Review Article

Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100

A systematic review

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Summary

It was the aim of the present study to perform a systematic review of the published studies that estimated the prevalence of non-responders to aspirin, as assessed by the closure time of PFA-100®, a point-of-care device, and to analyse: 1) some major clinical and methodological factors that can influence it and 2) its possible association with vascular outcomes. The prevalence of non-responders to aspirin in 64 populations from 53 studies, comprising 6,450 subjects, had a median value of 0.27. A higher number of aspirin non-responders was found among older patients, those with acute vascular events, or those treated for more than one month. Aspirin non-response was more frequently associated with the use of "home-established" cut-offs or when closure time was only assessed after aspirin (rather than both before and after). Among risk factors, type 2 diabetes appeared to be associated with a higher prevalence of aspirin non-responders. The latter was also higher in less recent publications and in studies that used 3.2% rather than 3.8% Na-citrate as an anticoagulant. In eight studies comprising 847 subjects, aspirin non-responders were more likely to have vascular events than responders (relative risk: 1.63; 95% CI 1.16–2.28). In conclusion, although there appears to be heterogeneity among the studies analysed, this review indicates that about one quarter of people receiving aspirin would be identified – as an average – as aspirin non-responders by PFA-100. As this is a simple, widely available point-of-care test, efforts to better standardize it and to control for its major methodological variables might be useful to improve monitoring of platelet performance under aspirin treatment and to firmly establish the observed association with clinical vascular events.

Keywords

Aspirin resistance, aspirin variability, PFA-100, diabetes, point-of-care test, platelet function, clinical outcome

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Introduction

Aspirin has been shown to be effective in both primary and secondary prevention of atherothrombotic disease (1, 2). However, some patients experience recurrent vascular events despite treatment with aspirin, a phenomenon referred to as "treatment failure" (3–6).

Several tests have been developed to evaluate laboratory response to aspirin. This is best evaluated by techniques that isolate cyclooxygenase (COX)-1 activity, the biochemical target of aspirin, such as arachidonic acid-induced platelet aggregation, platelet, serum, or urinary thromboxane measurements. It has also been evaluated, however, by tests dependent on other platelet activation pathways besides COX-1. They include turbidometric and impedance aggregometry, Ultegra Rapid Platelet Function Analyzer, or activation-dependent changes on the platelet surface (P-selectin expression, GPIIb-IIIa activation), or cessation of blood flow by a platelet plug either *in vivo* or *in vitro* (bleeding time and Platelet Function Analyzer) (4, 6–8).

When evaluating platelet response to aspirin with these laboratory tests, "poor or no response to aspirin" would indicate that in a particular subject, on a given day, with a certain test, other

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platelet activation pathways predominate over thromboxane synthesis to give a normal or subnormal platelet function response.

Platelet Function Analyzer (PFA)-100® is one of the most employed tests to monitor aspirin response, because it provides a simple and rapid, point-of-care assessment of platelet function in whole blood in conditions of high shear. Indeed, this device measures the time ("closure time") needed for blood flow to cease through an aperture on a membrane coated with collagen and epinephrine (or collagen and ADP), that is present in the instrument's cartridge; it depends on different variables, such as von Willebrand factor levels, platelet count or haematocrit and is usually affected by aspirin intake, when using the Collagen/Epinephrine cartridge (9).

The first aim of this paper was to review the reports of aspirin non-response, as assessed by the PFA-100, and some major clinical and methodological factors that can influence it. A second aim was to assess whether aspirin non-response by PFA-100 would be associated with a higher risk of recurrent vascular events. To the best of our knowledge, this review is the largest effort to summarize the current literature on PFA-100 as a tool for monitoring platelet response to aspirin and its clinical relevance.

Methods

Search strategy

Studies in humans whose title and/or abstract contained the terms "aspirin resistance", "aspirin responder", "aspirin response", "aspirin responsiveness" or "aspirin variability" combined with "PFA-100", were searched in the PubMed database until October 15, 2007. To supplement the search, the above terms were also checked without "PFA-100" and citations in pertinent review articles were examined (3–16). Seventy-three publications that estimated aspirin response with the PFA-100 were identified (17–89). Studies were excluded if the criterion for aspirin response was not defined (34, 48), they were case-reports (38), it was impossible to know the number of aspirin non-responders (33, 50, 69, 74, 78, 89), aspirin was used in combination with clopidogrel (63, 72, 73, 84, 85) or they reported duplicate data (24, 39, 58, 64, 77, 87). A total of 53 studies, comprising 6,450 subjects, were selected for this review. All but two articles (19, 55) were in English.

Definition of aspirin non-response

For the purpose of this review, subjects not responding to aspirin were those who, after aspirin administration, had a closure time with the Collagen/Epinephrine cartridge equal to or shorter than the cut-off, as defined in each study. Cut-off values varied between 137 and 300 seconds (sec); about half of the studies defined the cut-off as upper limit of normal distribution in their own healthy controls obtained in the absence of aspirin, the remaining studies used a cut-off established either by previous studies or the PFA-100 manufacturer.

Identification of populations

Within each study, healthy subjects or patients (23, 41, 42, 52, 57, 68, 79), or patients with different clinical conditions (44, 49, 54, 60, 61) were defined as separate populations. In particular, in the study by Borna et al. (44) three different groups of patients (chest pain with no sign of cardiac disease, non ST-elevation myocardial infarction [NSTEMI] and STEMI), were reported; we included the first group as a population without vascular events and considered the other two as patients with coronary events; in the study by Fateh-Moghadam et al. (49), diabetic patients with coronary artery disease were separated from those without coronary artery disease; in the study of Hobikoglu et al. (54), only the population with stable coronary artery disease (CAD) was considered, because acute coronary syndrome (ACS) patients were included in a more recent study (81); in the study of Yilmaz et al. (60), patients with occluded saphenous vein grafts were considered a different population from those with a patent vein graft, in the study by Abaci et al. (61), patients with diabetes were regarded apart from patients with coronary artery disease and in the study of Gulmez et al. (79) individuals with CAD were considered apart from those with only risk factors for CAD.

In the studies by Christiaens et al. (29), Pamukcu et al. (57) and Gulmez et al. (79) the response to aspirin considered was only that obtained before performing the stress test, in order to exclude the effect of physical exercise on platelet activation and responsiveness to aspirin (24, 90–92).

In several studies (27, 28, 30, 67, 71, 88), subjects or patients were only considered during treatment with aspirin alone.

In other studies (23, 25, 32, 43, 51, 80), subjects receiving the lowest aspirin dosage were considered. In a further analysis of the latter studies, the possible dose-related effect of prevalence of non-responders was also assessed (29, 36, 46, 56, 76, 80, 81, 86).

In the study by Golanski et al. (37), only patients with ischemic heart disease were considered, because the data on healthy volunteers were not informative.

In the study by McCabe et al. (56) data from patients after an ischaemic stroke or transient ischemic attack (TIA) were available both in the early and in the convalescent phase; we only considered the latter, as better characterized.

In the study by von Pape et al. (59), patients were evaluated three times, i.e. after a period of treatment, after a second one with reinforced compliance, and after both reinforced compliance and dosage increase: the aspirin response considered for our analysis was only the second one.

In the study by Sambola et al. (41), only data collected at the six-month follow-up were included.

The final material for this review comprised 64 populations.

Subgroup analysis

Subgroups were defined taking into account the variables listed in Table 1.

Subjects taking aspirin for primary prevention or diabetic patients free of CAD, were considered as populations without vascular events (42, 43, 49, 61, 66, 68, 79, 82). Obviously, the "stage of disease" subgroups only enclose populations with vascular events.

In gender and risk factor subgroups, studies were only reviewed that provided separate values of aspirin response in men versus women, smoking versus non smoking and so on (21, 24, 25, 29, 32, 45, 46, 49, 54, 61, 65, 66, 71, 75, 76, 81, 82, 86).

Table 1: Variables used to define subgroups.

Vascular events (presence or absence)
Stage of disease (acute or chronic)
Gender
Age
Aspirin treatment (dosage and duration)
PFA-100 test (closure time cut-off, reference range used, before and after aspirin or only after aspirin)
Risk factors (smoking, diabetes, hypertension and dyslipidemia)
Country
Year of publication
Anticoagulant concentration
Control of compliance

Analysis of clinical events in aspirin non-responders

Eight studies evaluating the occurrence of fatal and non fatal vascular events (myocardial infarction, sudden death, stroke, TIA, revascularization, occlusion of coronary bypass, restenosis/reocclusion after percutaneous transluminal angioplasty in peripheral arterial occlusive disease) in both aspirin responders and non-responders by PFA-100 were also included in a separate analysis to evaluate the clinical predictivity of this laboratory test among patients using aspirin (21, 27, 28, 31, 41, 60, 76, 81).

Statistical analysis

Pooled prevalences were calculated using an exact method (93, 94). Briefly, this approach used exact maximum likelihood binomial distribution for calculating pooled prevalences and 95% confidence intervals (CI). Homogeneity across studies was tested using the Breslow-Day test. The method provides stratum specific estimates and test of difference across subgroups, and accounts for sparseness of individual studies.

To evaluate the association of PFA-100 non response with clinical events, pooled relative risk (RR) was also calculated with the same approach.

Results

Fifty-three publications comprised 64 populations whose response to aspirin was investigated with the PFA-100 using the Collagen/Epinephrine cartridge, for a total of 6,450 subjects. Twenty-one populations (2,283 subjects) consisted of subjects without any current or previous clinical vascular event (apparently healthy subjects) and 43 populations (4,167 subjects) of patients affected by vascular events.

The main characteristics of all populations included in this review as well as the prevalence of non-responders to aspirin for each population are reported in Table 2 and Figure 1. The prevalence of non-responders to aspirin in the 64 populations considered had a wide range of variability, with a median value of 0.27.

Breslow-Day test ($p<0.0001$) suggested evidence of heterogeneity among studies; therefore a systematic review was considered to be the most appropriate approach to explore the role of major study characteristics in explaining the observed interstudy heterogeneity (Figs. 2–4).

Table 2: Summary of the characteristics of 21 populations without and 43 with vascular events (from 53 studies) included in the review.

Authors (year)	Country	Subjects (total n)	Aspirin non-responders (total n)	PR (95% CI)	Mean age (years)	Aspirin mean dosage (mg/daily)	PFA-100 CT cut-off (sec)	Na-citrate (%)	Ref.
No vascular events									
Marshall et al (1997)	UK	12	1	0.08 (0.01–0.59)	n. r.	2250	300	3.2	17
Homoncik et al (2000)	Austria	10	2	0.20 (0.05–0.80)	28	100	173	3.8	18
Kretschemer et al (2001)	Germany	5	1	0.20 (0.03–1.42)	n. r.	100	162	3.2	22
Peters et al (2001)	Germany	17	9	0.53 (0.28–1.02)	29	288	197	3.2	23
Golanski et al (2004)	Poland	61	27	0.44 (0.30–0.65)	37	150	n. r.	3.2	37
Sambola et al (2004)	Spain	7	1	0.14 (0.02–1.01)	32	125	137	3.8	41
Watala et al (2004) *	Poland	48	15	0.31 (0.19–0.52)	49	150	151	3.2	42
†		31	16	0.52 (0.32–0.84)	50	150	151	3.2	42
Abaci et al (2005)	Turkey	102	34	0.33 (0.24–0.47)	50	100	300	3.8	43
Borna et al (2005)	Sweden	67	6	0.09 (0.04–0.20)	66	98	193	3.8	44
Fateh-Moghadam et al (2005)	Germany	110	21	0.19 (0.12–0.29)	62	100	165	3.8	49

PR indicates prevalence; CI confidence intervals ; CT: closure time; n.r. : not reported; * Healthy; † Diabetes; ll Occluded saphenous vein graft; # Patent saphenous vein graft..

Table 2: Continued.

Authors (year)	Country	Subjects (total n)	Aspirin non-responders (total n)	PR (95% CI)	Mean age (years)	Aspirin mean dosage (mg/daily)	PFA-100 CT cut-off (sec)	Na-citrate (%)	Ref.
No vascular events									
Gonzalez-Conejero et al (2005)	Spain	24	8	0.33 (0.17–0.67)	36	100	300	3.8	51
Harrison et al (2005)	UK	10	1	0.10 (0.01–0.71)	n. r.	300	139	3.2	52
Pamukcu et al (2005)	Turkey	20	2	0.10 (0.03–0.40)	51	300	186	3.8	57
Abaci et al (2006)	Turkey	111	14	0.13 (0.07–0.21)	49	300	193	3.8	61
Faraday et al (2006)	USA	1311	267	0.20 (0.18–0.23)	45	81	193	3.2	66
Fontana et al (2006)	Switzerland	96	28	0.29 (0.20–0.42)	28	100	190	3.2	67
Gresner et al (2006) *	Poland	38	9	0.24 (0.12–0.46)	49	150	151	3.2	68
†		38	25	0.66 (0.44–0.97)	52	150	151	3.2	68
Gulmez et al (2007)	Turkey	55	12	0.22 (0.12–0.38)	n.r.	264	165	3.8	79
Kaharaman et al (2007)	Turkey	110	24	0.22 (0.15–0.33)	54	100	187	n.r.	82
Vascular events									
Feuring et al (1999)	Germany	48	33	0.69 (0.49–0.97)	67	100	137	3.2	18
Golanski et al (2000)	Poland	22	17	0.77 (0.48–1.24)	n.r.	150	150	3.2	19
Gum et al (2001)	USA	325	31	0.10 (0.07–0.14)	58	325	193	3.8	21
Peters et al (2001)	Germany	19	12	0.63 (0.36–1.11)	57	100	197	3.2	23
Roller et al (2002)	Austria	26	10	0.38 (0.21–0.71)	62	100	165	3.8	25
Sane et al (2002)	USA	88	49	0.56 (0.42–0.74)	65	325	193	3.8	26
Ziegler et al (2002)	Austria	52	5	0.10 (0.04–0.23)	n.r.	100	170	3.8	27
Andersen et al (2003)	Norway	71	25	0.35 (0.24–0.52)	66	160	196	3.8	28
Christiaens et al (2003)	France	50	10	0.20 (0.11–0.37)	61	187	186	3.8	29
Grau et al (2003)	Germany	31	5	0.16 (0.07–0.39)	63	285	193	3.2	30
Grundmann et al (2003)	Germany	53	12	0.23 (0.13–0.40)	68	100	165	3.2	31
Macchi et al (2003)	France	98	29	0.30 (0.21–0.43)	66	160	186	3.8	32
Alberts et al (2004)	USA	129	48	0.37 (0.28–0.49)	62	250	171	n.r.	35
Chakroun et al (2004)	France	55	28	0.51 (0.35–0.74)	52	126	200	3.8	36
Macchi et al (2004)	France	37	9	0.24 (0.13–0.47)	60	160	186	n.r.	40
Sambola et al (2004)	Spain	89	39	0.48 (0.35–0.66)	n.r.	113	137	3.8	41
Borna et al (2005)	Sweden	68	35	0.51 (0.37–0.72)	72	98	193	3.8	44
Coakley et al (2005)	UK	75	38	0.51 (0.37–0.70)	63	75	163	3.2	45
Coma-Canella et al (2005)	Spain	113	36	0.32 (0.23–0.44)	63	155	161	3.2	46
Crowe et al (2005)	Ireland	31	13	0.42 (0.24–0.72)	61	165	176	3.2	47
Fateh-Moghadam et al (2005)	Germany	62	16	0.26 (0.16–0.42)	62	100	165	3.8	49
Harrison et al (2005)	UK	78	26	0.33 (0.23–0.49)	n.r.	300	139	3.2	52

PR indicates prevalence; CI confidence intervals ; CT: closure time; n.r. : not reported; * Healthy; † Diabetes; ll Occluded saphenous vein graft; # Patent saphenous vein graft..

Table 2: Continued.

Authors (year)	Country	Subjects (total n)	Aspirin non-responders (total n)	PR (95% CI)	Mean age (years)	Aspirin mean dosage (mg/daily)	PFA-100 CT cut-off (sec)	Na-citrate (%)	Ref.
Vascular events									
Harrison et al (2005)	UK	100	22	0.22 (0.14–0.33)	72	77	164	3.2	53
Hobikoglu et al (2005)	Turkey	100	27	0.27 (0.19–0.39)	58	n.r.	170	3.8	54
Maly' et al (2005)	Czech Rep	342	53	0.16 (0.12–0.20)	67	100	160	n.r.	55
McCabe et al (2005)	UK	45	19	0.42 (0.27–0.66)	67	75	164	3.2	56
Pamukcu et al (2005)	Turkey	62	8	0.13 (0.06–0.26)	54	300	186	3.8	57
von Pape et al (2005)	Germany	212	22	0.10 (0.07–0.16)	66	100	170	3.8	59
Yilmaz et al (2005) ll	Turkey	14	7	0.50 (0.24–1.05)	64	214	193	n.r.	60
#		14	1	0.07 (0.01–0.51)	66	189	193	n.r.	60
Abaci et al (2006)	Turkey	73	14	0.19 (0.11–0.32)	49	300	193	3.8	61
Agarwal et al (2006)	UK	20	5	0.25 (0.10–0.60)	n.r.	75	163	3.2	62
Bernardo et al (2006)	Spain	76	25	0.33 (0.22–0.49)	62	100	193	3.8	65
Lepantalo et al (2006)	Finland	101	21	0.21 (0.14–0.32)	61	100	170	3.8	70
Mani et al (2006)	Germany	82	12	0.15 (0.08–0.26)	66	100	200	n.r	71
Wong et al (2006)	Australia	45	12	0.27 (0.15–0.47)	n.r.	100	158	3.2	75
Atiemo et al (2007)	USA	94	47	0.50 (0.38–0.67)	61	228	193	3.2	76
Gulmez et al (2007)	Turkey	46	6	0.13 (0.06–0.29)	n.r.	264	165	3.8	79
Gurbel et al (2007)	USA	120	32	0.27 (0.19–0.38)	65	81	193	3.2	80
Hobikoglu et al (2007)	Turkey	124	45	0.36 (0.27–0.49)	60	267	170	3.8	81
Lordkipanidzé et al (2007)	Canada	200	119	0.60 (0.50–0.71)	67	183	193	3.2	83
Narvaez et al (2007)	Spain	268	44	0.16 (0.12–0.22)	64	134	174	n.r.	86
Pamukcu et al (2007)	Turkey	417	96	0.23 (0.19–0.28)	59	237	186	3.8	88

PR indicates prevalence; CI confidence intervals ; CT: closure time; n.r. : not reported; * Healthy; † Diabetes; ll Occluded saphenous vein graft; # Patent saphenous vein graft..

The prevalence of aspirin non-responders appeared to be significantly higher in populations with vascular events (0.28, 95% CI: 0.26–0.30 vs. 0.23, 95% CI: 0.21–0.25) and among the former it was significantly higher in the acute (0.41, 95% CI: 0.37–0.47) than in the chronic (0.25, 95% CI: 0.24–0.27) phase of disease. No significant difference was found between men and women.

Populations with higher mean age had a significantly greater number of aspirin non-responders (0.29, 95% CI: 0.27–0.31) than those with lower mean age (0.24, 95% CI: 0.22–0.26).

Mean daily doses of aspirin used ranged between 75 and 2,250 mg. There was no obvious dose-related effect on the prevalence of aspirin non-responders in those studies in which several doses of aspirin were tested in the same population (29, 36, 46, 56, 76, 81, 86) (data not shown); however, the subgroup of subjects who received ≤100 mg/day aspirin had a prevalence of non-responders significantly lower than that of subjects receiving >100 mg/day aspirin (0.23, 95% CI: 0.21–0.25 vs. 0.30, 95% CI: 0.28–0.32).

The greatest majority of subjects was given aspirin for seven or more days: the prevalence of aspirin non-response was significantly higher for longer treatment periods (0.32, 95% CI: 0.29–0.35 vs. 0.25, 95% CI: 0.23–0.27).

The average PFA-100 closure time cut-off level used to distinguish between normal sensitivity or no response to aspirin was 174 sec. The prevalence of non-responders was not influenced by either this value of closure time cut-off, or by a widely employed cut-off of 193 sec (21). On the other hand, the prevalence of aspirin non-responders was significantly higher (0.28, 95% CI: 0.26–0.30) when the cut-off was experimentally established by the investigators themselves than when they used the cut-off suggested by the manufacturer or previous literature (0.25, 95% CI: 0.23–0.27).

When closure time was assessed in the same study both before and after aspirin, the prevalence of non-responders was significantly lower than when it was assessed after aspirin only (0.24, 95% CI: 0.22–0.27 vs. 0.28, 95% CI: 0.26–0.29) (Fig. 2).

Studies that quantified aspirin response separately for populations of smokers/non-smokers and presence/absence of other vascular risk factors, showed significantly greater number of aspirin non-responders among diabetics versus non-diabetics (0.26, 95% CI: 0.23–0.31 vs. 0.22, 95% CI: 0.20–0.23), while within the other three subgroups (smoking, hypertension, dyslipidaemia) the results were comparable (Fig. 3).

We also took into account several other variables, including

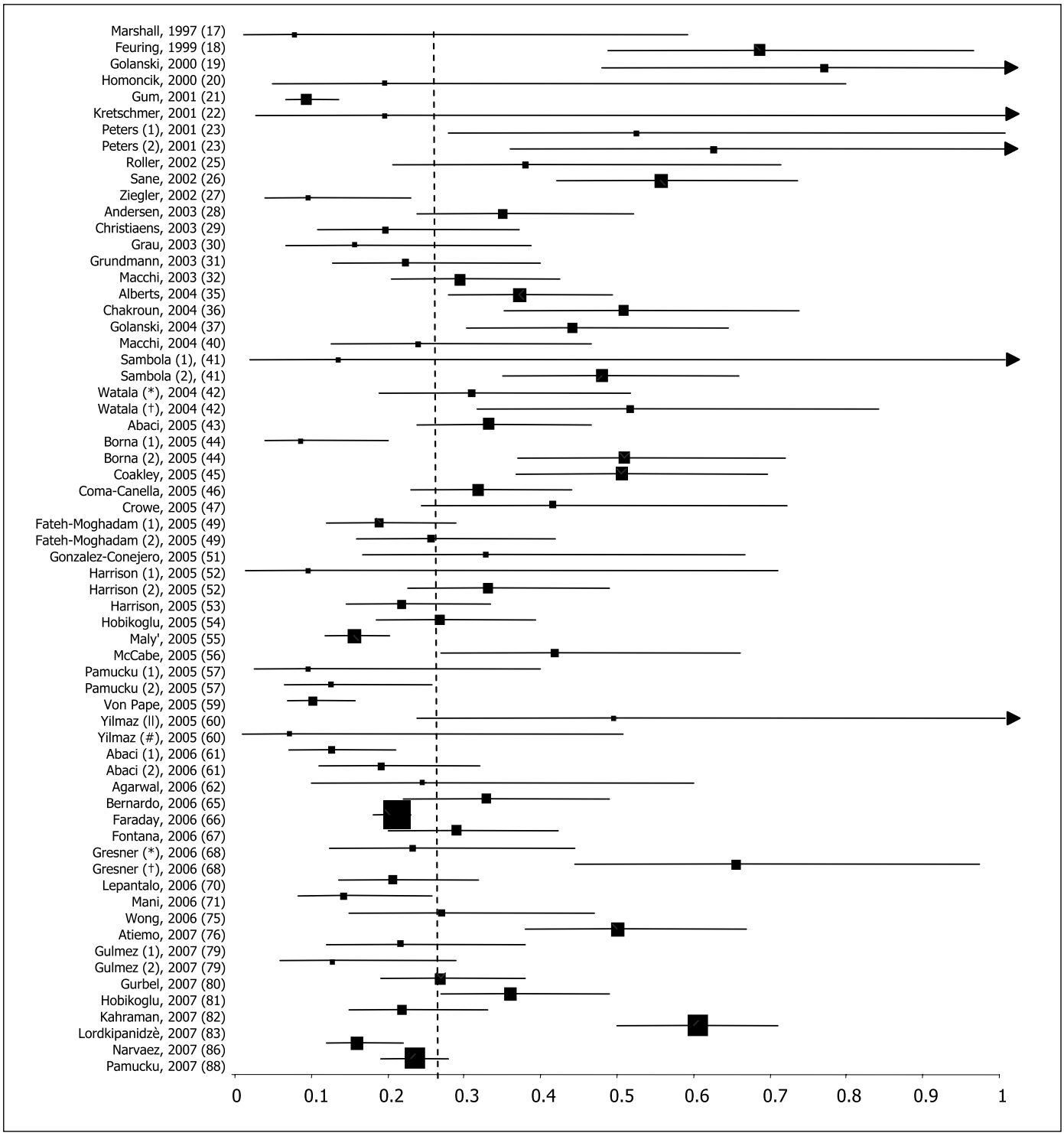

Figure 1: Prevalences of aspirin non-responders. Black squares indicate the prevalence in each study, with the square sizes inversely proportional to the standard error of prevalences. Studies with standard errors greater than 0.20 were represented with squares of the same size for graphic reasons. Horizontal lines represent the 95% CI. To facilitate reading of the figure, a vertical line indicating a prevalence of 0.27 (median value) has been included. 1 No vascular events; 2 Vascular events; *, †, II, # as in Table 2.

the country where each study was performed, the year of publication or the citrate concentration used to anticoagulate blood. The prevalence of aspirin non-responders was not different between European and North American populations, but it was significantly greater in less recent publications (0.32, 95% CI: 0.29–0.35 vs. 0.25, 95% CI: 0.23–0.26) or in studies that used 3.2% rather than 3.8% citrate (0.31, 95% CI: 0.29–0.33 vs. 0.24, 95% CI: 0.22–0.26).

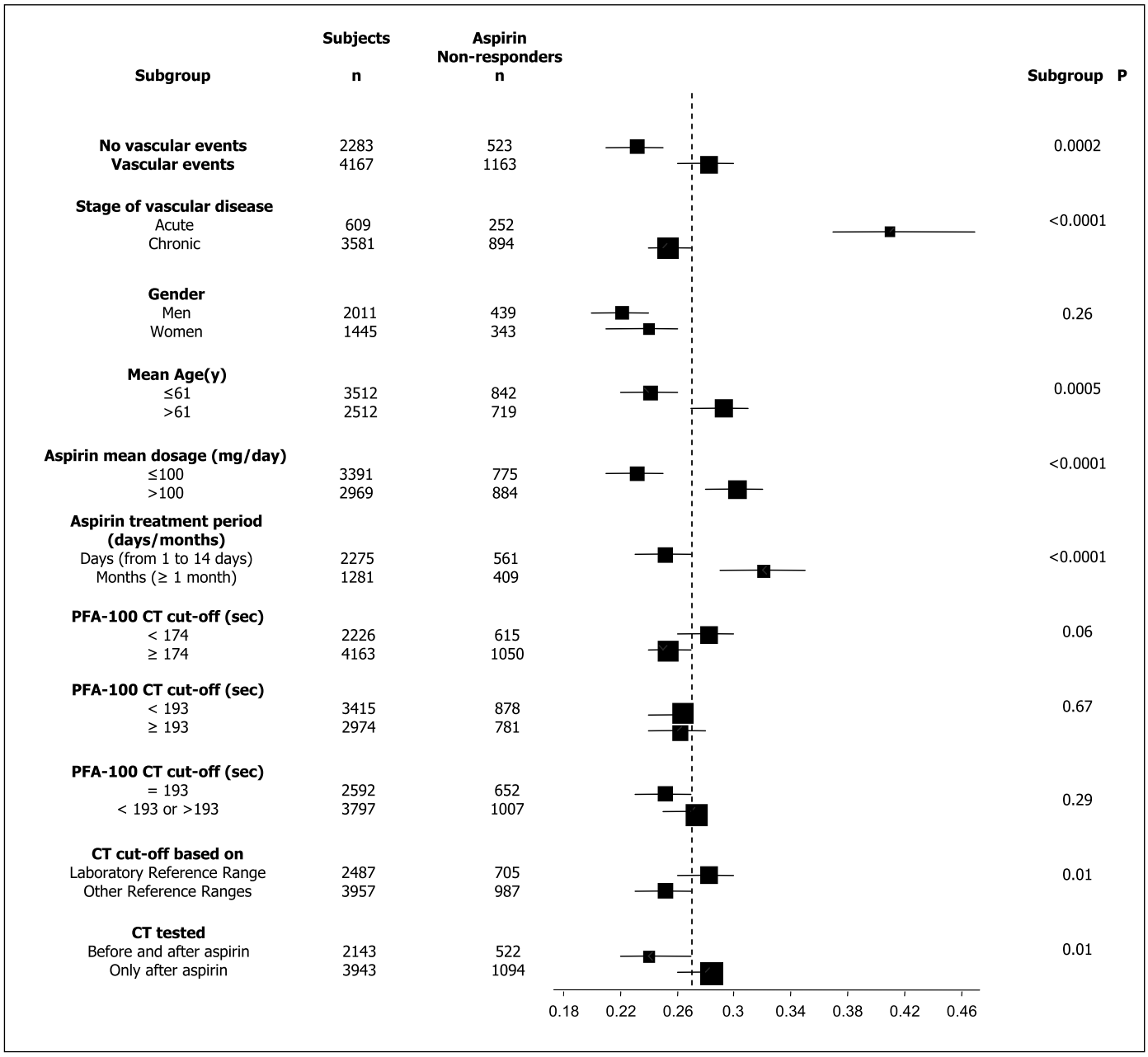

Figure 2: Prevalence of aspirin non-response in relation to clinical variables, aspirin treatment and cut-off values. Black squares indicate the prevalence in each subgroup, with the square sizes inversely proportional to the standard error of prevalences. Horizontal lines represent the 95% CI. A vertical line indicating a prevalence of 0.27 (median value) has been included.

The majority of studies either did not mention control of compliance, or declared to have controlled compliance in an objective way (aspirin medication was received under observation of a study nurse or aspirin intake was verified by personal interview, TxB2 dosage, measurement of systemic salicylate levels), but did not mention exclusion from analyses of non-compliant subjects; other studies in contrast used objective approaches (recruitment of inpatients or healthy subjects from medical staff, reinforcing the importance of regular aspirin intake, questioning the patients and his/her caregivers on aspirin intake, pills count, review of medical records and medication dispensing logs, check of patients drug chart, blood or urinary tests to detect aspirin metabolites, tests of platelet aggregation in response to arachidonic acid, TxB2 or salicylate measurements) to exclude from analyses non compliant subjects. Surprisingly, control for compliance did not appear to influence the PFA-100 response to aspirin (Fig. 4).

Association of aspirin response by PFA-100 with clinical vascular events

In eight studies the aspirin response by PFA-100 was related to the risk of vascular events. In these studies including 847 patients

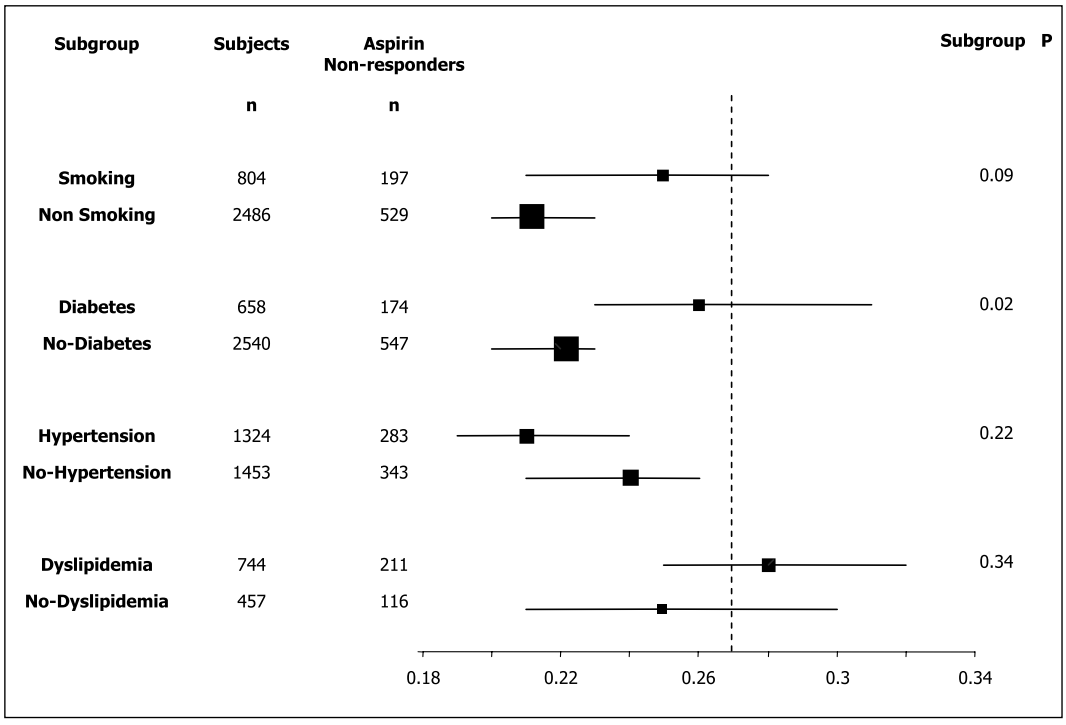

Figure 3: Prevalence of aspirin non-response and common vascular risk factors. Black squares indicate the prevalence in each subgroup, with the square sizes inversely proportional to the standard error of prevalences. Horizontal lines represent the 95% CI. A vertical line indicating a prevalence of 0.27 (median value) has been included.

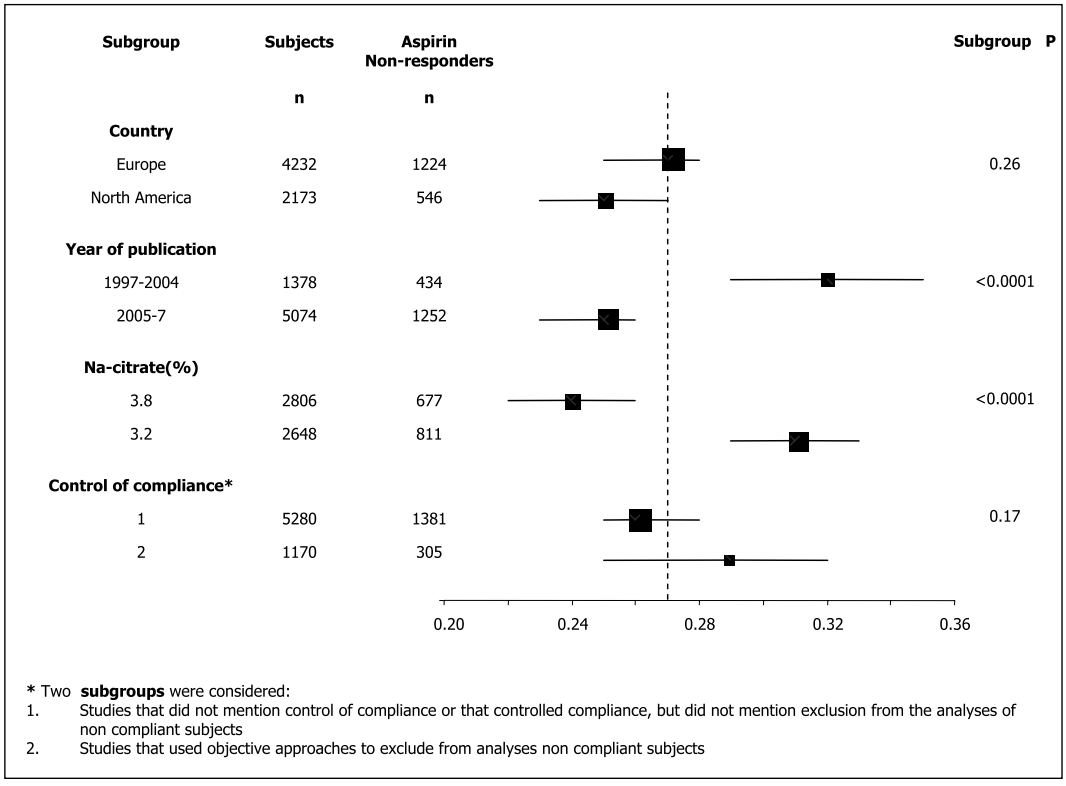

Figure 4: Prevalence of aspirin non-response in relation to country, year of publication, anticoagulant concentration and drug compliance. Black squares indicate the prevalence in each subgroup, with the square sizes inversely proportional to the standard error of prevalences. Horizontal lines represent the 95% CI. A vertical line indicating a prevalence of 0.27 (median value) has been included.

there were 129 events in 625 responders and 77 events in 222 non-responders.

Pooling these studies, the risk of vascular clinical events appeared to be significantly higher in non-responders to aspirin (RR: 1.63, 95% CI: 1.16–2.28) (Table 3).

Discussion

This review shows that aspirin non-responders, as detected by the PFA-100 device (Collagen/Epinephrine cartridge) were present among all 64 populations studied and their median preva-

Table 3: Occurrence of vascular events in aspirin responders and non-responders.

Authors (Year)	Design	Occurrence of vascular events		Clinical endpoints	Ref.
		Events/ aspirin responders	Events/ aspirin non-responders		
Gum et al (2001)	Prospective cohort	38/294	5/31	Death, MI, stroke	21
Ziegler et al (2002)	Prospective cohort	13/47	0/5	Restenosis/reocclusion after PTA in PAOD (Peripheral Arterial Occlusive Disease) patients	27
Andersen et al (2003)	Prospective cohort	11/46	9/25	Non-fatal events (MI, stroke, revascularization)	28
Grundmann et al (2003)*	Case-control	23/41	12/12	Stroke,TIA	31
Sambola et al (2004)	Prospective cohort	0/51	5/49	Sudden death, fatal ischemic events	41
Yilmaz et al (2005)*	Case-control	7/20	7/8	Occlusion of coronary bypass	60
Atiemo et al (2007)	Prospective cohort	24/47	23/47	Death, MI, revascularization	76
Hobikoglu et al (2007)	Prospective cohort	13/79	16/45	Death, MI, cerebrovascular accident, revascularization	81
Total (8 studies)		**129/625**	**77/222**		
Pooled Relative Risk: 1.63; 95% CI: 1.16–2.28					

* In the study by Grundmann et al 35 cases (patients with stoke or TIA) were studied and among them 12 were aspirin non-responders, in the study by Yilmaz et al 14 cases (patients with occluded coronary bypass) were studied and among them 7 were aspirin non-responders.

lence was 0.27. This value is comparable to the mean prevalence of persistent platelet reactivity despite use of aspirin measured by different laboratory tests, as reported in a recent meta-analysis (15). In our review, prevalence of non-response to aspirin appeared to be higher in the presence of an acute vascular event. Sex or the value of closure time cut-off did not affect response to aspirin, in contrast prevalence of non-response was higher in older subjects, or in those taking a dose of aspirin higher than 100 mg/day, or treated with aspirin for longer than one month. Also when the closure time cut-off was based on a laboratory reference range or when closure time was assessed after aspirin only, the number of non-responders was higher.

Type 2 diabetes, but not other common risk factors, was associated with higher aspirin non-response. Variables such as year of publication and concentration of citrate used as anticoagulant, but not the country where the study was performed, appeared to increase the prevalence of aspirin non-responders. On the other hand, the response to aspirin did not appear to depend on strict control of compliance.

Prevalence of aspirin non-response in relation to clinical variables, aspirin treatment and cut-off values

The finding of higher prevalence of aspirin non-response among patients with vascular events than apparently healthy subjects, suggests a possible association of aspirin non-response by PFA-100 with a higher risk of vascular events. Such a possibility has been formally tested and will be discussed in a following paragraph. The higher prevalence of aspirin non-responders observed in patients during the acute stage of different vascular diseases, could be due to high levels of proteins accompanying acute phase inflammation, such as von Willebrand factor. The PFA-100 closure time is known to be dependent on von Willebrand factor, as higher is this factor levels, shorter the PFA-100 closure time (9). Although aspirin non-response measured by PFA-100 was reported in few studies to be associated with increased von Willebrand factor levels (36, 53, 56, 62, 67), the majority of studies included in this review did not report von Willebrand factor levels; thus we can neither support nor dispute the hypothesis that high levels of von Willebrand factor may contribute to the higher prevalence of aspirin non-response in acute stage of vascular disease. Moreover, several other confounders which could not be controlled for, could also influence the association of aspirin non response with shorter closure time. However, the largest study included in this review showed that aspirin response measured by PFA-100 was not influenced by high levels of two possible confounders, such as CRP or fibrinogen (66).

Higher prevalence of aspirin non-responders was reported in older age populations, a finding consistent with shorter closure times in older men (95) and in the rise of von Willebrand factor with age (96, 97).

Insufficient dosage of aspirin is considered one of the possible mechanisms for its lack of effect (11); however, higher prevalence of aspirin non-responders was found in subjects taking more than 100 mg/day aspirin. This apparently counter intuitive finding is likely be due to the higher number of smaller studies that used aspirin dosage higher than 100 mg/day, as compared to larger studies using lower aspirin dosage. The caution in interpreting this data is reinforced by the observation that in the studies where different doses of aspirin were compared in the same population, no dose-response could be found.

Non-response to aspirin was apparently higher in patients treated longer than one month (up to 6.5 years), a finding apparently in line with the observation that inhibition of platelet aggregation by aspirin might progressively decrease within two years of follow-up (98).

Assembling in different ways studies that used different cut-off levels did not result in any difference of the prevalence of aspirin non-response. Caution should therefore be taken in interpreting results of studies where the closure time cut-off was

based on a reference range established by the investigators themselves (as compared to "independent" closure time cut-off established according to the manufacturer or previous authors). In the former case, indeed, the prevalence of aspirin non-response was higher than in the latter, indicating a possible bias, namely that the use of "home-made" cut-offs may help emphasizing a high prevalence of aspirin non-response.

The evaluation of closure time in the same subjects both before and after aspirin, allows a more realistic estimate of the drug efficacy; in this case the number of non-responders was lower than when closure time was assessed after aspirin only.

Prevalence of aspirin non-response and common vascular risk factors

Smokers tended to be less sensitive to aspirin than non-smokers, in agreement with previous studies testing the effect of aspirin on platelet function measured by other methods (99, 100) and with the greater clinical efficacy of aspirin in non-smokers as compared to current smokers, as found in the Women's Health Study (101).

Whether diabetics may represent a special case of aspirin non-responders is a matter of debate (102, 103). The meta-analysis by the Antithrombotic Trialists' Collaboration suggested that diabetic patients receive lower cardioprotective benefit from aspirin than non-diabetic ones (2). More recently, a subgroup analysis of diabetic patients in the Primary Prevention Project (PPP) showed that low dose aspirin only marginally reduced the risk of major cardiovascular events (104). Our review supports the latter findings, showing a higher prevalence of aspirin non-responders among diabetics as detected by PFA-100. Several potential mechanisms underlying an inadequate blockade of platelet function by aspirin are very likely to occur in patients with diabetes (11). These include "priming" and hypersensitivity of blood platelets to agonists (105, 106), and altered prostanoid metabolism (107–112). Diabetes is also often associated with other cardiovascular risk factors, such as hypertension and hypercholesterolemia. Although elevated values of systolic blood pressure and total cholesterol were associated with lower benefit from aspirin (113–114), this review does not support a higher prevalence of aspirin non-responders by PFA-100 in patients with hypertension or dyslipidemia. Similarly, recent findings obtained with the Ultegra Rapid Platelet Function Analyzer – another point-of-care test – failed to find any association of these factors with increased aspirin non-response (115).

Prevalence of aspirin non-response in relation to country, year of publication, anticoagulant concentration and drug compliance

An intriguing observation is that in more recently published studies (2005–2007) the prevalence of aspirin non-response declined as compared with studies performed in previous years; the former studies included larger populations than that reported in earlier studies.

In agreement with previous data that 3.8% citrate increases the prolongation of closure time by aspirin (116), we observed a higher prevalence of aspirin non-response when using 3.2% versus 3.8% citrate. A possible explanation for the latter finding is that a higher citrate concentration more effectively lowers calcium levels and reduces the primary response of platelets to aggregating agents (117), thus increasing the aspirin inhibitory effect.

Poor compliance with aspirin is a common explanation why aspirin is apparently ineffective in the laboratory and clinically (4, 5, 11); however, our review reveals that strict drug compliance did not appear to influence aspirin response as no obvious difference could be measured between studies that excluded or not non-compliant subjects from the analysis. We cannot, however, be sure that the studies excluding non-compliant subjects correctly identified all the subjects non adherent to the prescribed medication. Compliance is a critical issue, especially in chronic therapies, including aspirin (118, 119). Thus, our observation is surprising and requires further investigation.

Aspirin non-response and clinical outcomes: Should we trust this point-of-care test to predict vascular events in aspirin treated subjects?

In the first part, our study concluded that about one quarter of people receiving aspirin would be identified by PFA-100 as aspirin non-responders.

In the second part of our study, we investigated whether aspirin non-response by this point-of-care device would predict high risk of (recurrent) cardiovascular events.

We found that, pooling the results from eight studies comprising 847 patients, those who were aspirin non-responders by PFA-100 had significantly increased risk of vascular events (pooled RR: 1.63; 95% CI:1.16–2.28). This data confirms and extends recent findings (16) showing a significant association between persistent platelets reactivity despite use of aspirin, measured by different laboratory tests including the PFA-100 device, and occurrence of vascular events. As in the meta-analysis by Snoep et al (16), the studies included in our meta-analysis differed in several aspects, such as cardiovascular diseases, aspirin dosage, duration of follow-up and definition of outcome. Moreover, two were case-control and six perspective studies. At variance with Snoep et al. (16), PFA-100 device was only used in all studies and patients were only given aspirin. Despite several limitations, including the fact that laboratory aspirin response was only determined on a single occasion in all but one study (21), our review provides the first overview of available studies on vascular outcome of laboratory aspirin response by PFA-100 in patients with vascular diseases. The significant association between aspirin non-response and recurrent events should encourage to pursue intensive investigation to firmly establish whether laboratory aspirin non-response is a real phenomenon of important clinical relevance.

The estimated prevalence of more than 25% laboratory non-response to aspirin observed in this review is sufficiently high to adequately test in a large prospective trial the hypothesis that PFA-100 predicts the clinical outcome of aspirin treatment. If so, the use of a readily available, simple point-of-care device will hopefully help more easily translating population-based therapeutic results to individual patients (11, 120, 121).

Conclusions

In conclusion, this review has been performed on 53 studies that appeared to be heterogeneous under several aspects. The intra- and inter-individual variability of the assay was largely unex-

plored. The range of normality and the definition of the threshold of responsiveness to aspirin differed among studies: it was thus important to evidentiate possible bias in many studies, namely that cut-offs different from that suggested by the manufacturer were associated with higher prevalence of aspirin poor response.

On the basis of the data of this review, studies to standardize the clinical use of PFA-100 device should clearly distinguish between healthy subjects and patients with vascular disease; among the latter, acute and chronic conditions should also be clearly separated. While no difference was apparent between men and women, age should be taken into account, as the prevalence of aspirin non-response was significantly higher in older people. As far as the choice of the best cut-off level is concerned, we suggest to consider aspirin non-responders those subjects showing a closure time shorter than 193 sec. In any case, "objective" cut-off levels (such as that mentioned above) rather than "home-made" cut-off levels are preferable.

Diabetic patients and, possibly, smokers, hypertensive or dyslipidemic patients should be studied as separate groups. As the prevalence of aspirin non-response was significantly lower when 3.8% citrate was used as anticoagulant, the latter concentration should be preferred to 3.2%, that could be associated to an excessively high number of aspirin non-responders. The compliance of aspirin intake should be checked by objective methods. Moreover, to test closure time after aspirin only, could lead to underestimate the effect of the drug.

Recommendations against the use of PFA-100 assay to monitor aspirin response have been released on the basis of inconsistent evidence from selected literature (6, 9), at a moment when no systematic review of all studies was still available. Despite the limitations of PFA-100 to test platelet performance under aspirin treatment, the present analysis may contribute to improve the quality of data that will derive from future trials designed to answer the important question of clinical predictivity of laboratory platelet tests (122, 123).

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