three groups of daily aspirin dose (≤100 mg, 101–299 mg and ≥300 mg).

Two additional meta analyses focussed on trials where aspirin non-responders were identified by the PFA-100 method. Crescente et al. analysed 53 publications reporting aspirin responses (34). The analysis included 64 patient populations with a total of 6,450 subjects. Twenty-one populations (2,283 subjects) consisted of apparently healthy subjects and 43 populations (4,167 subjects) of patients with cardiovascular disease. The prevalence of aspirin non-responders identified by the PFA-100 was significantly higher in the patients. There was no obvious relation between aspirin dose and aspirin non-response. While the prevalence of non-responders was not significantly influenced by the cut-off value of the PFA-100 closure time, the prevalence of non-responders was significantly higher when the cut-off values were established by the investigators themselves, compared with studies using the cut-off level of the PFA-100 manufacturer or from literature. Overall, the PFA-100 response to aspirin was related to the risk of vascular events, with a significantly higher relative risk of 1.63 in the aspirin non-responders.

Another meta-analysis by Reny et al. analysed seven non-prospective studies (1,466 patients) and eight prospective studies (1,227 patients) (35). In the non-prospective studies, the prevalence of aspirin non-responders ranged from 10–22% on the basis of PFA-100 cut-off values of 130–193 seconds, but a funnel plot identified publication bias. In the prospective studies, the prevalence of aspirin nonresponders ranged from 9.5–49%, on the basis of cut-off values of 170–203 seconds. The pooled

analysis of the prospective studies showed that aspirin non-response was significantly associated with an about two-fold increase of the relative risk for recurrence of ischemic events.

Therefore, both meta-analyses agreed that PFA-100-defined aspirin non-responders were more likely to have vascular events than responders. Because this assay is probably not purely dependent on platelet activation by TXA_2, it remains unproven whether this results from an absent or insufficient pharmacological effect of aspirin or simply reflects that patients with more active platelets have an impaired cardiovascular prognosis.

Taken together, there is a remarkable heterogeneity of the data, which besides methodological issues probably reflects the vast heterogeneity of the manifestations of vascular disease. The following discussion, therefore, appraises laboratory aspirin resistance in relation to the manifestations of cardiovascular disease.

Coronary artery disease (CAD)

A total of nine studies investigated the outcome of CAD patients with aspirin resistance (36–44). Of these, two trials analysed patients with a long-term follow-up (years) after MI or ACS, respectively (36, 39). Additional studies evaluated the short term outcome in the early period of ACS, elective PCI and elective CABG (45–48). Moreover, case-control studies investigated the impact of aspirin resistance on the occurrence of ACS (49, 50), on stent thrombosis (51, 52) and on bypass graft thrombosis (53). In most of the trials, platelet function was assessed by PFA-100 (7 studies), LTA (4 studies), RPFA (2 studies), thromboxane (2 studies), and bleeding time (1 study). Only two studies investigated aspirin resistance by more than one method.

In a group of 326 stable CAD patients taking 325 mg/day aspirin for at least seven days, Gum et al. noted laboratory aspirin resistance in 17 patients (5.2%) (37). This prospective, blinded study determined aspirin resistance by LTA. After a mean follow up of 1.86 years, major events (all-cause death, MI, or stroke) occurred in four of 17 patients in the aspirin-resistant group, compared with 30 of 309 patients in the aspirin-sensitive group. This difference was statistically significant ($p=0.03$). After adjustment for prognostic factors, a statistically significant 4.1-fold excess hazard was calculated ($p=0.009$), which was independent of age, gender and other conventional risk factors.

Similar results were reported by Chen et al. in a one-year follow up of 468 CAD patients receiving 80–325 mg/day aspirin (43). The prevalence of aspirin resistance was 27.4% (RPFA assay). Patients identified as aspirin-resistant had a 3.1-fold increased risk of the composite outcome of cardiovascular death, MI, unstable angina requiring hospitalization, stroke or transient ischemic attack (TIA), compared with aspirin-sensitive patients (15.6% vs. 5.3%, $p<0.001$). After correction for other risk factors, aspirin resistance was associated with a hazard ratio of 2.5.

A smaller trial by Pamukcu et al. (42) in 234 patients with stable CAD taking 100–300 mg/day aspirin identified 22.2% as aspirin-resistant using the PFA-100 (epinephrine). After a mean follow up of 20.6 months, major adverse events (MI, unstable angina, stroke, cardiac death) occurred in 15.4% of the subjects with laboratory aspirin resistance and in 11.0% aspirin-sensitive patients, which was not statistically significant ($p=0.269$). The study assigned 28 aspirin-resistant patients to additional clopidogrel therapy (75 mg/day). These experienced less frequently major adverse events compared with those not on clopidogrel or who stopped clopidogrel (see also chapter *Treatment of aspirin resistance*).

Two additional trials studied the role of aspirin resistance in secondary prevention with previous MI. Cotter et al. (38) identified 52 MI survivors with stable CAD as aspirin responders (100 mg/day), while nine patients were classified as non-compliant or aspirin-resistant, based on TXB_2 formation in response to platelet stimulation by collagen *in vitro*. Reference values of TXB_2 were obtained from patients whose adherence to aspirin was known. After one-year follow up, cardiac events (cardiovascular death, MI, unstable angina) were more common in the non-adherent/resistant group (1 of 9; 11%), compared with aspirin responders (3 of 52; 6%). While a multivariate logistic regression analysis identified aspirin non-response/resistance as risk factor for severe ischemic events (OR 5.15; $p=0.006$), the low patient number was an important limitation. The second study was a retrospective substudy of the WARIS-II trial (36), which included 129 survivors of MI receiving 160 mg/day aspirin. This study observed only a non-significant trend for a higher incidence of adverse vascular events in aspirin non-responders (PFA-100, epinephrine).

Four studies evaluated the outcome after previous ACS, among which two evaluated early (41, 44) and two long-term outcome (39, 40).

The clinical outcome in 106 ACS patients without ST-elevation undergoing PCI and stenting was evaluated by Cuisset et al. (41). The patients were divided into quartiles according to the maximal intensity of platelet aggregation (ADP and arachidonic acid-induced LTA). Those in the highest quartile were defined as "low-responders". Twelve recurrent cardiovascular events occurred within one month despite oral aspirin and clopidogrel therapy. Low responders showed a significant, almost six-fold increased risk of cardiovascular events during one month of follow-up. Valles et al. studied a group of 62 patients with ST-elevation myocardial infarction (STEMI) who received 100–500 mg/day aspirin before hospitalization or upon arrival to the hospital. Among these, 20 patients were already on chronic aspirin treatment. Partial inhibition of platelet TXA_2 synthesis after collagen stimulation in whole blood (<95% inhibition compared with aspirin-free healthy subjects) occured in 21 patients (34%). This group had significantly higher troponin T and CK-MB levels within 48 hours after hospital admission (44).

Stejsdal et al. treated a cohort of 103 CAD patients with previous ACS with 100 mg/day aspirin and measured platelet function by LTA, using the relatively nonspecific agonist propyl gallate (39). Aspirin responsiveness was defined by agonist-induced and spontaneous aggregation. Aspirin resistance occurred in 55% and was associated with an 88% incidence of recurrent events during the four-year follow up. The event rate was significantly lower (46%) in aspirin responders ($p<0.01$). More patients were aspirin-resistant at follow-up in the subgroup with recurrent cardiovascular events than in those without (72% vs. 8%; $p<0.01$). In another chronic study, 20 out of 105 ACS patients were aspirin-resistant, as identified by the PFA-100 (epinephrine) (40). During one-year follow up, aspirin was prescribed in doses of 100–300 mg/day. Major cardiovascular events occurred in nine patients (45%) with aspirin resistance and in 10 patients

(11.7%) without (p=0.001). Multivariate analysis identified PFA-100-defined aspirin resistance as an independent predictor of major cardiovascular events.

Two other studies determined platelet responses to aspirin in patients undergoing elective PCI, who also routinely received clopidogrel. Lev et al. studied 150 PCI patients taking aspirin (80–325 mg/day) for at least one week and observed a prevalence of laboratory aspirin resistance of 12.7% (46). Aspirin resistance was defined by at least two of three criteria: RPFA score ≥550, ADP-induced aggregation ≥70% and arachidonic acid-induced aggregation ≥20%. Within 24 hours after intervention, elevation of the CK-MB after stenting occurred more frequently in the aspirin-resistant patients (38.9% vs. 18.3%; p=0.04). Another study by Chen et al. examined aspirin responsiveness (RPFA assay) in patients undergoing elective PCI, receiving 80–325 mg/day aspirin for at least seven days (45). Of 151 enrolled PCI patients, 19.2% were aspirin-resistant and CK-MB was elevated in 51.7% of them, twice as high as in aspirin responders (24.6%, p=0.006). The troponin I levels achieved similar results. Aspirin resistance (OR 2.9) and bifurcation lesion (OR 2.8) were the statistically significant, independent predictors of myonecrosis in this study.

Patients who undergo CABG are at high risk of thrombotic graft occlusion and laboratory aspirin resistance seems to occur frequently in this setting (30). The Benefits and Risks of ASA on Thrombosis (BRAT) study recruited 289 patients after elective CABG taking 325 mg aspirin (47). Aspirin responses were assessed by bleeding time and platelet-derived TXB_2 in blood samples after clotting. Aspirin non-responders were defined by absence of prolongation of bleeding time. Surprisingly, platelet TXB_2 was similarly inhibited in aspirin responders and non-responders, and a two-year follow up failed to show significant differences in MI, unstable angina, cardiac death, or stroke, although there was a trend for more events among nonresponders. The authors concede that the aspirin status may have been misclassified in some patients by bleeding times and that the study was underpowered. More recently, Poston et al. evaluated thrombelastography, collagen-stimulated LTA and arachidonic acid-stimulated whole blood flow cytometry among 225 patients, who underwent elective off-pump CABG (48). Laboratory aspirin resistance was observed only in 10 patients (4%) at baseline. On days 1, 3 and 30 after CABG, 22 (10%), 67 (30%) and seven (2%) patients, respectively, were classified aspirin-resistant. During the 30 day follow-up, the incidence of thrombotic bypass graft occlusions was highest in the patients with aspirin resistance, especially in those with endothelial lesions of the graft. Multivariate logistic regression showed that aspirin resistance on day 1 was a significant, independent predictor of postoperative graft thrombosis (OR 2.59).

In addition to the cohort trials summarized above, several case-control studies demonstrated an impact of aspirin resistance on CAD complications, including ACS, stent thrombosis and thrombotic bypass graft occlusion. Among 135 patients admitted for chest pain and taking aspirin (75–300 mg/day) for at least one week, aspirin resistance (PFA-100) was 9.7% in a subgroup without cardiac disease, compared with 26.0% (n.s.) in non-ST-elevation myocardial infarction (NSTEMI) and 83.3% (p<0.001) in STEMI (49). Another study compared 104 ACS patients with 100 patients with stable CAD (54). Both groups used aspirin for the last seven days (dose unknown). Aspirin resistance (PFA-100) was significantly (p=0.04) more prevalent in ACS patients (40.3%) than in patients with stable CAD (27%). A case-control study observed aspirin resistance (LTA) more frequently in patients who developed stent thromboses after coronary stenting (11/23) than in matched controls with patent stents (16/50), but this was not statistically significant (51). A similar study analyzed 204 CAD patients with previous stent implantation among which 102 had intracoronary stent restenosis (52). Upon treatment with 100–300 mg/day aspirin, 32 patients (31.3%) with intracoronary stent restenosis and 11 patients (10.7%) with patent stents were resistant to aspirin, as detected by the PFA-100 method (epinephrine). Finally, a case-control study screened 14 CABG patients with at least one occluded saphenous vein graft after surgery for aspirin resistance (PFA-100, epinephrine) and compared them with 14 age- and gender-matched patients with well-functioning grafts on late postoperative coronary angiograms (mean follow-up 6.5 years) (53). Aspirin resistance was observed in 50% and 7.1% of the patients with and without graft occlusion, respectively (p=0.03). Being aspirin resistant increased the risk of vein graft occlusion 13-fold and multivariate linear regression analysis identified the PFA-100 closure time as an independent predictor.

In summary, laboratory aspirin resistance appears to be linked to worse long-term outcomes in patients with all manifestations of CAD, including those with previous PCI or CABG. Different methods to study the effect of aspirin, heterogeneous endpoints and the often low numbers of enrolled patients limit the comparability of the studies. The reported event ratios and outcome data are summarized in Table 2. An extended version of this table is available as supplementary material online at www.thrombosis-online.com.

Cerebrovascular disease

The clinical outcome of aspirin resistance has also been studied in patients with cerebrovascular disease. Grotemeyer et al. determined a platelet reactivity index by an assay for platelet-platelet and platelet-blood cell adhesion, performed 12 hours after an oral dose of 500 mg/day aspirin (55). Of 180 stroke patients, 33% were classified aspirin-resistant. All were prescribed 500 mg aspirin three times daily and were followed for 24 months. Among 174 patients with complete two-year follow up, the aspirin non-responders had a 9.1-fold increased incidence of stroke, MI, or vascular death in comparison with aspirin responders. Similarly, in a case-control study enrolling 53 patients treated with 100 mg/day aspirin for secondary prevention of TIA or stroke over 60 months or longer, the rate of aspirin resistance (PFA-100, epinephrine) was significantly higher in those with recurrent cerebrovascular events during a follow up period of >5 years, compared with those without recurrence (34% vs. 0%) (56).

Hence, much less data are available in patients with cerebrovascular disease than in CAD. Nevertheless, laboratory aspirin resistance seems also associated with an impaired clinical outcome in these patients.

Peripheral arterial disease (PAD)

In contrast to CAD and cerebrovascular disease, peripheral atherothrombotic events are rarely life threatening but exert a high impact on quality of life. Ziegler et al. studied 52 PAD patients

Table 2: Characteristics of clinical trials in defined patient populations which studied the clinical impact of laboratory aspirin resistance. In case control studies, prevalences of aspirin resistance are given insteead of outcome. A more extensive version which also includes trials without outcome data and includes additional information on methodology is accessible online at www.thrombosis-online.com.

Sample size	Aspirin dose / day	Clinical impact of aspirin resistance	Reference
Coronary artery disease (CAD)			
– Stable coronary artery disease			
326	325 mg	3.1-fold increased risk for composite of all-cause death, MI, or stroke (p=0.03); composite of all-cause death, MI, or stroke: – 23.5 % in aspirin resistant; – 9.7 % in aspirin sensitive (mean follow up: 1.86 years)	Gum et al, 2003 (37)
468	80 – 325 mg	3.12–fold increased risk for composite of cardiovascular death, MI, unstable angina requiring hospitalization, stroke, or TIA: – 15.6 % in aspirin resistant; – 5.3 % in aspirin sensitive (p<0.001) (mean follow up: 379 days)	Chen et al, 2007 (43)
234	100 – 300 mg (+ clopidogrel 75 mg in 28 aspirin resistant patients)	major adverse events (myocardial infarction, unstable angina, stroke, or cardiac death): – 15.4 % in aspirin resistant – 11.0 % in aspirin sensitive (p=0.269) (mean follow up: 20.6 months)	Pamukcu et al, 2007 (42)
61 (plus 12 patients with confirmed nonadherence)	100 mg	5.1–fold increased risk for severe ischemic events (p=0.006); adverse events (cardiovascular death, MI, unstable angina): – 11% (1 of 9) in aspirin resistant – 6 % (3 of 52) in aspirin sensitive cardiac readmission rate: – 11% (1 of 9) in aspirin resistant – 17% (9 of 52) in aspirin sensitive (mean follow up: 1 year)	Cotter et al, 2004 (38)
129 aspirin: 71; aspirin plus warfarin: 58	160 mg, or 75 mg plus warfarin, or warfarin alone	event rate (nonfatal MI, stroke, revascularisation): – 36 % in aspirin resistant; – 24 % in aspirin sensitive (p=0.28) (mean follow up: 4 years)	Andersen et al, 2002 (36)
103	100 mg	event rate (MI, stroke, unstable angina): – 88 % in aspirin resistant – 46 % in aspirin sensitive (p<0.01) (mean follow up: 4 years)	Stejskal et al, 2006 (39)
105	100 – 300 mg	event rate (cardiovascular death, MI, stroke, unstable angina): – 45 % in aspirin resistant – 11.7 % in aspirin sensitive (p=0.001) (mean follow up: 1 year)	Pamukcu et al, 2006 (40)
– Acute coronary syndrome (ACS)			
106	160 mg (+ clopidogrel 75 mg)	5.76–fold increased risk for recurrent cardiovascular events (95% CI: 1.54 to 35.61) (mean follow up: 1 month)	Cuisset et al, 2006 (41)
62	100 – 500 mg/d (chronic aspirin treatment in 20 patients)	CK-MB [U/L]: – 2740 ± 610* in aspirin resistant; – 1470 ± 250* in aspirin sensitive (p<0.05) TnI [ng/mL]: – 6.3 ± 1.5* in aspirin resistant; – 3.5 ± 0.7* in aspirin sensitive (p<0.05) (mean follow up: 24 – 48 h) * values estimated from figure	Valles et al, 2007 (44)

Table 2: continued

Sample size	Aspirin dose / day	Clinical impact of aspirin resistance	Reference
135 NSTEMI: 38, STEMI: 30, control group without cardiac disease: 67	75 – 300 mg (use of aspirin for at least 1 week before enrolment)	case-control–study – 9.7% aspirin resistant in control group – 26,0% aspirin resistant in NSTEMI (n.s. vs. control) – 83.3% aspirin resistant in STEMI (p<0.001 vs. control) Chronic aspirin (<1 week) reduced platelet aggregation in control and NSTEMI groups, but not in STEMI	Borna et al, 2005 (49)
204 ACS: 104, control group with stable CAD: 100	dose not mentioned (use of aspirin for at least 1 week before enrolment)	case-control–study – 27.0% aspirin resistant in control group – 40.3% aspirin resistant in ACS patients (p=0.04)	Hobikoglu et al, 2005 (54)
– Elective percutaneous coronary intervention (PCI)			
150	80 – 325 mg (+ clopidogrel 75 mg) (use of aspirin for at least 1 week before enrolment)	CK-MB – elevation: – 38.9% in aspirin resistant; – 18.3% in aspirin sensitive (p=0.04) (follow up: 24 h)	Lev et al, 2006 (46)
151	80 – 325 mg (+ clopidogrel 75 mg) (use of aspirin for at least 1 week before enrolment)	2.9–fold increased risk for myonecrosis (CK-MB =16 U/L) early after PCI (p=0.015); CK-MB – elevation: – 51.7% in aspirin resistant; – 24.6% in aspirin sensitive (p=0.006) TnI – elevation: – 65.5% in aspirin resistant; – 38.5% in aspirin sensitive (p=0.012) (mean follow up: 24 h)	Chen et al, 2004 (45)
73 stent thrombosis: 23, control group with patent stents: 50	100 mg (+ clopidogrel 75 mg)	case-control – study – 32.0% aspirin resistant in control group – 48% aspirin resistant in patients with stent thrombosis (p=n.s.)	Wenaweser et al, 2005 (51)
204 stent thrombosis: 102, control group with patent stents: 102	100 – 300 mg	case-control – study 31.3 % (32 of 102) in in-stent restenosis; 10.7 % (11 of 102) in patent stents (p<0.05)	Pamukcu et al, 2005 (52)

receiving 100 mg/day aspirin for one year after percutaneous transluminal angioplasty. Using the PFA-100 method (epinephrine), five aspirin non-responders were identified, but they did not significantly differ from responders in peripheral arterial Doppler indices (57). Unfortunately, the number of aspirin non-responders was very low. Another study included 70 men and 30 women undergoing peripheral angioplasty for intermittent claudication, and complications were evaluated for up to 18 months (58). Aspirin was prescribed at a dose of 100 mg/day. Platelet function was assessed using whole blood aggregometry with arachidonic acid, ADP and collagen as agonists. Depending on the change of platelet function, patients were divided into four classes, and a score was calculated that stratified the results into expected (aspirin responders) and unexpected low or no antiplatelet effect (aspirin non-responders). During the one-year follow up, platelet responsiveness to aspirin fluctuated considerably. The incidence of laboratory aspirin resistance was as high as 60% at weeks 4, 8 and 52. Eight re-occlusions occurred in the patients for whom aggregometry had failed to prove an antiplatelet effect of aspirin. After an 18-month follow-up period, the risk of arterial reocclusion in the subgroup of aspirin-nonresponsive men was 87% higher compared with the aspirin responders (p=0.0093). This study is interesting because all patients showed a completely inhibited platelet response to arachidonic acid stimulation. Consequently, activation pathways other than arachidonic acid-stimulated TXA_2 formation seemed to have an important impact on clinical outcome in this aspirin-treated population.

Various vascular diseases

In a post-hoc study of aspirin-treated patients from the Heart Outcomes Prevention Evaluation (HOPE) trial, Eikelboom et al. reported a higher risk of vascular events in patients with increased urinary 11-dehydro-TXB_2 (59). The study included data from 976 aspirin-treated patients at high risk of cardiovascular disease, with a follow up for five years. Aspirin responsiveness was stratified in quartiles according to urinary 11-dehydro-TXB_2. The adjusted odds for the composite endpoint MI, stroke, or cardiovascular death increased with the quartile of 11-dehydro-TXB_2 levels. Patients in the highest quartile (indicating as-

pirin resistance) had a 1.8-fold higher risk of the composite endpoint than those in the lowest quartile. There was a two-fold higher risk of MI and a 3.5-fold increased risk of cardiovascular death in the upper versus lower quartile. This association was independent of conventional risk factors for vascular disease. Similarly, Ohmori et al. divided patients with stable coronary disease (n=50) or previous stroke (n=86) into quartiles according to collagen-induced aggregation (LTA). The upper quartile level of aggregate formation was associated with an eight-fold increased risk for cardiovascular events within a 12-month follow-up (60).

Overall assessment of clinical trials

The vast majority of the aforementioned studies illustrate that an impaired responsiveness to aspirin, as detected by numerous different laboratory methods, is associated with adverse spontaneous (cardiovascular death, ACS, stroke, peripheral arterial occlusion) or procedural (myonecrosis after PCI, reocclusion after angioplasty, graft thrombosis after CABG) clinical events in diverse patient populations with an increased risk of atherothrombosis. Thus, patients having laboratory aspirin resistance seem to be at a higher risk of cardiovascular events.

Unfortunately, the trials examining clinical outcomes have often been retrospective, underpowered and sometimes without a precise definition of aspirin resistance. Few studies assessed adherence to treatment. Many used composite endpoints, without showing associations with individual outcome parameters. Moreover, many studies rely on exclusively functional assays, without considering TXA_2 formation, the pharmacological target of aspirin.

Most studies assessed laboratory aspirin resistance only at one timepoint by one single in-vitro test of platelet function, usually without control measurements before aspirin was given. Without an estimation of the actual antiplatetelet effect achieved by aspirin in an individual patient, compared with a pre-treatment measurement, it is difficult to obtain a reliable readout about the true effect of aspirin therapy. Thus, one may ask whether these studies rather demonstrate that an increased platelet reactivity was associated with an increased incidence of cardiovascular events, which would not be too surprising. Certainly, the dilemma is that withholding aspirin treatment for control measurements would be an ethical problem.

Treatment of aspirin resistance

Although the evidence for an association of laboratory aspirin resistance with an increased risk of atherothrombotic events is increasing, there are no established guidelines for its diagnosis and treatment. Theoretically, the adjustment of antiplatelet treatment to an individually diagnosed laboratory or clinical aspirin resistance may include an increased aspirin dose or the addition of (or replacement by) other antiplatelet drugs. Both strategies will certainly cause more and/or additional side effects.

Increasing the dose of aspirin

Several studies demonstrated that laboratory aspirin resistance can be overcome by a higher dose, both in patients and in healthy subjects. Using arachidonic acid-induced platelet aggregation, the individual antiplatelet dose of aspirin was evaluated in 108 patients with cardiovascular disease (61). A dose of 30 mg/day blocked platelet aggregation near-completely only in 40%. Fifty percent required a dose of 100 mg/day and the remaining 10% needed 300–500 mg/day for complete aggregation inhibition. A similar heterogeneity was observed by Helgason et al., who administered increasing doses of aspirin (325–1300 mg/day) to 306 patients with previous ischemic stroke and determined platelet aggregation (arachidonic acid, collagen, epinephrine, ADP) after two weeks and at six-month intervals (62). Most patients (74.5%) had complete and 25.5% partial inhibition of aggregation at initial testing. Repeat testing in the patients with partial platelet inhibition revealed that 67% achieved complete inhibition either by higher aspirin doses or by response fluctuation at the same dose. Overall, 8.2% of patients remained aspirin-resistant even at 1,300 mg/day. Moreover, platelet inhibition by a fixed aspirin dose was inconstant over time. Any modification of aspirin treatment should therefore consider that aspirin resistance can be transient. Another example for a dose-dependent effect has been provided by Bornstein et al., who compared 129 patients with a second stroke, whose aspirin therapy (100–500 mg/day) had clinically failed, with a matched control group with a first ischemic stroke and subsequent aspirin therapy (63). Here, the average period between first and recurrent events increased from 10 to 24 months with aspirin doses increasing from 100 to 500 mg/day. Some other studies provided inconsistent results probaby due to low subject numbers (64–66). While a recent meta-analysis concluded that the prevalence of aspirin resistance is associated with aspirin doses ≤100 mg (31), there is presently not enough evidence for the concept that antithrombotic therapeutic effects of aspirin can be restored or improved by an increased dose of aspirin.

Additional or alternative antiplatelet drugs

Clopidogrel

Large trials in patients with ACS (CURE [67]), PCI (CREDO [68]) or with symptomatic carotid stenosis (CARESS [69]) showed a clinical benefit of adding clopidogrel to aspirin as compared with aspirin alone. Two other multicenter trials (MATCH and CHARISMA) showed no advantage of dual therapy, despite an increased rate of severe bleeding (70, 71). Only a subgroup of CHARISMA with prior cardiovascular events did benefit from dual antiplatelet treatment (72). Although it is not proven that the benefit of dual therapy is related to laboratory aspirin resistance, it is interesting that the frequency of aspirin resistance was higher in patients who develop ACS (where the addition of clopidogrel to aspirin seems of advantage) than that in patients with stable coronary disease (54).

More recently, Pamukcu et al. prospectively investigated clopidogrel in addition to aspirin in stable CAD patients who were laboratory resistant to 100–300 mg/day aspirin (PFA-100) (42). Of the 234 enrolled patients, 52 were aspirin resistant. Twenty eight of the latter were randomized to additional clopidogrel (75 mg/day) for 6–12 months. Patients receiving aspirin plus clopidogrel experienced significantly fewer major cardiovascular events compared with those without clopidogrel or who stopped clopidogrel prematurely. The

duration of clopidogrel therapy was a significant predictor of major events. This prospective study was limited by a small number of patients and missing data concerning cessation of aspirin.

It must also be considered that a subset of patients do not respond to clopidogrel with the expected inhibition of platelet function (clopidogrel resistance) (73, 74). There are even patients who do not respond to either of these treatments: Lev et al. identified almost 50% of laboratory aspirin-resistant patients (undergoing elective PCI) as clopidogrel-resistant (46).

Dipyridamole

Some studies assessed the efficacy of dipyridamole in addition to aspirin in patients at risk of vascular events. While the meta-analysis of the Antithrombotic Trialists' Collaboration showed no difference between the combination of aspirin- and dipyridamole versus aspirin alone in high-risk patients (6), the ESPS-2 study reported a reduction of vascular events (but not vascular death) by adding dipyridamole to a very low dose of aspirin (50 mg/day) (75). However, ESPS-2 was the only trial showing a significant reduction of the risk of vascular events among 11 trials comparing dipyridamole plus aspirin with aspirin alone (76). Unfortunately, none of the studies with dipyridamol in addition to aspirin reported laboratory results on the antiplatelet effect of these drugs.

Altogether, the available clinical data are insufficient to answer whether increasing the dose of aspirin or the addition of another antiplatelet drug, such as clopidogrel or dipyridamole, has a beneficial impact on the clinical outcome of patients with laboratory aspirin resistance.

Implications for clinical practice

On the basis of the mentioned studies, non-responsiveness or resistance to aspirin measured in the laboratory may be associated with increased atherothromboisis. However, there are important shortcomings in the available data, including different definitions, small sample sizes, uncertain compliance, insufficient data on pretreatment platelet activity and methodological problems. The following short statements can be made:

1. Routine evaluation of aspirin resistance is not recommended until more data are available (Guideline of the International Society on Thrombosis and Haemostasis (ISTH) Working Group on Aspirin Resistance [77]). It is presently unclear which treatment is safe and effective in aspirin non-responders.
2. Most laboratory and point-of-care diagnostic tools are suspect. Single measurements indicate (at best) the current status of platelet reactivity towards an agonist. They do not tell how much inhibition has been achieved by aspirin treatment.
3. If it is decided to assess for aspirin resistance in an individual patient, assays using aspirin-sensitive activation pathways, such as arachidonic acid-induced platelet responses, are preferable.
4. Thromboxane measurements in urine are contaminated by platelet-independent sources, while serum thromboxane is more platelet-specific. Rapid and easy, inexpensive assays to determine serum thromboxane need to be developed.
5. If aspirin resistance is suspected or diagnosed, dose and compliance should be examined first. Poor compliance with aspirin is often neglected.
6. Aspirin resistance may be transient. There is little evidence for relevant genetic defects causing aspirin to fail permanently.
7. An established cause of aspirin resistance is the adverse interaction with other drugs, such as non-steroidal antiinflammatory drugs (NSAIDs), which are regularly consumed by many patients. Paracetamol and COX-2 selective inhibitors are unlikely to interfere with aspirin, but have their own side effects.
8. There is presently not enough evidence that replacement of aspirin with an agent having a different mechanism of action, or its addition to aspirin, improves outcome when laboratory aspirin resistance is present. These options should be based on clinical judgement.
9. Higher aspirin doses (e.g. 300 instead of 100 mg/day) may increase the laboratory response to aspirin, but are not proven to be more clinically active.
10. Slow-release formulations of aspirin may have a lower bioavailability. Laboratory aspirin resistance may resolve by switching to conventional formulations.

Future research

A key question for future research is to compare the extent to which the different assays of platelet function (Table 1) and aspirin´s antiplatelet action independently predict cardiovascular events in patients prescribed antiplatelet doses of aspirin. We need better standardised and validated laboratory or bedside tests, which are really able to quantify the antiplatelet effect of aspirin and do not just measure platelet reactivity to a non-specific agonist. This will probably lead to a more stringent definition of aspirin resistance. Validated threshold levels of functional or biochemical parameters are necessary. Since many potential mechanisms can account for the failure of aspirin to prevent cardiovascular events (see chapter *Supposed mechanisms of aspirin resistance*), their further characterization is a necessary task, as well as the search for strategies to preserve or restore the antiplatelet activity of aspirin.

Clinical studies on larger populations using TXA_2 (and thereby aspirin) -specific assays are also required to better correlate laboratory aspirin resistance with clinical outcomes than has been achieved today (see chapter *Prevalence of aspirin resistance and impact on clinical outcome*, Table 2). These should also evaluate potential improvements by systematic alterations in antiplatelet treatment, such as aspirin dose adjustments or addition of or replacement by other antiplatelet drugs, of which the current data are very limited (see chapter *Treatment of aspirin resistance*).

Two prospective randomized trials are currently underway: The ASpirin nonresponsiveness and Clopidogrel Endpoint Trial (ASCET) is a prospective clinical trial where aspirin non-responders among CAD patients are randomized to continue with aspirin or to switch to clopidogrel (78). The Research Evaluation to Study Individuals who Show Thromboxane Or P2Y12 Receptor resistance (RESISTOR) evaluates if point-of-care diagnosis

of non-responsiveness to oral antiplatelet agents (aspirin or clopidogrel) and subsequent delivery of eptifibatide can prevent myonecrosis in CAD patients undergoing coronary interventions (79).

Finally, it remains open if there are defined patient populations who have an elevated incidence of aspirin resistance. How about gender, racial variation and comorbidities, such as diabetes? It is intriguing how many questions still remain unanswered about this well-studied drug.

Abbreviations

ACS, acute coronary syndrome; ADP, adenosine diphosphate; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CK-MB, creatine kinase-myocardial band; COX, cyclooxygenase; HR, hazard ratio; LTA, light transmission aggregation; MI, myocardial infarction; n.s., not significant; NSAID, non-steroidal anti-inflammatory drug; NSTE, non-ST-elevation; OR, odds ratio; PCI, percutaneous coronary intervention; PFA-100, Platelet Function Analyser; RPFA, Rapid Platelet Function Assay; SVG, saphenous vein graft; TIA, transient ischemic attack; TX, thromboxane.

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