Use of the PFA-100™ closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a systematic review and meta-analysis

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Summary. Background: PFA-100™ is a point-of-care assay that evaluates platelet reactivity in high-shear-stress conditions by measuring the closure time (CT) of a membrane aperture. When determined with a collagen/epinephrine cartridge (CEPI), the CT is usually prolonged by aspirin. Studies of the predictive value of a short PFA-100™CT_{CEPI} for ischemic events in aspirin-treated patients have given variable results. Objectives: To conduct a systematic review and meta-analysis of studies on the clinical predictive value of a short PFA-100™CT_{CEPI} in aspirin-treated cardiovascular patients. Patients and methods: Relevant studies were identified by scanning electronic databases. Studies were selected if they included aspirin-treated patients with symptomatic atherosclerosis, measured the PFA-100™CT_{CEPI}, used a CT cut-off value to define aspirin 'responders' and 'non-responders', and reported ischemic events. Results: We selected seven non-prospective studies (1466 patients) and eight prospective studies (1227 patients). In non-prospective studies, the PFA-100™CT_{CEPI} was performed after the ischemic clinical endpoint, and a publication bias was identified. In prospective studies, the global odds ratio (OR) for the recurrence of an ischemic event in 'aspirin non-responders' relative to 'aspirin responders' was 2.1 [95% confidence interval (CI) 1.4–3.4, \( P < 0.001 \)]. Pooled analysis with a random effect model revealed no heterogeneity (Q Cochran \( P = 0.36 \) and \( \hat{I}^2 = 9.4\% \)). Conclusions: A short PFA-100™CT_{CEPI} is associated with increased recurrence of ischemic events in aspirin-treated cardiovascular patients. This finding needs to be confirmed in stable ischemic patients, and the PFA-100™CT_{CEPI} cut-off needs to be refined in these patients.

Keywords: aspirin, atherosclerosis, ischemia, PFA-100™, platelet reactivity.

Aspirin is part of the first-line treatment regimen for atherothrombosis [1]. The benefit of low-dose aspirin in cardiovascular patients is related to permanent inactivation of cyclooxygenase (COX)-1, which results in the inhibition of platelet thromboxane (Tx)A2 production, a major pathway amplifying platelet activation [2]. However, the inhibitory effect of aspirin on platelet reactivity varies among individuals, leading to the concept of 'aspirin resistance' [3].

Specific pharmacodynamic resistance to aspirin, i.e. the inability of aspirin to inhibit COX-1-dependent Tx\(_A2\) generation, is uncommon [4], as shown by studies of residual Tx\(_A2\) production based on the measurement of serum or urinary levels of Tx\(_B2\) (a stable Tx\(_A2\) metabolite) or arachidonic acid-induced platelet aggregation [5,6]. In contrast, studies of platelet function based on non-specific tests dependent on other platelet amplification pathways or biological parameters suggest that aspirin leaves platelet reactivity globally intact in 20–30% of subjects [7,8]. Among these latter tests, the Platelet Function Analyzer (PFA)-100™ (Dade-Behring, Marburg, Germany) is of particular interest. This device implements a whole-blood test that evaluates platelet function in vitro in high-shear-rate conditions by measuring platelet occlusion of a membrane coated with platelet agonists. Aspirin usually prolongs the PFA-100™ closure time (CT) when the collagen/epinephrine (CEPI) cartridge is used, whereas conditions such as high von Willebrand factor (VWF) levels tend to shorten it [9]. As high-shear-stress conditions prevail in stenotic arteries, and as VWF is a marker of the cardiovascular risk [10], the PFA-100™CT could potentially serve to detect high residual platelet reactivity despite aspirin therapy, and thereby
to predict the risk of ischemic events. However, recent studies have given conflicting results [11,12], and their statistical power is often limited by their small size. Here, we made a systematic review and quantified the clinical relevance of a short PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} in aspirin-treated cardiovascular patients by performing a meta-analysis of prospective studies comparing the frequency of ischemic events in aspirin ‘responders’ and ‘non-responders’ identified on the basis of the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI}.

**Material and methods**

**Search strategy**

We scanned the MEDLINE, Web of Science and Cochrane Central Register of Controlled Trials databases for relevant studies. The search was confined to articles in English [13] published up to July 2007 (Appendix). We also examined the reference lists of the studies thus identified, as well as meeting abstracts. To be included, studies had to: (i) involve aspirin-treated patients with symptomatic atherosclerosis; (ii) use the PFA-100\textsuperscript{TM} device and CEPI cartridge to evaluate platelet function; (iii) use a PFA-100\textsuperscript{TM}CT cut-off value to define aspirin ‘responders’ and ‘non-responders’; and (iv) evaluate the occurrence or recurrence of arterial ischemic events as a prespecified endpoint. The reviewers were not blinded to the journal, authors, or institution of the publications, as this has been shown to be unnecessary [14].

**Data extraction**

To assess the specificity of the identified studies, we used a data collection form to abstract information from each report, regarding the year of publication, the duration and setting of the study, the study design, completeness of follow-up, blinding, case definition and matching of patients, total sample size, aspirin dosage, study population, and the PFA-100\textsuperscript{TM}CT cut-off used to define aspirin responsiveness. Absolute numbers of true positives (aspirin ‘non-responder’ patients with an ischemic event), true negatives (aspirin ‘responder’ patients without an ischemic event), false positives (aspirin ‘non-responder’ patients without an ischemic event) and false negatives (aspirin ‘responder’ patients with an ischemic event) were extracted from the articles to prepare $2 \times 2$ tables. When the control group of a non-prospective study included patients without atherosclerotic disease, only data for atherosclerotic controls were used. Two authors (J. L. Reny and P. Fontana) of this systematic review selected the studies and abstracted the data independently, and disagreements were resolved by discussion among all authors. Studies with retrospective, case-control or cross-sectional designs (referred to below as ‘non-prospective’) were analyzed separately from studies with a true prospective design, as defined elsewhere [15]. We pooled all the prospective studies for the determination of a global odds ratio (OR).

**PFA-100\textsuperscript{TM}**

The PFA-100\textsuperscript{TM} [16] is a Food and Drug Administration-approved device usually used to evaluate acquired and congenital platelet dysfunction [17]. The PFA-100\textsuperscript{TM} device measures the time (CT) required for platelets to plug an aperture simulating an injured vessel, after platelet activation by relevant stimuli, namely collagen and epinephrine (CEPI), or collagen and ADP (CADP). The maximum possible CT is 300 s, values above 300 s corresponding to non-closure. Aspirin influences the CT\textsubscript{CEPI} value, whereas the CT\textsubscript{CADP} is unaffected [17,18]. The reference range of the CEPI cartridge is based on values for 127 healthy unmedicated subjects, and is 85–165 s when blood is collected in tubes containing 0.129 M citrate (3.8%, package insert), and 82–105 s when blood is collected in tubes containing 0.105 M citrate (3.2%) [19].

**Statistical analysis**

The characteristics of the patients were recorded as means and standard deviations for continuous variables, and counts and percentages for categorical variables. Comparisons were made with Student’s unpaired $t$-test for continuous variables. Relationships between continuous variables were assessed with simple linear regression. Groups of aspirin ‘responders’ and ‘non-responders’ were defined on the basis of the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off used in each study: aspirin ‘responders’ were reported to have a CT\textsubscript{CEPI} value greater than or equal to the cut-off, and aspirin ‘non-responders’ a CT\textsubscript{CEPI} value lower than or equal to the cut-off. The endpoint was the occurrence (non-prospective studies) or recurrence (prospective studies) of an arterial ischemic event. The results of each study were summarized in $2 \times 2$ tables. As in meta-analyses of therapeutic trials [20], given that there was no reason to favor a particular effect model, we used various methods based on both fixed and random effect models (combined logitnom of the odds ratio, Mantel–Haenszel, Cochran, and Peto). As the results obtained with the different methods were similar, only the ORs calculated with the logitnom of the OR are reported here, with the corresponding 95% confidence intervals (CIs). Association and heterogeneity tests were used for each analysis [21]. Heterogeneity was considered to exist when the $P$-value of the heterogeneity test was 0.15 or less. Heterogeneity was also quantified with the $I^2$ statistic [22]. The causes of heterogeneity were always sought. We assessed potential publication bias graphically, using funnel plots and distribution of the ORs according to the study sizes. A $P$-value of 0.05 or less in an association test was considered significant. Statistical analyses were performed using Stata (College Station, TX, USA) software release 7. The meta-analysis was implemented with EasyMA, a program for meta-analysis of clinical trials (available at http://www.spc.univ-lyon1.fr/easyma.dos/).
Results

Study selection

The flowchart of the meta-analysis is shown in Fig. 1. The main reasons for excluding studies on the basis of the information contained in their abstracts were as follows: assessment of platelet function prior to surgery without ischemic endpoints; studies of biological effects of antiplatelet agents on platelet function without clinical endpoints; studies of the prevalence of low aspirin responsiveness without clinical endpoints; and studies of non-cardiovascular patients. On the basis of their abstracts, 26 studies met the inclusion criteria. Eight studies were prospective and seven were non-prospective. The reasons for excluding the other 11 studies, of which nine were non-prospective, are given in Fig. 1.

Non-prospective studies (Table 1)

The study by Malek et al. [30] was classified as non-prospective, despite a short follow-up period during index hospitalization, because, relative to admission, the median interval before the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} assay and before the ischemic endpoint was the same (6 days).

All selected non-prospective studies involved patients with coronary artery disease, except one that included only patients with stroke as the qualifying event [23]. Platelet reactivity was evaluated close to the acute ischemic event in five studies [23,26,30–32]. Whether or not an acute ischemic event occurred close to the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} assay was not mentioned in two studies [27,28]. The total number of patients in the non-prospective studies was 1466 (28–588 patients per study), and their mean ages ranged from 56 to 69 years. The aspirin doses ranged from 75 to 325 mg (not mentioned in one study [26]). The prevalence of aspirin 'non-responders' ranged from 10% to 22%, on the basis of PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off values of 130–193 s. When measured (one study), the VWF level correlated with the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} [31]. However, in the multivariate analysis of this latter study, the association between the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} and ischemic events was independent of the VWF level [31]. The funnel plot representation for non-prospective studies (not shown) was in favor of a publication bias. Indeed, three small studies [23,28,30], with 53, 28 and 91 patients, respectively, reported high ORs (Table 1) that accounted for an asymmetric graphical representation.

Prospective studies (Table 2)

The total number of patients in the prospective studies was 1227 (47–325 patients per study), and their mean age ranged from 54 to 66 years. All selected prospective studies involved patients with coronary artery disease. Follow-up lasted 20 months on average, and ranged from 6 to 48 months. In one study, follow-up was limited to the index hospitalization [12]. The prevalence of aspirin 'non-responders' ranged from 9.5% to 49%, on the basis of cut-off values of 170–203 s. The daily aspirin dose ranged from 81 to 500 mg. Patients were enrolled with stable atherosclerotic disease, at some distance from an acute ischemic event, in three studies [24,25,34], whereas the PFA-100\textsuperscript{TM} assay was performed in the acute setting in the other studies (within 48 h of admission or of percutaneous coronary revascularization). There was no significant difference between prospective and non-prospective studies as regards the number of patients per study (153 \(\pm\) 91 and 209 \(\pm\) 193 respectively, \(P=0.5\)) or their mean age (61 \(\pm\) 4 and 63 \(\pm\) 5 years respectively, \(P=0.3\)). Although the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off values were higher in prospective than in non-prospective studies (190 \(\pm\) 10 and 171 \(\pm\) 22 s, respectively; \(P=0.049\)), the mean prevalence of aspirin 'non-responders' was similar in the two types of study (30 \(\pm\) 13% and 25 \(\pm\) 8%, respectively; \(P=0.4\)).

Pooled analysis of the prospective studies (Fig. 2) showed that aspirin 'non-responder' status was significantly associated with the recurrence of ischemic events (OR = 2.1, 95% CI 1.4–3.4, \(P<0.001\)). The heterogeneity test was significant in this latter analysis using fixed effect models (Q Cochran, \(P=0.10\)). We then used a random effect model, which yielded a low level of heterogeneity (\(I^2 = 9.4\%\)) that was not statistically significant (Q Cochran, \(P=0.36\)).

Plasma VWF was measured in only one study, in which it had no significant impact on the recurrence of major adverse cardiovascular events during the follow-up period [29].

Fig. 1. Flowchart of the meta-analysis.
Potential determinants of aspirin ‘non-responder’ status

The choice of the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off value in these studies was variously based on the authors’ own results [30], receiver operating characteristic curve analysis [32], the 95th percentile for aspirin-untreated patients [25], the mean + 2 SD for healthy subjects [33], and the manufacturer’s normal range [23]. Seven study reports mentioned that the biologists and/or clinicians were blinded to clinical and/or biological data. The mean prevalence of aspirin...

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'non-responders' was similar in studies whose reports mentioned and did not mention the use of blinded assessments (25 ± 10% and 30 ± 11%, respectively; \( P = 0.3 \)). The prevalence of aspirin 'non-responders' was slightly but not significantly lower in studies with higher aspirin doses \( (R = 0.64, P = 0.2) \) and lower PFA-100\(^{TM}\)CT\(_{CEPI}\) cut-offs \( (R = 0.29, P = 0.3) \) and when PFA-100\(^{TM}\) was performed during stable ischemic disease (only three studies) rather than during acute ischemia \( (22 ± 7\% vs. 30 ± 11\%, P = 0.3) \).

Interestingly, the PFA-100\(^{TM}\)CT\(_{CEPI}\) cut-off values correlated negatively with the number of patients included in each study \( (R = 0.7, P = 0.005) \), indicating that the cut-off value was higher in small studies than in larger ones. The prevalence of aspirin 'non-responders' was also higher in small studies \( (R = 0.6, P = 0.02) \).

**Discussion**

This meta-analysis shows that, on the basis of prospective studies, high residual platelet reactivity in cardiovascular patients treated with aspirin (designated as aspirin 'non-responders' in most studies), as evaluated in vitro with PFA-100\(^{TM}\)CT\(_{CEPI}\), is associated with recurrent ischemic events, with an OR of 2.1 \( (95\% CI 1.4-3.4, P < 0.001) \). Pooled analysis with a random effect model revealed no heterogeneity. This absence of statistical heterogeneity may be due to the fact that the selected studies involved the same biological assay, even though this absence of statistical heterogeneity does not necessarily reflect a real absence of clinical heterogeneity among studies. In contrast, a recent meta-analysis [36] included studies with a wide variety of biological assays and showed statistically significant heterogeneity, even with random effect models, both in the overall analysis and in subgroup analyses [37]. Additionally, in their meta-analysis, Snoep et al. were not able to include studies published in late 2006 and 2007. In total, they included only three prospective studies using PFA-100\(^{TM}\)CT\(_{CEPI}\), thus limiting the conclusions that could be drawn on this specific assay. Our meta-analysis included eight prospective studies with a total of more than 1200 patients tested with the same PFA-100\(^{TM}\)CT\(_{CEPI}\).

Several methodological limitations of the studies included in our meta-analysis should be underlined. Patient compliance with treatment was not assessed in some studies. Non-compliance has been shown to be an important cause of biological 'aspirin resistance' [38,39], but it should not have affected the significant association found in this meta-analysis between the aspirin 'non-responder' status and the occurrence or recurrence of ischemic events. In most non-prospective studies, platelet reactivity was measured with PFA-100\(^{TM}\) in an acute ischemic setting. Acute arterial thrombosis has been linked to elevated levels of platelet activation markers such as circulating monocyte-platelet aggregates, circulating neutrophil-platelet aggregates, and activated glycoprotein IIb–IIIa expression on the platelet surface, factors that could influence the results of the assay [32]. PFA-100\(^{TM}\)CT\(_{CEPI}\) was recently shown to correlate with coronary artery disease stability, and may thus reflect not only aspirin non-responsiveness but also plaque instability or ongoing arterial thrombosis [32]. In this meta-analysis, we found no major difference in the prevalence of aspirin 'non-responders' when PFA-100\(^{TM}\) was used during stable ischemic disease (three studies) or during acute ischemia. However, the choice of the PFA-100\(^{TM}\)CT\(_{CEPI}\) cut-off and of the aspirin dose may interfere with the definition, and thus the prevalence, of aspirin 'non-responders'.

Interestingly, small studies had significantly higher PFA-100\(^{TM}\)CT\(_{CEPI}\) cut-off values and, thus, higher prevalence of aspirin 'non-responders'. This potential bias may be explained by the need, in smaller studies, to have a sufficient number of aspirin 'non-responders' for meaningful statistical analysis. The observed tendency for smaller studies to yield higher ORs is also a known bias of small association studies [40]. Finally, non-prospective studies performed PFA-100\(^{TM}\)CT\(_{CEPI}\) during or after the ischemic clinical endpoint, thus preventing any interpretation of its potential predictive value. For this reason and because of the obvious lack of a truly prospective follow-up design, the meta-analysis and global OR of these non-prospective studies are not provided.

Although plasma VWF influences the PFA-100\(^{TM}\)CT value [9] and the cardiovascular risk [10], this parameter was rarely evaluated in the studies selected for this meta-analysis, raising the possibility that VWF may be a confounding factor. It is noteworthy, however, that a multivariate analysis showed no impact of the plasma VWF level on the relationship between the PFA-100\(^{TM}\)CT\(_{CEPI}\) value and the recurrence of ischemic events in one study involving 146 patients [29].

Aspirin-treated subjects with short CT\(_{CEPI}\) values are considered to be aspirin 'resistant' or aspirin 'non-responders', but we and others have shown that TxA\(_2\) production is similarly inhibited in the vast majority of these individuals [25,41]. This suggests that residual COX-1 activity is not the primary explanation for short PFA-100\(^{TM}\) values in these patients. The relative importance of the TxA\(_2\) pathway in platelet activation varies considerably among individuals and, although global methods such as PFA-100\(^{TM}\) may identify patients with high residual platelet reactivity, they do not necessarily identify patients who are 'resistant' to aspirin [4]. However, this assay provides a global assessment of non-vascular primary hemostasis and therefore has the advantage of taking compensatory mechanisms into account. Aspirin may thus unveil other biological factors that contribute to shortening the CT\(_{CEPI}\) value. Indeed, aspirin-treated healthy subjects and patients with short CT\(_{CEPI}\) values have higher VWF levels than individuals with higher CT\(_{CEPI}\) values [9,41]. We have shown that high plasma growth arrest specific gene 6 product levels are also associated with short CT values in healthy subjects taking aspirin [42].

This meta-analysis has several limitations. In particular, each individual study had a different PFA-100\(^{TM}\)CT\(_{CEPI}\) cut-off value. However, at least in prospective studies, this parameter was fairly homogeneous: in all but one study,
PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off values ranged from 186 to 203 s, which is within the range of the coefficient of variation of the assay [43]. For previously mentioned reasons, the lack of studies assessing platelet function in stable atherosclerotic disease means that no firm conclusions, particularly with regard to a causal relationship between short PFA-100\textsuperscript{TM} values and the recurrence of ischemic events, can be drawn in this particular setting. Finally, the relatively small number of patients included in each study design limits the evaluation of several potentially interesting associations, including the impact of the dose of aspirin and the citrate concentration on the prevalence of aspirin ‘non-responders’ (2 vs. 11 studies for this latter comparison). However, our study was not designed to identify parameters influencing the prevalence of aspirin ‘non-responders’, which were recently reviewed elsewhere [44,45].

In conclusion, this meta-analysis shows that high residual platelet reactivity evaluated with the PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} method in aspirin-treated cardiovascular patients is associated with recurrence of ischemic events. These findings now need to be confirmed in stable ischemic patients. The question of whether aspirin-treated patients with low CT\textsubscript{CEPI} values warrant more aggressive antiplatelet therapy needs to be evaluated in a prospective interventional study. The PFA-100\textsuperscript{TM}CT\textsubscript{CEPI} cut-off also needs to be refined. These issues have to be addressed before revising the position of the Working Group on Aspirin Resistance [46].

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Appendix

The following terms (MeSH and free text) were used in our search strategy.

\textbf{MEDLINE} search: (platelet function analyzer AND aspirin) OR (platelet function analyser AND aspirin) OR (PFA AND aspirin) OR (PFA-100 AND aspirin).

Web of Science: (TS = PFA AND TS = aspirin) OR (TS = platelet function analyzer AND TS = aspirin) OR (TS = platelet function analyser AND TS = aspirin) OR (TS = PFA-100 AND TS = aspirin).

Cochrane Central Register of Controlled Trials: PFA AND aspirin (search all text); PFA-100 AND aspirin (search all text); Platelet function analyzer AND aspirin; Platelet function analyser AND aspirin.

References
