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Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of MACE in patients on clopidogrel: IPD meta-analysis

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Title: Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data

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Abstract

Prior studies have shown an association between high on-clopidogrel platelet reactivity (PR) and the risk of major adverse cardiovascular events (MACE). However, large intervention trials on PR-tailored treatments have been neutral. The role and usefulness of PR with regard to levels of cardiovascular risk are unclear. We undertook a systematic review and meta-analysis of individual patient data on MACE outcomes (acute coronary syndromes (ACS), ischemic strokes, and vascular deaths) in relation to PR and its interaction with cardiovascular risk levels. PR was determined using ADP-induced light transmission aggregometry with a primary concentration of 20µM ADP.Thirteen prospective studies totaled 6,478 clopidogrel-treated patients who experienced 421 MACE (6.5%) during a median follow-up of 12 months. The strength of the association between the risk of MACE and PR increased significantly (p=0.04) with the number of risk factors present (age>75 years, ACS at inclusion, diabetes, and hypertension). No association was detected in patients with no risk factor (p=0.48). In patients presenting one risk factor, only high-PR was associated with an increased risk of MACE (HR 3.2, p=0.001). In patients presenting ≥ 2 risk factors, the increase of risk started from medium-PR (medium-PR: HR=2.9, p=0.0004; high-PR: HR=3.7, p=0.0003). PR allowed the reclassification of 44% of the total population to a different risk level for the outcome of MACE, mostly in intermediate or high risk patients.

In conclusion, the magnitude of the association between PR and MACE risk is strongly dependent on the level of cardiovascular risk faced by patients on clopidogrel.

Keywords: clopidogrel, drug response, platelets, cardiovascular diseases, ischemic events.

Introduction

Atherosclerotic diseases account for more than 40% of deaths in Western countries, and antiplatelet therapy is a major preventive strategy in this setting(1). Clopidogrel, a $P2Y_{12}$ receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is widely prescribed for secondary prevention in patients with atherosclerotic diseases. When combined with aspirin, clopidogrel is particularly effective in patients with acute coronary syndromes (ACS)(2), and has proved superior to aspirin alone in several other large randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide inter-individual variability(3, 4). Numerous cohort studies, often performed on patients with acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions (PCI), have shown an association between high on-treatment platelet reactivity (PR) and the risk of recurrent major adverse cardiovascular events (MACE)(5-7). However, recent studies in cohorts of stable cardiovascular outpatients(8, 9) or in medically managed ACS patients(10) failed to confirm these results. Several randomized trials aimed at reducing the recurrence of ischemic events have compared standard clopidogrel treatment to a $P2Y_{12}$-inhibitor strategy tailored according to the presence of high PR. Although initial small trials were promising(11, 12) more recent larger trials showed no benefit from adjusting clopidogrel doses or switching to prasugrel based on PR testing in low-risk coronary patients undergoing PCI(13, 14). These contrasting results, both from observational studies and randomized intervention trials, may be explained by different patient characteristics including the level of risk, but to date few data substantiate these hypotheses. We previously showed, in a study-level meta-analysis, that the risk of recurrent MACE associated with high PR was greater in studies using GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did not(7). Another meta-regression from a study-level meta-analysis of randomized trials suggested that the higher the incidence of coronary stent thrombosis in a given study, the larger the net clinical benefit from a PR-tailored strategy(15). Finally, the ADAPT-DES registry of patients undergoing PCI showed that high PR was predictive of stent thrombosis mostly in ACS patients, but there was no interaction reported between PR and the presence

of an ACS at inclusion(16). This information suggests the hypothesis that high PR might be more relevant in high-risk populations, but convincing data at the individual level are lacking. To date, the only meta-analysis on individual patient data performed on 6 studies totaling 3,059 patients assessed with the VerifyNow P2Y12 assay did not explore this hypothesis(17). Similarly, one of the largest and more recent meta-analysis on 8 studies and 4817 patients did not explore this interaction due to the lack of individual data(18). To further investigate this interaction on a larger population we performed a collaborative meta-analysis of individual patient data and focused on the interaction between relevant vascular risk factors and PR, assessed with ADP induced light transmission aggregometry (LTA), in order to better define the risk of MACE. ADP-induced LTA is the assay upon which all $P2Y_{12}$ receptor inhibitors have been developed, thus supporting its use in the present meta-analysis. In addition, among several available assays to evaluate PR, LTA is the historical gold standard with which most platelet function assays were compared.

Methods

Data sources

Literature review, confined to articles in English(19), was based on electronic databases (Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC). A free-text search was conducted using an 'ADP' and 'aggregation' and 'clopidogrel' key-word combination. Articles were selected on the basis of abstracts, before examination of the full text. Reference lists of selected articles were also hand-searched to identify additional relevant reports. Reviewers (JLR and PF) were not blinded to the journal, authors or institutions in the publications as this has been shown to be unnecessary(20). The electronic database search was last updated on 31 July, 2013. The objective of this individual patients' data meta-analysis was described in a project that was part of French ministry of health's initiative to encourage meta-analyses (PHRC 15-07 to JL Reny "*Etudes prospectives sur la réponse biologique au clopidogrel et évènements ischémiques chez les patients athérothrombotiques : Métaanalyse sur données individuelles et résumées*" http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf). Protocol in French available upon request.

Study selection

Selected studies met the following criteria: (a) patients were treated with clopidogrel and had symptomatic atherothrombosis (clinical signs related to vascular atherothrombotic lesions); (b) pharmacodynamic response to clopidogrel was evaluated using the maximal aggregation value from LTA on platelet-rich plasma with 20, 10, or 5 µM ADP as an agonist; (c) LTA was performed remote from platelet function interfering drugs such as GpIIb/IIIa inhibitors; (d) patients were prospectively monitored for MACE for at least 30 days, defined using at least one of the following items: acute coronary syndrome (unstable angina, myocardial infarction with/without ST segment elevation), ischemic stroke (acute neurological deficit due to a cerebral infarction), and vascular death; (e) studies involved either a prospective cohort or a

randomised therapeutic trial, but one in which treatment was allocated independently of the response to clopidogrel. When studies were suspected of including the same patients, the authors were asked to provide data from the largest possible number of independent patients.

Data extraction

The corresponding authors or principal investigators of eligible studies were contacted and asked to participate in the CLOpidogrel and Vascular ISchemic events – Individual Patient Data (CLOVIS-IPD) meta-analysis group. Investigators provided individual data on: the qualifying cardiovascular condition and clinical setting at inclusion (ACS or stable disease); MACE and date of occurrence during follow-up; platelet reactivity (PR) with ADP 20, 10, and/or 5 μM and its timing relative to loading dose of clopidogrel; age, gender, height, and weight; current smoking status, diabetes, hypercholesterolemia, and hypertension; left ventricular ejection fraction; platelet count; PCI; use of GpIIb/IIIa inhibitors and timing; concomitant medications; and bleeding events and timing during follow-up. Data were checked for completeness and consistency with published reports. Any discrepancies were resolved with the corresponding authors. After format harmonization, data were compiled for statistical analysis. All studies were approved by their respective institutional review boards.

Quality assessment of studies

A new quality assessment tool for prognostic studies called PROBAST (see Acknowledgements) was used to estimate risks of bias and concerns about applicability. As PROBAST is not customized for meta-analyses of individual patient data, items were adapted accordingly. Based on the present study's list of relevant criteria, risks of bias, and concerns about applicability are rated as low, unclear, or high. Supplemental Figure 1 shows the list of criteria.

Primary outcomes and measures

The primary clinical outcome was the occurrence of MACE, as defined above (see Study selection (d)). The primary biological outcome was maximal aggregation with 20 µM ADP, as it is a better concentration for analyzing the effects of clopidogrel than lower ones. PR was categorized in three strata. The higher cut-offs were selected on the basis of previously published cut-offs (59% to 64% for 20 µM ADP, and 43% to 46% for 5 µM ADP)(21), and to keep relatively balanced numbers of patients in each PR categories. Three pre-specified categories allowed a better description of the dose-dependent effects of PR on the risk of MACE compared to the usual dichotomous high and low PR categorization. Three categories were also chosen to better parallel the analysis with a therapeutic PR window that has been associated with optimal net clinical benefit(22). A surrogate for the level of cardiovascular risk was defined as the number of factors with homogeneous definitions across studies, and these were markers of MACE in the meta-analysis. The factors were selected from among age, diabetes, hypertension, smoking, hypercholesterolemia, and the presence of an ACS at inclusion (as defined in study selection (d)), and were all provided at the time of inclusion and PR testing.

Statistical analysis

MACE-free survival curves were derived from individual patient data using the Kaplan-Meier estimator; curves were compared using log-rank tests stratified by study. Associations between conventional risk factors, PR strata, and risk of MACE were analyzed using multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the size of the random effects (Tau^2) which is an estimate of the between study variability(23). The presence of heterogeneity was tested by comparing models with and without random effects (likelihood ratio test). The interactions between the level of risk and PR strata were tested. MACE-free survival according to PR, as a continuous variable, was assessed using the R package prodlim using the symmetrical nearest neighborhoods method.(24) Sensitivity analyses were conducted to check the robustness of the findings with respect to: the risks of bias and concerns about the applicability of studies; the definition of MACE, including target

vessel revascularization or PCI at inclusion, and; the influence of a given specific study. The net reclassification index (NRI) for survival data(25) was computed to quantify the contribution of PR testing for the prediction of the 6-month risk of MACE in patients with increasing numbers of traditional risk factors. The event and non-event continuous NRIs were reported. Potential publication bias was checked for. P-values below 0.05 were considered significant and all tests were two-sided. Published guidelines for meta-analysis of observational studies in epidemiology (MOOSE) and their reporting(26) were followed. Details on statistical methods are given in the online data supplement.

Results

Characteristics of included studies

The Figure 1 flow-chart details how 13 of 20 qualifying studies were included, totaling 6,478 patients(8, 27-38). Table 1 shows their characteristics. Data on body mass index, concomitant medications, left ventricular ejection fraction, or the occurrence of target and non-target vessel revascularization during follow-up were only available in some studies. All studies provided individual data allowing a homogeneous definition of MACE, current smoking status, ACS, diabetes (fasting plasma glucose ≥ 7.0 mmol/l, 2-h plasma glucose ≥ 11.1 mmol/l after 75g oral glucose load or background therapy for diabetes), and hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or a documented history of hypertension). Hypercholesterolemia was not defined in a homogeneous fashion across studies and plasma LDL-cholesterol levels were not available for more than 2,000 patients. Overall, risks of bias and concerns about applicability were low (online data supplement further details study characteristics, bias, and applicability). Information on bleeding was limited to five studies, with only 67 major and 20 moderate/minor bleedings.

MACE and level of risk

Overall, 421 MACE occurred in 6,478 patients (6.5%), the majority being ACS (n = 383). There were 83 stent thromboses, including 79 definite or probable and four possible ones, all included in the composite outcome of MACE. The MACE-free survival rate across the different studies at the end of follow-up ranged from 77.4% to 97.3%. In a multivariate analysis, four factors were found relevant to determining patients' levels of risk: age greater than 75 years, diabetes, ACS at inclusion, and hypertension (Table 2). The number of these factors was used as a surrogate for the individual risk of MACE. Patients with none of these factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with two or more factors 'high-risk' (global p-value <0.0001 for the trend).

MACE and PR

Nine studies (n = 4,438 patients) performed LTA using 20 µM ADP, four studies (n = 2,144 patients) used 10 µM ADP, and eight studies (n = 3,317 patients) used 5 µM ADP. Figure 2 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE increased significantly with PR with 20 µM ADP, 10 µM ADP, and 5 µM ADP.

With adjustment, high PR was still significantly associated with an increased risk of MACE (Table 3). However, for PR evaluated using 10 µM ADP, risk only increased for the highest PR category, corresponding to LTA values greater than 60%.

Interaction between risk level and PR for the outcome of MACE

Platelet reactivity assessed with 20 µM ADP. Patients with none of the four risk factors showed no significantly increased risk associated with PR, while for patients with one risk factor only, the higher strata of PR was associated with an increased risk of MACE. Patients with two or more risk factors showed an increased risk of MACE for both the medium and higher strata of PR. (Figure 3). In a Cox model, the interaction between PR strata and the risk level was statistically significant (p=0.04). The corresponding hazard ratios (HRs) are shown in Figure 3. Heterogeneity was not detected for the overall interaction (p=0.81), as

well as when it was restricted to each risk level category (intermediate versus low risk level, p=0.45, and high versus low risk level, p=0.90). Additional results on heterogeneity are provided in the supplemental material. Figure 4A shows that PR, when analyzed in a continuous fashion, barely affects the risk of MACE at 6 months in patients with no risk factors: the risk is close to 2% at six months, irrespective of the level of platelet reactivity. Conversely, patients with one risk factor and an overall 4.1% risk of MACE at six months have in fact a 2% risk of MACE when they have a low PR, or a 6% risk of MACE when they have a high PR (Figure 4B). Similarly, patients with two or more risk factors and an overall 6% risk of MACE at six months can indeed have a 2% risk of MACE when they have a low PR (Figure 4C). The reclassification of the 6-month risk of MACE, according to the three categories of platelet reactivity, in patients with no, one and two or more risk factors, is shown in Table 4. Overall, PR allowed the reclassification of 44% of the total population (1837/4193 patients) included in a 6-month follow-up to a different level, mostly in patients originally identified as intermediate or high risk on the basis of the number of risk factors only. In patients experiencing MACE in the first 6 months of follow-up, the risk predicted by the combination of PR and risk factors was on average increased compared with the risk predicted from risk factors only: the continuous event net reclassification index (NRI) was 0.39 (95%CI 0.23 to 0.62). Conversely, in patients free of MACE at 6 months, the measure of PR did not modify the predicted risk: the continuous non-event NRI was 0.01 (95%CI -0.16 to 0.09). The overall NRI was 0.39 (95%CI 0.22 to 0.57).

Platelet reactivity assessed with 10 µM ADP. A total of only five low-risk patients in four studies performing 10 µM ADP LTA to assess PR precluded an analysis of this low-risk group. Furthermore, the surrogate for risk level failed to demonstrate an association with the observed risk of MACE in these studies. Figure 4B shows that the risk of MACE increased in both intermediate- and high-risk patients for PR values above 40%, without any obvious relation with the level of risk.

Platelet reactivity assessed with 5 µM ADP. The direction of interaction between PR using 5 µM ADP and the risk level was similar to that observed for PR using 20 µM ADP, even though overall interaction did not reach the significance level (p=0.17). Of note there were 980 fewer patients in the studies performing 5 µM ADP than in those using 20 µM ADP. The increased risk of MACE as PR increases is indeed similar for intermediate- and high-risk patients; for low-risk patients PR is not associated with a MACE outcome (online data supplement). Heterogeneity was not detected for the overall interaction (p=0.19). Figure 4C shows that the risk of MACE was unaffected by PR in low-risk patients while it increased for PR values above 30% in intermediate-risk patients and for PR values above 10%–20% in high-risk patients.

Sensitivity analyses

Sensitivity analyses were performed for PR using 20 µM ADP to assess: the robustness of the association between PR and risk of MACE and its interaction with the level of cardiovascular risk; the robustness of the results in the population of PCI patients and when target vessel revascularization is added to the composite outcome. All analyses showed that the sizes of the effects remained similar, and whilst in some instances the statistical significance of the interactions could be lost, there was no impact on their magnitudes (supplemental Tables 1 and 2). Notably, when PR was categorized in quartiles (20 µM ADP maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%. 51.4%–63.0%, 63.1%–100%) the interaction between PR and the number of risk factors remained significant (p=0.01). When restricted to the population of 3,564 patients treated with PCI and tested using 20 µM ADP the interaction was of similar magnitude but no longer significant (supplemental Table 3).

Publication and availability biases

A check for potential publication bias was made for PR using 20 µM ADP, on which the main analyses were performed. A funnel plot was obtained by representing the HR of PR using 20

μM ADP and the standard error, assessed in each separate study (supplemental Figure 4). Two studies with a negative association between PR using 20 μM ADP and the risk of MACE (with small sample sizes) were detected as missing using the 'trim and fill' method for making the funnel plot symmetrical. When these missing studies were added, the pooled HR was not significantly modified. These findings suggested that the publication bias in our meta-analysis was minor.

Seven qualifying studies could not provide individual patient data. It is of note that in five of these, the relation between clopidogrel non-response and ischemic events was not a study objective (pharmacokinetic-pharmacodynamic studies or randomized trials of different clopidogrel loading doses). The two remaining studies (n = 101 and 111 patients) were specifically interested in the prognostic value of PR for MACE.

Discussion

In the present meta-analysis of individual patient data conducted in a representative panel of clopidogrel-treated patients we demonstrated that the association between PR and the risk of MACE depended strongly on the level of cardiovascular risk. When using 20 µM ADP, the most commonly used concentration in LTA, the risk of MACE associated with PR increased with the level of cardiovascular risk. Indeed, PR did not affect the risk of MACE in patients presenting no risk factors, however it gradually increased the risk of MACE as the number of cardiovascular risk factors increased, reaching a 3.7 times greater risk in high-risk patients with a high PR. The measure of PR with 20 µM ADP, in addition to risk factors, modified the interpretation of the 6-month risk of MACE in 44% of patients, mainly in patients with at least one risk factor.

Interestingly, smoking and hypercholesterolemia were not associated with the outcome of MACE and were not included in the analysis of the interaction between PR and risk factors. In randomized controlled trials, the benefit of clopidogrel in reducing the incidence of MACE is primarily seen in smokers, with little benefit to non-smokers(39). With regard to the cohort studies of clopidogrel-treated patients included in this meta-analysis, this differential effect suggests that the increased risk of MACE related to smoking is offset by the benefit clopidogrel provides to smokers; it thereby weakens any possible analysis of the interaction between smoking and PR for outcomes of MACE. Regarding hypercholesterolemia, this conventional risk factor is likely to be confounded by indications for statin treatment. Indeed, in the ADAPT-DES registry(16) hyperlipidemia was protective against mortality with a HR=0.60 (0.41–0.86) and was not prognostic of MACE in post-ACS patients with optimal medical therapy(40). In addition, hypercholesterolemia was not homogeneously defined across the studies in the present meta-analysis and other markers, such as plasma LDL-cholesterol levels, were not widely available.

When PR was evaluated using 5 µM ADP, its interaction with the level of cardiovascular risk for the prediction of MACE was of a similar magnitude, although non-significant. These findings may reflect the lower number of patients available in studies using 5 µM ADP, and a

corresponding loss of power. Moreover, it was previously shown that ADP-induced platelet aggregation in citrated plasma was dependent on the artifactual generation of TxA2 that was modulated by aspirin, at least at lower ADP concentrations(41). This may be associated with an additional background noise in which the interaction between the identified risk factors and PR to predict MACE is blurred, as seen with the lowest 5 µM ADP concentrations and partially also with the intermediate 10 µM ADP concentrations. Only four of the studies analysed used 10 µM ADP, and two of these had a follow-up limited to 30 days; with only 124 MACEs during follow-up, this accounts for a limitation in power to reliably study interactions. Overall, the concentration of ADP used is of limited significance since the influence of risk factors appears in all three ADP concentration groups (table 3 and figure 2). Which laboratory assay and which platelet agonist concentration are best suited for the clinical evaluation of platelet function is the matter of some debate. ADP-induced LTA is highly reproducible within a given laboratory, but its lack of standardization across studies may have slightly weakened the positive findings or lower the level of significance for the interactions found in the present meta-analysis. Of note, the present meta-analysis does not aim to promote the use of LTA to tailor antiplatelet therapy but it rather relied on a historical gold standard in platelet function testing to evidence an interaction with patients' characteristics that should be considered for a tailored approach. The point-of care VerifyNow $P2Y_{12}$ assay, used in several intervention trials, correlates well with ADP-induced LTA(42, 43) and we speculate that the main findings of the present meta-analysis would have been similar, had PR been evaluated using the VerifyNow $P2Y_{12}$ assay.

Several intervention trials have compared conventional clopidogrel treatment to an antiplatelet strategy tailored according to PR. Early, small randomized trials(11, 12) that utilized vasodilator-stimulated phosphoprotein phosphorylation level measurement to indicate $P2Y_{12}$ receptor reactivity, showed a protective effect for repeat 600 mg clopidogrel loading doses in ACS patients prior to PCI. However, recent larger trials utilizing the VerifyNow $P2Y_{12}$ assay were negative. Indeed, the GRAVITAS(13) and ARCTIC(14) studies failed to show the benefit of a PR-tailored antiplatelet strategy after PCI. Various limitations of these trials were

addressed in a recent consensus publication(22). The event rate of the GRAVITAS study was low compared to the one used for power calculation, and the antiplatelet effect of the high-dose regimen may have been suboptimal as it reduced the prevalence of high PR by only 22%. Similarly, the ARTIC study population was also at a low absolute risk of subsequent cardiovascular events because the prevalence of ACS patients was low, and the composite endpoint also included other events that may not be related to platelet function. The interaction of PR and the number of risk factors, as identified in the present meta-analysis, substantiates the hypothesis that the risk associated with high PR was not clinically relevant in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a beneficial reduction of MACE for these low-risk patients. Based on these observations we speculate that higher risk patients are more likely to benefit from a therapy tailored to their initial PR. This may explain why early interventions designed to efficiently blunt high PR in ACS patients with multiple conventional risk factors translated into a reduction of MACE(11, 12, 22).

In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk. However, a recent cost-effectiveness analysis for six European perspectives showed that the universal use of newer $P2Y_{12}$ inhibitors for ACS patients is probably not as cost-effective as strategies based on PR(44). It should also be kept in mind that ticagrelor and prasugrel increase the risk of bleeding and that a therapeutic medium-PR window is associated with optimal net clinical benefit(22). The net benefits of newer $P2Y_{12}$ inhibitors could also probably be improved not only by testing for PR, but also by incorporating patient risk levels in the decision-making process. Although ongoing trials on tailored $P2Y_{12}$ strategies, including TROPICAL-ACS (ClinicalTrials.gov identifier: NCT01959451) and ANTARCTIC(45) partly include this concept of risk levels, further efforts in this direction are needed.

This meta-analysis has several strengths, such as the good overall quality of the studies included, as assessed using a quality tool specifically adapted to prognostic studies. The availability of individual patient data allowed a reliable evaluation of the risk associated with

PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a secondary prevention population were thus identified. The consistency of results across the different ADP concentrations used in the different studies to assess PR, as well as the sensitivity analyses, indicated that the results were robust.

Despite the advantages related to the availability of individual patient data, this meta-analysis also had some limitations, including a low proportion of women (25%). This did not allow a stratification of the analyses by gender, as is usually the case in risk assessment tools such the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is not considered as one of traditional risk factors, but is rather presented in separate charts for women and men. There were incomplete data on concomitant medications or other relevant risk factors such as the left ventricular ejection fraction, cholesterol levels or renal insufficiency. Finally, information on bleeding was limited to five studies and a low number of events, thus precluding a reliable analysis of bleeding events and their relation to PR.

In conclusion, high PR in patients on clopidogrel is associated with an increased risk of MACE in patients with vascular risk factors, but not in low-risk patients. These findings suggest that trials on tailored PR treatment strategies should be primarily stratified on the individual vascular risk factors in order to assess a truly personalized approach.

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Table 1. Main characteristics of published studies

Study	Years of publication	Patients (n)	Age (y)	Male (%)	Diabetics (%)	Smokers (%)	Hyper-tension (%)	Hypercholes-terolemia (%)	ACS at inclusion (%)	PCI (%)	GpIIb/IIIa inhibitor (%)	Follow-up (months)*	ADP (μM)
Campo et al.(27)	2006	70	64±13	69	19	37	63	34	100	100	100	10 (15)	5, 20
Hochholzer et al.(28)	2006	765	66±9	78	24	11	82	92	0	100	0	12 (12)	5, 20
Angiolillo et al.(29)	2007	173	67±9	65	100	13	65	68	0	0	0	24 (36)	20
Cuisset et al.(30)	2007	190	65±12	76	33	48	58	53	87.4	100	14.7	1 (1)	10, 20
Geisler et al.(31)	2008	1,092	67±11	74	33	39	80	59	51.7	100	7.7	1 (1)	20
Gurbel et al.(32)	2008	297	65±12	65	41	55	74	82	0	100	42	24 (24)	5, 20
Cuisset et al.(33)	2009	598	65±12	78	35	39	56	55	100	100	9.9	1 (1)	10
Yong et al.(34)	2009	248	63±12	71	22	27	53	52	100	55	39.7	6 (21)	5, 10, 20
Breet et al.(35)	2010	1,069	64±11	75	81	11	77	80	0	100	7.0	12 (12)	5, 20
Marcucci et al.(36)	2010	1,108	69±10	75	24	23	66	55	100	100	26.0	12 (12)	10
Beigel et al.(37)	2011	174	59±12	83	27	41	51	45	100	100	-	6 (6)	5
Aradi et al.(38)	2012	160	62±9	63	38	36	84	50	0	100	0	12 (12)	5
Reny et al.(8)	2012	534	62±12	82	21	20	56	63	0	0	0	32 (50)	5, 20

Age, mean ± standard deviation; CAD, coronary artery disease; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP, adenosine diphospate concentration used for the evaluation of platelet reactivity

* Median (maximum)

Table 2: Multivariate analysis to assess the associations between the risk factors and the composite outcome of MACE. This analysis was conducted on the patients of the 13 studies of the meta-analysis (n=6,256 after exclusion of missing data). MACE were observed in 412 patients. Hazard ratios (HR) greater than one show an increased risk of MACE in patients having the corresponding risk factor.

Factors collected in studies	Adjusted HR [95% CI]	p	Level of risk of MACE *	HR [95% CI]	p
Current smoking status	0.92 [0.71;1.18]	0.50	*Low risk (n=579)*	1	
Age (> 75)	1.56 [1.25;1.95]	<0.0001	*Intermediate risk (n=2444)*	1.61 [1.05;2.45]	0.03
Diabetes	1.58 [1.27;1.96]	<0.0001	*High risk (n=3435)*	2.58 [1.69;3.94]	<0.0001
Hypercholesterolemia	0.86 [0.69;1.06]	0.15			
Hypertension	1.23 [0.98;1.54]	0.07			
ACS at inclusion	2.00 [1.27;3.16]	0.003			
Gender (Male)	1.11 [0.89;1.40]	0.35			

*: a surrogate for the level of risk was defined as the number of risk factors (among age, diabetes, hypertension, and ACS at inclusion): low risk for no risk factor, intermediate risk for one risk factor and high risk for two or more risk factors).

Table 3: Associations between the ADP induced-aggregation categories and the composite outcome of MACE with adjustment on the factors collected in the studies of the meta-analysis (factors shown in Table 2).

	ADP 20 µM		**ADP 10 µM**		**ADP 5 µM**	
	N		**N**		**N**	
Studies	9		4		8	
Events	287		124		229	
Patients (after exclusion of missing data)	4,140		2,077		3,160	
	HR [95% CI]	**p**	**HR [95% CI]**	**p**	**HR [95% CI]**	**p**
ADP induced-aggregation categories		0.0003**		0.03**		0.02**
*Lower category **	1		1		1	
*Intermediate category **	1.85 [1.26;2.73]	0.002	1.31 [0.79;2.17]	0.30	1.79 [1.02;3.14]	0.04
*Higher category **	2.91 [1.78;4.74]	<0.0001	2.61 [1.64;4.16]	<0.0001	2.79 [1.50;5.22]	0.001

HR, Hazard Ratio; CI, Confidence Interval

* Categories for ADP 20 and 10 µM are 0%-40%, 41%-60%, 61%-100%, and for ADP 5 µM are 0%-30%, 31%-50%, 51%-100%

**: global p-values for testing the hypothesis that both HRs (intermediate- and higher-ADP induced-aggregation category) equal 1

Table 4: Reclassification of the 6-month risk of MACE when the individual risk was predicted from platelet reactivity measured by 20µM ADP in addition to risk factors. The predicted risk was stratified in three levels (low: ≤3%, intermediate: >3% and ≤5%, high: >5%) in agreement with the 6-month risk observed in patients with none, one and two or more risk factors (2.3%, 4.1% and 6.2% respectively). Patients were stratified according to their number of risk factors and to the level of the predicted risk. The numbers of patients and, in brackets, the corresponding observed 6-month risk of MACE in each stratum.

	Risk predicted by the combination of risk factors and platelet reactivity measured by 20µM ADP			
Risk predicted by the number of risk factors only	Low risk (≤3%)	Intermediate risk (>3% and ≤5%)	High risk (>5%)	Total
Low risk - no risk factor	524 * (2.4% **)	26 *	0 *	550 * (2.3% **)
Intermediate risk - one risk factor	625 * (2.1% **)	576 * (3.7% **)	622 * (6.3% **)	1823 * (4.1% **)
High risk - two or more risk factors	102 * (0.0% **)	462 * (3.0% **)	1256 * (7.6% **)	1820 * (6.2% **)
Total	1251 * (2.1% **)	1064 * (3.4% **)	1878 * (7.1% **)	4193 * (4.7% **)

*: number of patients

**: observed 6-month risk of MACE

Figure 1: Flow chart of the meta-analysis

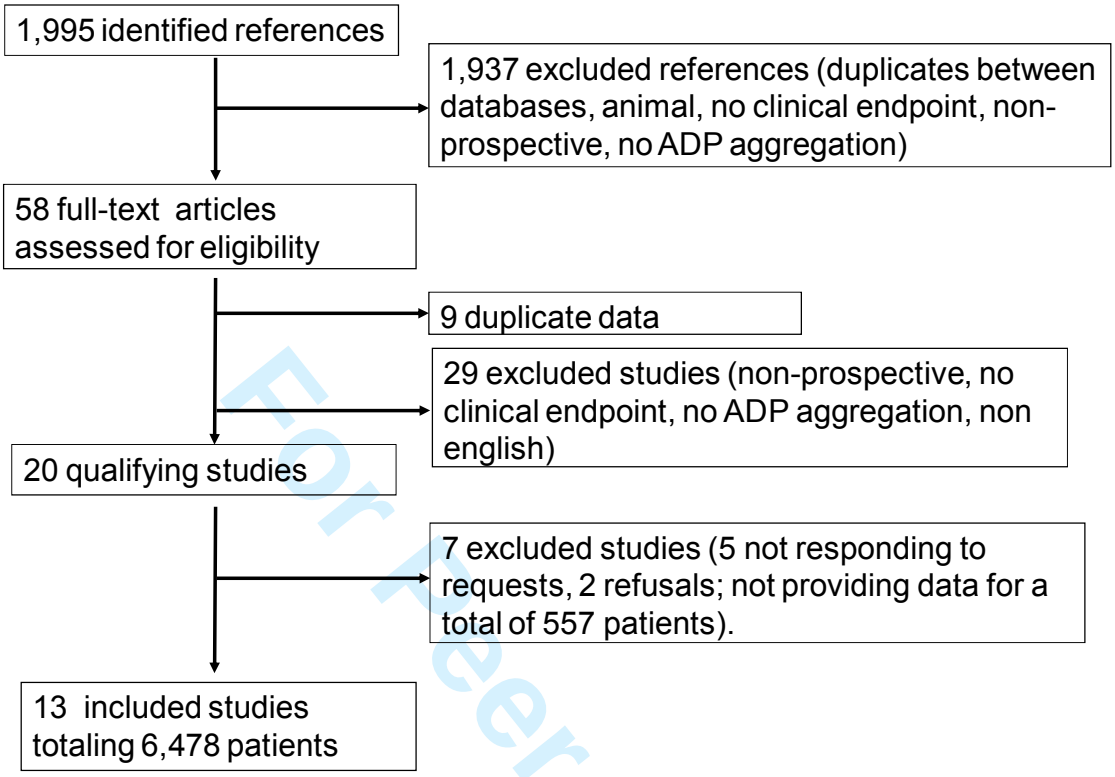


Figure 2: Kaplan-Meier survival curve for the occurrence of MACE

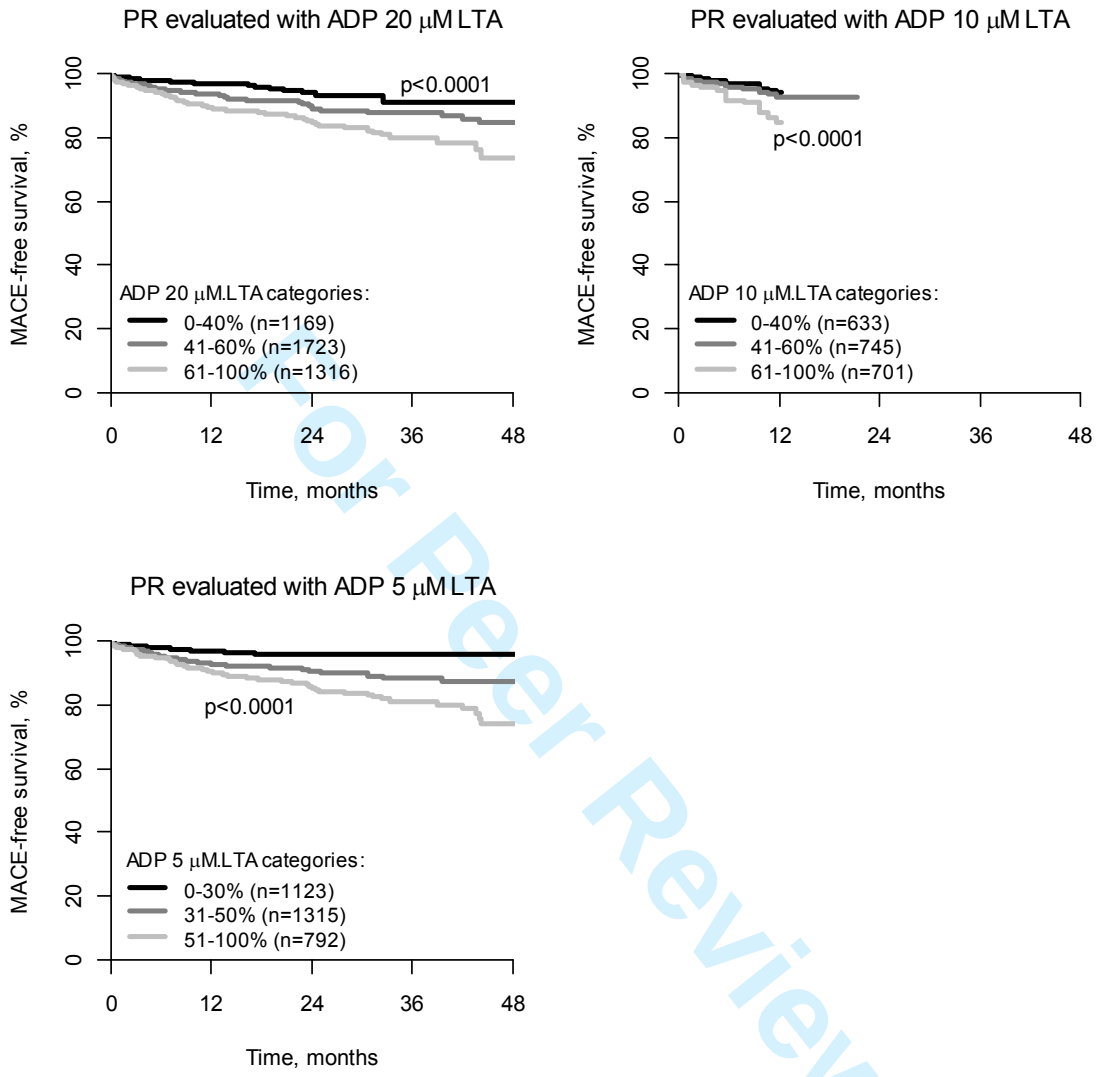


Figure 3: Association between platelet reactivity and the occurrence of MACE according to the level of risk. Low-risk patients have none of the risk factors (among age > 75 years, acute coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have one risk factor and high-risk patients have two or more risk factors. PR was assessed with 20 µM ADP LTA.

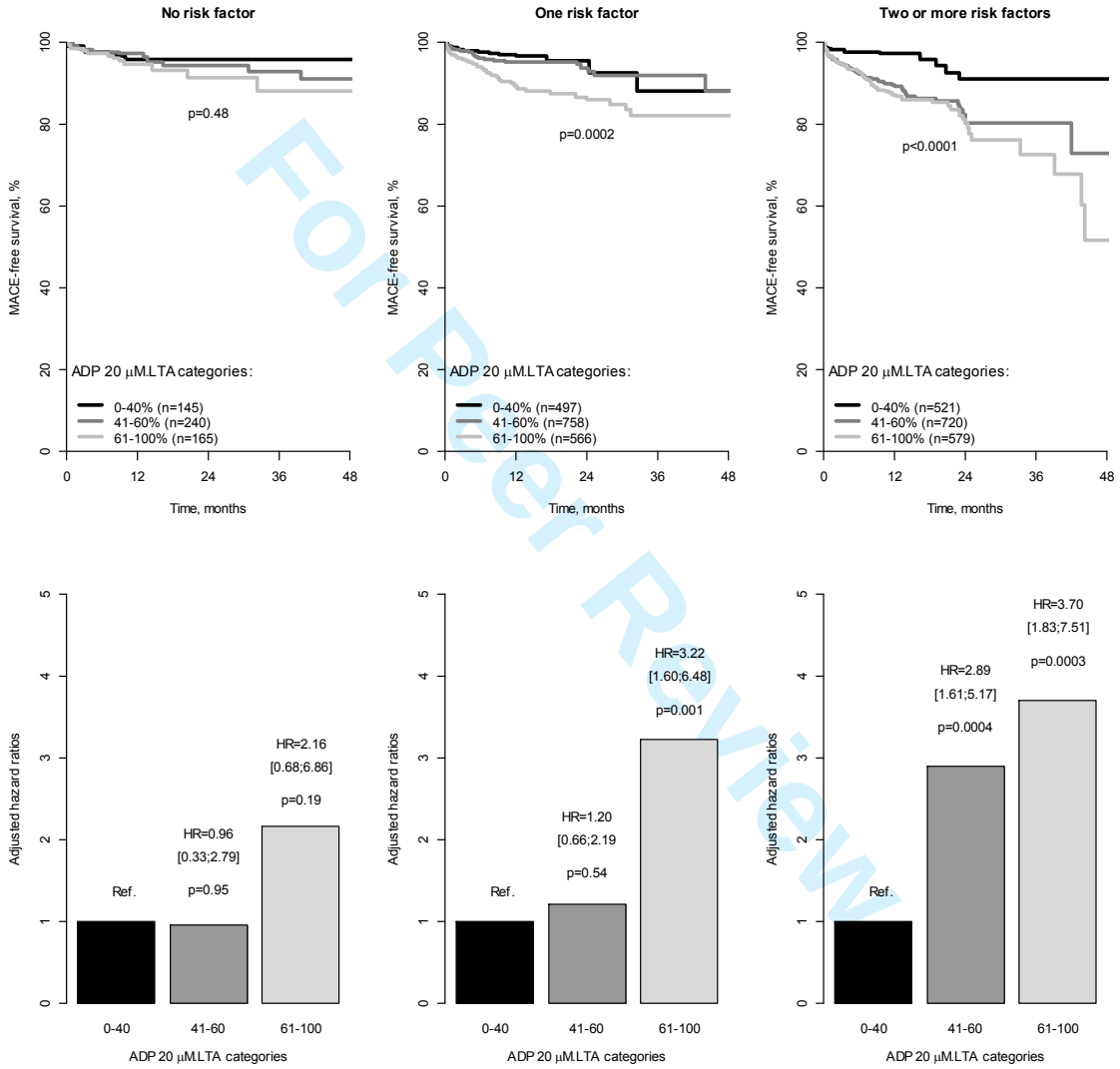
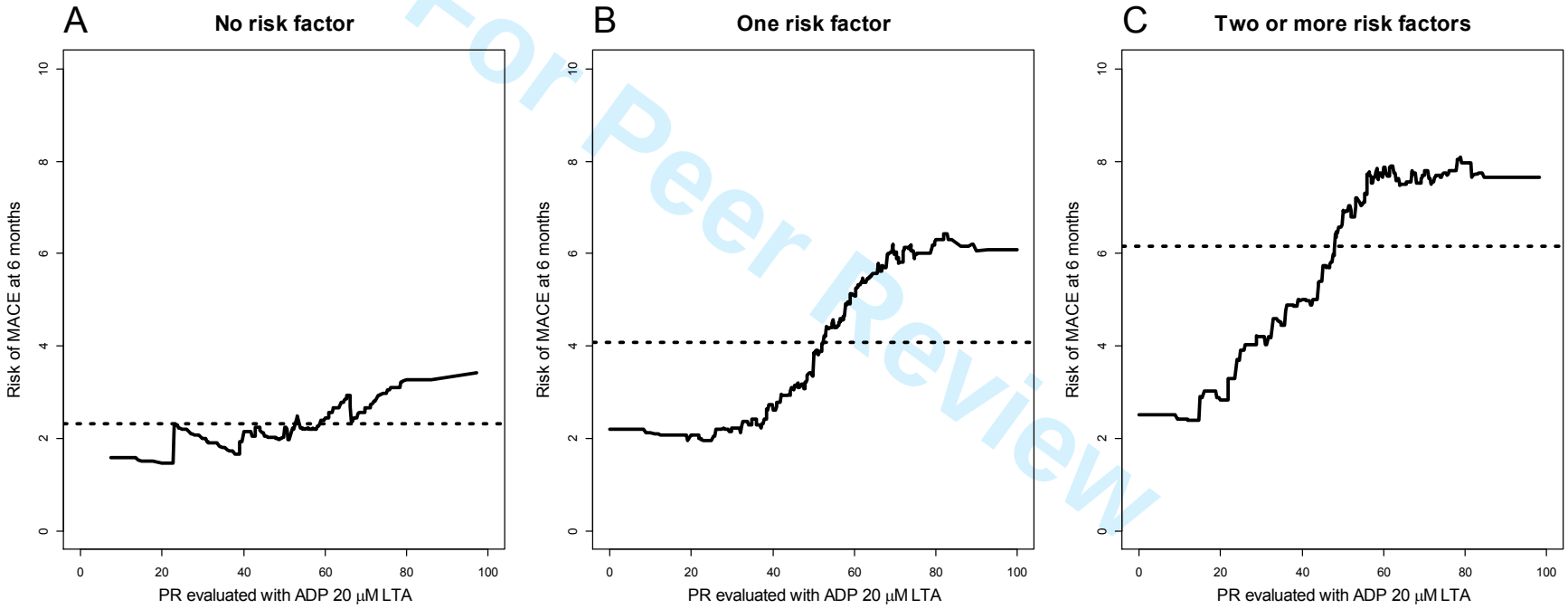


Figure 4: 6-month risk of MACE according to platelet reactivity in the different risk groups. The dashed line represents the overall risk, ignoring platelet reactivity and the black line shows the risk according to the platelet reactivity assessed with 20 µM ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).



Title: Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data

Running head: Vascular risk, platelet reactivity, and prognosis

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Abstract

Prior studies have shown an association between high on-clopidogrel platelet reactivity (PR) and the risk of major adverse cardiovascular events (MACE). However, large intervention trials on PR-tailored treatments have been neutral. The role and usefulness of PR with regard to levels of cardiovascular risk are unclear. We undertook a systematic review and meta-analysis of individual patient data on MACE outcomes (acute coronary syndromes (ACS), ischemic strokes, and vascular deaths) in relation to PR and its interaction with cardiovascular risk levels. PR was determined using ADP-induced light transmission aggregometry with a primary concentration of 20µM ADP.Thirteen prospective studies totaled 6,478 clopidogrel-treated patients who experienced 421 MACE (6.5%) during a median follow-up of 12 months. The strength of the association between the risk of MACE and PR increased significantly (p=0.04) with the number of risk factors present (age>75 years, ACS at inclusion, diabetes, and hypertension). No association was detected in patients with no risk factor (p=0.48). In patients presenting one risk factor, only high-PR was associated with an increased risk of MACE (HR 3.2, p=0.001). In patients presenting ≥ 2 risk factors, the increase of risk started from medium-PR (medium-PR: HR=2.9, p=0.0004; high-PR: HR=3.7, p=0.0003). PR allowed the reclassification of 44% of the total population to a different risk level for the outcome of MACE, mostly in intermediate or high risk patients.

In conclusion, the magnitude of the association between PR and MACE risk is strongly dependent on the level of cardiovascular risk faced by patients on clopidogrel.

Keywords: clopidogrel, drug response, platelets, cardiovascular diseases, ischemic events.

Comment [jlr1]: Significant downsizing to comply with the 250 word requirement. No added or altered content.

Comment [jlr2]: « suggesting that PR-tailored strategies may be most effective in higher-risk patients." : this end of the sentence has been deleted from the conclusion, as suggested by reviewer 2

Introduction

Atherosclerotic diseases account for more than 40% of deaths in Western countries, and antiplatelet therapy is a major preventive strategy in this setting(1). Clopidogrel, a $P2Y_{12}$ receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is widely prescribed for secondary prevention in patients with atherosclerotic diseases. When combined with aspirin, clopidogrel is particularly effective in patients with acute coronary syndromes (ACS)(2), and has proved superior to aspirin alone in several other large randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide inter-individual variability(3, 4). Numerous cohort studies, often performed on patients with acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions (PCI), have shown an association between high on-treatment platelet reactivity (PR) and the risk of recurrent major adverse cardiovascular events (MACE)(5-7). However, recent studies in cohorts of stable cardiovascular outpatients(8, 9) or in medically managed ACS patients(10) failed to confirm these results. Several randomized trials aimed at reducing the recurrence of ischemic events have compared standard clopidogrel treatment to a $P2Y_{12}$-inhibitor strategy tailored according to the presence of high PR. Although initial small trials were promising(11, 12) more recent larger trials showed no benefit from adjusting clopidogrel doses or switching to prasugrel based on PR testing in low-risk coronary patients undergoing PCI(13, 14). These contrasting results, both from observational studies and randomized intervention trials, may be explained by different patient characteristics including the level of risk, but to date few data substantiate these hypotheses. We previously showed, in a study-level meta-analysis, that the risk of recurrent MACE associated with high PR was greater in studies using GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did not(7). Another meta-regression from a study-level meta-analysis of randomized trials suggested that the higher the incidence of coronary stent thrombosis in a given study, the larger the net clinical benefit from a PR-tailored strategy(15). Finally, the ADAPT-DES registry of patients undergoing PCI showed that high PR was predictive of stent thrombosis mostly in ACS patients, but there was no interaction reported between PR and the presence

of an ACS at inclusion(16). This information suggests the hypothesis that high PR might be more relevant in high-risk populations, but convincing data at the individual level are lacking. To date, the only meta-analysis on individual patient data performed on 6 studies totaling 3,059 patients assessed with the VerifyNow P2Y12 assay did not explore this hypothesis(17). Similarly, one of the largest and more recent meta-analysis on 8 studies and 4817 patients did not explore this interaction due to the lack of individual data(18). To further investigate this interaction on a larger population we performed a collaborative meta-analysis of individual patient data and focused on the interaction between relevant vascular risk factors and PR, assessed with ADP induced light transmission aggregometry (LTA), in order to better define the risk of MACE. ADP-induced LTA is the assay upon which all $P2Y_{12}$ receptor inhibitors have been developed, thus supporting its use in the present meta-analysis. In addition, among several available assays to evaluate PR, LTA is the historical gold standard with which most platelet function assays were compared.

Methods

Data sources

Literature review, confined to articles in English(19), was based on electronic databases (Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC). A free-text search was conducted using an 'ADP' and 'aggregation' and 'clopidogrel' key-word combination. Articles were selected on the basis of abstracts, before examination of the full text. Reference lists of selected articles were also hand-searched to identify additional relevant reports. Reviewers (JLR and PF) were not blinded to the journal, authors or institutions in the publications as this has been shown to be unnecessary(20). The electronic database search was last updated on 31 July, 2013. The objective of this individual patients' data meta-analysis was described in a project that was part of French ministry of health's initiative to encourage meta-analyses (PHRC 15-07 to JL Reny "*Etudes prospectives sur la réponse biologique au clopidogrel et évènements ischémiques chez les patients athérothrombotiques : Métaanalyse sur données individuelles et résumées*" http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf). Protocol in French available upon request.

Study selection

Selected studies met the following criteria: (a) patients were treated with clopidogrel and had symptomatic atherothrombosis (clinical signs related to vascular atherothrombotic lesions); (b) pharmacodynamic response to clopidogrel was evaluated using the maximal aggregation value from LTA on platelet-rich plasma with 20, 10, or 5 µM ADP as an agonist; (c) LTA was performed remote from platelet function interfering drugs such as GpIIb/IIIa inhibitors; (d) patients were prospectively monitored for MACE for at least 30 days, defined using at least one of the following items: acute coronary syndrome (unstable angina, myocardial infarction with/without ST segment elevation), ischemic stroke (acute neurological deficit due to a cerebral infarction), and vascular death; (e) studies involved either a prospective cohort or a

randomised therapeutic trial, but one in which treatment was allocated independently of the response to clopidogrel. When studies were suspected of including the same patients, the authors were asked to provide data from the largest possible number of independent patients.

Data extraction

The corresponding authors or principal investigators of eligible studies were contacted and asked to participate in the CLOpidogrel and Vascular ISchemic events – Individual Patient Data (CLOVIS-IPD) meta-analysis group. Investigators provided individual data on: the qualifying cardiovascular condition and clinical setting at inclusion (ACS or stable disease); MACE and date of occurrence during follow-up; platelet reactivity (PR) with ADP 20, 10, and/or 5 μM and its timing relative to loading dose of clopidogrel; age, gender, height, and weight; current smoking status, diabetes, hypercholesterolemia, and hypertension; left ventricular ejection fraction; platelet count; PCI; use of GpIIb/IIIa inhibitors and timing; concomitant medications; and bleeding events and timing during follow-up. Data were checked for completeness and consistency with published reports. Any discrepancies were resolved with the corresponding authors. After format harmonization, data were compiled for statistical analysis. All studies were approved by their respective institutional review boards.

Quality assessment of studies

A new quality assessment tool for prognostic studies called PROBAST (see Acknowledgements) was used to estimate risks of bias and concerns about applicability. As PROBAST is not customized for meta-analyses of individual patient data, items were adapted accordingly. Based on the present study's list of relevant criteria, risks of bias, and concerns about applicability are rated as low, unclear, or high. Supplemental Figure 1 shows the list of criteria.

Primary outcomes and measures

The primary clinical outcome was the occurrence of MACE, as defined above (see Study selection (d)). The primary biological outcome was maximal aggregation with 20 µM ADP, as it is a better concentration for analyzing the effects of clopidogrel than lower ones. PR was categorized in three strata. The higher cut-offs were selected on the basis of previously published cut-offs (59% to 64% for 20 µM ADP, and 43% to 46% for 5 µM ADP)(21), and to keep relatively balanced numbers of patients in each PR categories. Three pre-specified categories allowed a better description of the dose-dependent effects of PR on the risk of MACE compared to the usual dichotomous high and low PR categorization. Three categories were also chosen to better parallel the analysis with a therapeutic PR window that has been associated with optimal net clinical benefit(22). A surrogate for the level of cardiovascular risk was defined as the number of factors with homogeneous definitions across studies, and these were markers of MACE in the meta-analysis. The factors were selected from among age, diabetes, hypertension, smoking, hypercholesterolemia, and the presence of an ACS at inclusion (as defined in study selection (d)), and were all provided at the time of inclusion and PR testing.

Statistical analysis

MACE-free survival curves were derived from individual patient data using the Kaplan-Meier estimator; curves were compared using log-rank tests stratified by study. Associations between conventional risk factors, PR strata, and risk of MACE were analyzed using multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the size of the random effects (Tau^2) which is an estimate of the between study variability(23). The presence of heterogeneity was tested by comparing models with and without random effects (likelihood ratio test). The interactions between the level of risk and PR strata were tested. MACE-free survival according to PR, as a continuous variable, was assessed using the R package prodlim using the symmetrical nearest neighborhoods method.(24) Sensitivity analyses were conducted to check the robustness of the findings with respect to: the risks of bias and concerns about the applicability of studies; the definition of MACE, including target

vessel revascularization or PCI at inclusion, and; the influence of a given specific study. The net reclassification index (NRI) for survival data(25) was computed to quantify the contribution of PR testing for the prediction of the 6-month risk of MACE in patients with increasing numbers of traditional risk factors. The event and non-event continuous NRIs were reported. Potential publication bias was checked for. P-values below 0.05 were considered significant and all tests were two-sided. Published guidelines for meta-analysis of observational studies in epidemiology (MOOSE) and their reporting(26) were followed. Details on statistical methods are given in the online data supplement.

Results

Characteristics of included studies

The Figure 1 flow-chart details how 13 of 20 qualifying studies were included, totaling 6,478 patients(8, 27-38). Table 1 shows their characteristics. Data on body mass index, concomitant medications, left ventricular ejection fraction, or the occurrence of target and non-target vessel revascularization during follow-up were only available in some studies. All studies provided individual data allowing a homogeneous definition of MACE, current smoking status, ACS, diabetes (fasting plasma glucose ≥ 7.0 mmol/l, 2-h plasma glucose ≥ 11.1 mmol/l after 75g oral glucose load or background therapy for diabetes), and hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or a documented history of hypertension). Hypercholesterolemia was not defined in a homogeneous fashion across studies and plasma LDL-cholesterol levels were not available for more than 2,000 patients. Overall, risks of bias and concerns about applicability were low (online data supplement further details study characteristics, bias, and applicability). Information on bleeding was limited to five studies, with only 67 major and 20 moderate/minor bleedings.

MACE and level of risk

Overall, 421 MACE occurred in 6,478 patients (6.5%), the majority being ACS (n = 383). There were 83 stent thromboses, including 79 definite or probable and four possible ones, all included in the composite outcome of MACE. The MACE-free survival rate across the different studies at the end of follow-up ranged from 77.4% to 97.3%. In a multivariate analysis, four factors were found relevant to determining patients' levels of risk: age greater than 75 years, diabetes, ACS at inclusion, and hypertension (Table 2). The number of these factors was used as a surrogate for the individual risk of MACE. Patients with none of these factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with two or more factors 'high-risk' (global p-value <0.0001 for the trend).

MACE and PR

Nine studies (n = 4,438 patients) performed LTA using 20 µM ADP, four studies (n = 2,144 patients) used 10 µM ADP, and eight studies (n = 3,317 patients) used 5 µM ADP. Figure 2 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE increased significantly with PR with 20 µM ADP, 10 µM ADP, and 5 µM ADP.

With adjustment, high PR was still significantly associated with an increased risk of MACE (Table 3). However, for PR evaluated using 10 µM ADP, risk only increased for the highest PR category, corresponding to LTA values greater than 60%.

Interaction between risk level and PR for the outcome of MACE

Platelet reactivity assessed with 20 µM ADP. Patients with none of the four risk factors showed no significantly increased risk associated with PR, while for patients with one risk factor only, the higher strata of PR was associated with an increased risk of MACE. Patients with two or more risk factors showed an increased risk of MACE for both the medium and higher strata of PR. (Figure 3). In a Cox model, the interaction between PR strata and the risk level was statistically significant (p=0.04). The corresponding hazard ratios (HRs) are shown in Figure 3. Heterogeneity was not detected for the overall interaction (p=0.81), as

well as when it was restricted to each risk level category (intermediate versus low risk level, p=0.45, and high versus low risk level, p=0.90). Additional results on heterogeneity are provided in the supplemental material. Figure 4A shows that PR, when analyzed in a continuous fashion, barely affects the risk of MACE at 6 months in patients with no risk factors: the risk is close to 2% at six months, irrespective of the level of platelet reactivity. Conversely, patients with one risk factor and an overall 4.1% risk of MACE at six months have in fact a 2% risk of MACE when they have a low PR, or a 6% risk of MACE when they have a high PR (Figure 4B). Similarly, patients with two or more risk factors and an overall 6% risk of MACE at six months can indeed have a 2% risk of MACE when they have a low PR (Figure 4C). The reclassification of the 6-month risk of MACE, according to the three categories of platelet reactivity, in patients with no, one and two or more risk factors, is shown in Table 4. Overall, PR allowed the reclassification of 44% of the total population (1837/4193 patients) included in a 6-month follow-up to a different level, mostly in patients originally identified as intermediate or high risk on the basis of the number of risk factors only. In patients experiencing MACE in the first 6 months of follow-up, the risk predicted by the combination of PR and risk factors was on average increased compared with the risk predicted from risk factors only: the continuous event net reclassification index (NRI) was 0.39 (95%CI 0.23 to 0.62). Conversely, in patients free of MACE at 6 months, the measure of PR did not modify the predicted risk: the continuous non-event NRI was 0.01 (95%CI -0.16 to 0.09). The overall NRI was 0.39 (95%CI 0.22 to 0.57).

Platelet reactivity assessed with 10 μM ADP. A total of only five low-risk patients in four studies performing 10 μM ADP LTA to assess PR precluded an analysis of this low-risk group. Furthermore, the surrogate for risk level failed to demonstrate an association with the observed risk of MACE in these studies. Figure 4B shows that the risk of MACE increased in both intermediate- and high-risk patients for PR values above 40%, without any obvious relation with the level of risk.

Platelet reactivity assessed with 5 µM ADP. The direction of interaction between PR using 5 µM ADP and the risk level was similar to that observed for PR using 20 µM ADP, even though overall interaction did not reach the significance level (p=0.17). Of note there were 980 fewer patients in the studies performing 5 µM ADP than in those using 20 µM ADP. The increased risk of MACE as PR increases is indeed similar for intermediate- and high-risk patients; for low-risk patients PR is not associated with a MACE outcome (online data supplement). Heterogeneity was not detected for the overall interaction (p=0.19). Figure 4C shows that the risk of MACE was unaffected by PR in low-risk patients while it increased for PR values above 30% in intermediate-risk patients and for PR values above 10%–20% in high-risk patients.

Sensitivity analyses

Sensitivity analyses were performed for PR using 20 µM ADP to assess: the robustness of the association between PR and risk of MACE and its interaction with the level of cardiovascular risk; the robustness of the results in the population of PCI patients and when target vessel revascularization is added to the composite outcome. All analyses showed that the sizes of the effects remained similar, and whilst in some instances the statistical significance of the interactions could be lost, there was no impact on their magnitudes (supplemental Tables 1 and 2). Notably, when PR was categorized in quartiles (20 µM ADP maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%. 51.4%–63.0%, 63.1%–100%) the interaction between PR and the number of risk factors remained significant (p=0.01). When restricted to the population of 3,564 patients treated with PCI and tested using 20 µM ADP the interaction was of similar magnitude but no longer significant (supplemental Table 3).

Publication and availability biases

A check for potential publication bias was made for PR using 20 µM ADP, on which the main analyses were performed. A funnel plot was obtained by representing the HR of PR using 20

μM ADP and the standard error, assessed in each separate study (supplemental Figure 4). Two studies with a negative association between PR using 20 μM ADP and the risk of MACE (with small sample sizes) were detected as missing using the 'trim and fill' method for making the funnel plot symmetrical. When these missing studies were added, the pooled HR was not significantly modified. These findings suggested that the publication bias in our meta-analysis was minor.

Seven qualifying studies could not provide individual patient data. It is of note that in five of these, the relation between clopidogrel non-response and ischemic events was not a study objective (pharmacokinetic-pharmacodynamic studies or randomized trials of different clopidogrel loading doses). The two remaining studies (n = 101 and 111 patients) were specifically interested in the prognostic value of PR for MACE.

Discussion

In the present meta-analysis of individual patient data conducted in a representative panel of clopidogrel-treated patients we demonstrated that the association between PR and the risk of MACE depended strongly on the level of cardiovascular risk. When using 20 μM ADP, the most commonly used concentration in LTA, the risk of MACE associated with PR increased with the level of cardiovascular risk. Indeed, PR did not affect the risk of MACE in patients presenting no risk factors, however it gradually increased the risk of MACE as the number of cardiovascular risk factors increased, reaching a 3.7 times greater risk in high-risk patients with a high PR. The measure of PR with 20 μM ADP, in addition to risk factors, modified the interpretation of the 6-month risk of MACE in 44% of patients, mainly in patients with at least one risk factor.

Interestingly, smoking and hypercholesterolemia were not associated with the outcome of MACE and were not included in the analysis of the interaction between PR and risk factors. In randomized controlled trials, the benefit of clopidogrel in reducing the incidence of MACE is primarily seen in smokers, with little benefit to non-smokers(39). With regard to the cohort studies of clopidogrel-treated patients included in this meta-analysis, this differential effect suggests that the increased risk of MACE related to smoking is offset by the benefit clopidogrel provides to smokers; it thereby weakens any possible analysis of the interaction between smoking and PR for outcomes of MACE. Regarding hypercholesterolemia, this conventional risk factor is likely to be confounded by indications for statin treatment. Indeed, in the ADAPT-DES registry(16) hyperlipidemia was protective against mortality with a HR=0.60 (0.41–0.86) and was not prognostic of MACE in post-ACS patients with optimal medical therapy(40). In addition, hypercholesterolemia was not homogeneously defined across the studies in the present meta-analysis and other markers, such as plasma LDL-cholesterol levels, were not widely available.

When PR was evaluated using 5 μM ADP, its interaction with the level of cardiovascular risk for the prediction of MACE was of a similar magnitude, although non-significant. These findings may reflect the lower number of patients available in studies using 5 μM ADP, and a

corresponding loss of power. Moreover, it was previously shown that ADP-induced platelet aggregation in citrated plasma was dependent on the artifactual generation of TxA2 that was modulated by aspirin, at least at lower ADP concentrations(41). This may be associated with an additional background noise in which the interaction between the identified risk factors and PR to predict MACE is blurred, as seen with the lowest 5 µM ADP concentrations and partially also with the intermediate 10 µM ADP concentrations. Only four of the studies analysed used 10 µM ADP, and two of these had a follow-up limited to 30 days; with only 124 MACEs during follow-up, this accounts for a limitation in power to reliably study interactions. Overall, the concentration of ADP used is of limited significance since the influence of risk factors appears in all three ADP concentration groups (table 3 and figure 2). Which laboratory assay and which platelet agonist concentration are best suited for the clinical evaluation of platelet function is the matter of some debate. ADP-induced LTA is highly reproducible within a given laboratory, but its lack of standardization across studies may have slightly weakened the positive findings or lower the level of significance for the interactions found in the present meta-analysis. Of note, the present meta-analysis does not aim to promote the use of LTA to tailor antiplatelet therapy but it rather relied on a historical gold standard in platelet function testing to evidence an interaction with patients' characteristics that should be considered for a tailored approach. The point-of care VerifyNow $P2Y_{12}$ assay, used in several intervention trials, correlates well with ADP-induced LTA(42, 43) and we speculate that the main findings of the present meta-analysis would have been similar, had PR been evaluated using the VerifyNow $P2Y_{12}$ assay.

Several intervention trials have compared conventional clopidogrel treatment to an antiplatelet strategy tailored according to PR. Early, small randomized trials(11, 12) that utilized vasodilator-stimulated phosphoprotein phosphorylation level measurement to indicate $P2Y_{12}$ receptor reactivity, showed a protective effect for repeat 600 mg clopidogrel loading doses in ACS patients prior to PCI. However, recent larger trials utilizing the VerifyNow $P2Y_{12}$ assay were negative. Indeed, the GRAVITAS(13) and ARCTIC(14) studies failed to show the benefit of a PR-tailored antiplatelet strategy after PCI. Various limitations of these trials were

addressed in a recent consensus publication(22). The event rate of the GRAVITAS study was low compared to the one used for power calculation, and the antiplatelet effect of the high-dose regimen may have been suboptimal as it reduced the prevalence of high PR by only 22%. Similarly, the ARTIC study population was also at a low absolute risk of subsequent cardiovascular events because the prevalence of ACS patients was low, and the composite endpoint also included other events that may not be related to platelet function. The interaction of PR and the number of risk factors, as identified in the present meta-analysis, substantiates the hypothesis that the risk associated with high PR was not clinically relevant in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a beneficial reduction of MACE for these low-risk patients. Based on these observations we speculate that higher risk patients are more likely to benefit from a therapy tailored to their initial PR. This may explain why early interventions designed to efficiently blunt high PR in ACS patients with multiple conventional risk factors translated into a reduction of MACE(11, 12, 22).

In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk. However, a recent cost-effectiveness analysis for six European perspectives showed that the universal use of newer $P2Y_{12}$ inhibitors for ACS patients is probably not as cost-effective as strategies based on PR(44). It should also be kept in mind that ticagrelor and prasugrel increase the risk of bleeding and that a therapeutic medium-PR window is associated with optimal net clinical benefit(22). The net benefits of newer $P2Y_{12}$ inhibitors could also probably be improved not only by testing for PR, but also by incorporating patient risk levels in the decision-making process. Although ongoing trials on tailored $P2Y_{12}$ strategies, including TROPICAL-ACS (ClinicalTrials.gov identifier: NCT01959451) and ANTARCTIC(45) partly include this concept of risk levels, further efforts in this direction are needed.

This meta-analysis has several strengths, such as the good overall quality of the studies included, as assessed using a quality tool specifically adapted to prognostic studies. The availability of individual patient data allowed a reliable evaluation of the risk associated with

PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a secondary prevention population were thus identified. The consistency of results across the different ADP concentrations used in the different studies to assess PR, as well as the sensitivity analyses, indicated that the results were robust.

Despite the advantages related to the availability of individual patient data, this meta-analysis also had some limitations, including a low proportion of women (25%). This did not allow a stratification of the analyses by gender, as is usually the case in risk assessment tools such the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is not considered as one of traditional risk factors, but is rather presented in separate charts for women and men. There were incomplete data on concomitant medications or other relevant risk factors such as the left ventricular ejection fraction, cholesterol levels or renal insufficiency. Finally, information on bleeding was limited to five studies and a low number of events, thus precluding a reliable analysis of bleeding events and their relation to PR.

In conclusion, high PR in patients on clopidogrel is associated with an increased risk of MACE in patients with vascular risk factors, but not in low-risk patients. These findings suggest that trials on tailored PR treatment strategies should be primarily stratified on the individual vascular risk factors in order to assess a truly personalized approach.

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Author contributions:

Reny JL, Fontana P, and Combescure C are guarantors for the study, had full access to the data and take responsibility for the integrity of the data and the accuracy of its analysis

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Acquisition of data: Reny JL, Fontana P, Hochholzer W, Neumann FJ, Ten Berg J, Janssen PW, Geisler T, Gawaz M, Marcucci R, Gori AM, Cuisset T, Alessi MC, Berdagué P, Gurbel P, Yong G, Angiolillo D, Aradi D, Beigel R, Campo G.

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Table 1. Main characteristics of published studies

Study	Years of publication	Patients (n)	Age (y)	Male (%)	Diabetics (%)	Smokers (%)	Hyper-tension (%)	Hypercholes-terolemia (%)	ACS at inclusion (%)	PCI (%)	GpIIb/IIIa inhibitor (%)	Follow-up (months)*	ADP (μM)
Campo et al.(27)	2006	70	64±13	69	19	37	63	34	100	100	100	10 (15)	5, 20
Hochholzer et al.(28)	2006	765	66±9	78	24	11	82	92	0	100	0	12 (12)	5, 20
Angiolillo et al.(29)	2007	173	67±9	65	100	13	65	68	0	0	0	24 (36)	20
Cuisset et al.(30)	2007	190	65±12	76	33	48	58	53	87.4	100	14.7	1 (1)	10, 20
Geisler et al.(31)	2008	1,092	67±11	74	33	39	80	59	51.7	100	7.7	1 (1)	20
Gurbel et al.(32)	2008	297	65±12	65	41	55	74	82	0	100	42	24 (24)	5, 20
Cuisset et al.(33)	2009	598	65±12	78	35	39	56	55	100	100	9.9	1 (1)	10
Yong et al.(34)	2009	248	63±12	71	22	27	53	52	100	55	39.7	6 (21)	5, 10, 20
Breet et al.(35)	2010	1,069	64±11	75	81	11	77	80	0	100	7.0	12 (12)	5, 20
Marcucci et al.(36)	2010	1,108	69±10	75	24	23	66	55	100	100	26.0	12 (12)	10
Beigel et al.(37)	2011	174	59±12	83	27	41	51	45	100	100	-	6 (6)	5
Aradi et al.(38)	2012	160	62±9	63	38	36	84	50	0	100	0	12 (12)	5
Reny et al.(8)	2012	534	62±12	82	21	20	56	63	0	0	0	32 (50)	5, 20

Age, mean ± standard deviation; CAD, coronary artery disease; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP, adenosine diphospate concentration used for the evaluation of platelet reactivity

* Median (maximum)

Table 2: Multivariate analysis to assess the associations between the risk factors and the composite outcome of MACE. This analysis was conducted on the patients of the 13 studies of the meta-analysis (n=6,256 after exclusion of missing data). MACE were observed in 412 patients. Hazard ratios (HR) greater than one show an increased risk of MACE in patients having the corresponding risk factor.

Factors collected in studies	Adjusted HR [95% CI]	p	Level of risk of MACE *	HR [95% CI]	p
Current smoking status	0.92 [0.71;1.18]	0.50	*Low risk (n=579)*	1	
Age (> 75)	1.56 [1.25;1.95]	<0.0001	*Intermediate risk (n=2444)*	1.61 [1.05;2.45]	0.03
Diabetes	1.58 [1.27;1.96]	<0.0001	*High risk (n=3435)*	2.58 [1.69;3.94]	<0.0001
Hypercholesterolemia	0.86 [0.69;1.06]	0.15			
Hypertension	1.23 [0.98;1.54]	0.07			
ACS at inclusion	2.00 [1.27;3.16]	0.003			
Gender (Male)	1.11 [0.89;1.40]	0.35			

*: a surrogate for the level of risk was defined as the number of risk factors (among age, diabetes, hypertension, and ACS at inclusion): low risk for no risk factor, intermediate risk for one risk factor and high risk for two or more risk factors).

Table 3: Associations between the ADP induced-aggregation categories and the composite outcome of MACE with adjustment on the factors collected in the studies of the meta-analysis (factors shown in Table 2).

	ADP 20 µM		**ADP 10 µM**		**ADP 5 µM**	
	N		**N**		**N**	
Studies	9		4		8	
Events	287		124		229	
Patients (after exclusion of missing data)	4,140		2,077		3,160	
	HR [95% CI]	**p**	**HR [95% CI]**	**p**	**HR [95% CI]**	**p**
ADP induced-aggregation categories		0.0003**		0.03**		0.02**
*Lower category **	1		1		1	
*Intermediate category **	1.85 [1.26;2.73]	0.002	1.31 [0.79;2.17]	0.30	1.79 [1.02;3.14]	0.04
*Higher category **	2.91 [1.78;4.74]	<0.0001	2.61 [1.64;4.16]	<0.0001	2.79 [1.50;5.22]	0.001

HR, Hazard Ratio; CI, Confidence Interval

* Categories for ADP 20 and 10 µM are 0%-40%, 41%-60%, 61%-100%, and for ADP 5 µM are 0%-30%, 31%-50%, 51%-100%

**: global p-values for testing the hypothesis that both HRs (intermediate- and higher-ADP induced-aggregation category) equal 1

Table 4: Reclassification of the 6-month risk of MACE when the individual risk was predicted from platelet reactivity measured by 20µM ADP in addition to risk factors. The predicted risk was stratified in three levels (low: ≤3%, intermediate: >3% and ≤5%, high: >5%) in agreement with the 6-month risk observed in patients with none, one and two or more risk factors (2.3%, 4.1% and 6.2% respectively). Patients were stratified according to their number of risk factors and to the level of the predicted risk. The numbers of patients and, in brackets, the corresponding observed 6-month risk of MACE in each stratum.

	Risk predicted by the combination of risk factors and platelet reactivity measured by 20µM ADP			
Risk predicted by the number of risk factors only	Low risk (≤3%)	Intermediate risk (>3% and ≤5%)	High risk (>5%)	Total
Low risk - no risk factor	524 * (2.4% **)	26 *	0 *	550 * (2.3% **)
Intermediate risk - one risk factor	625 * (2.1% **)	576 * (3.7% **)	622 * (6.3% **)	1823 * (4.1% **)
High risk - two or more risk factors	102 * (0.0% **)	462 * (3.0% **)	1256 * (7.6% **)	1820 * (6.2% **)
Total	1251 * (2.1% **)	1064 * (3.4% **)	1878 * (7.1% **)	4193 * (4.7% **)

*: number of patients

**: observed 6-month risk of MACE

Figure 1: Flow chart of the meta-analysis

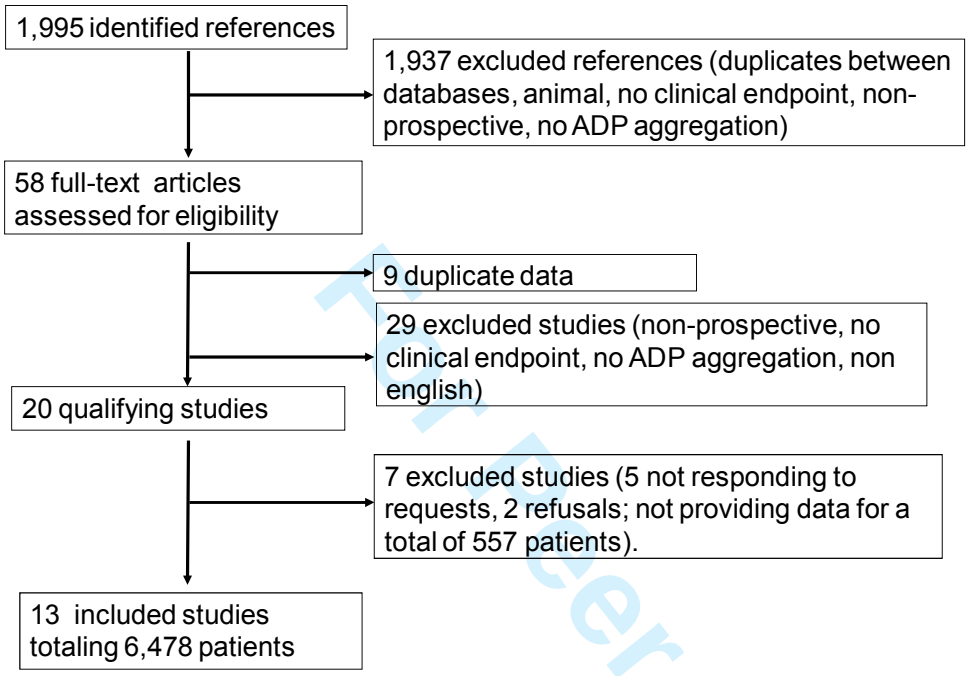


Figure 2: Kaplan-Meier survival curve for the occurrence of MACE

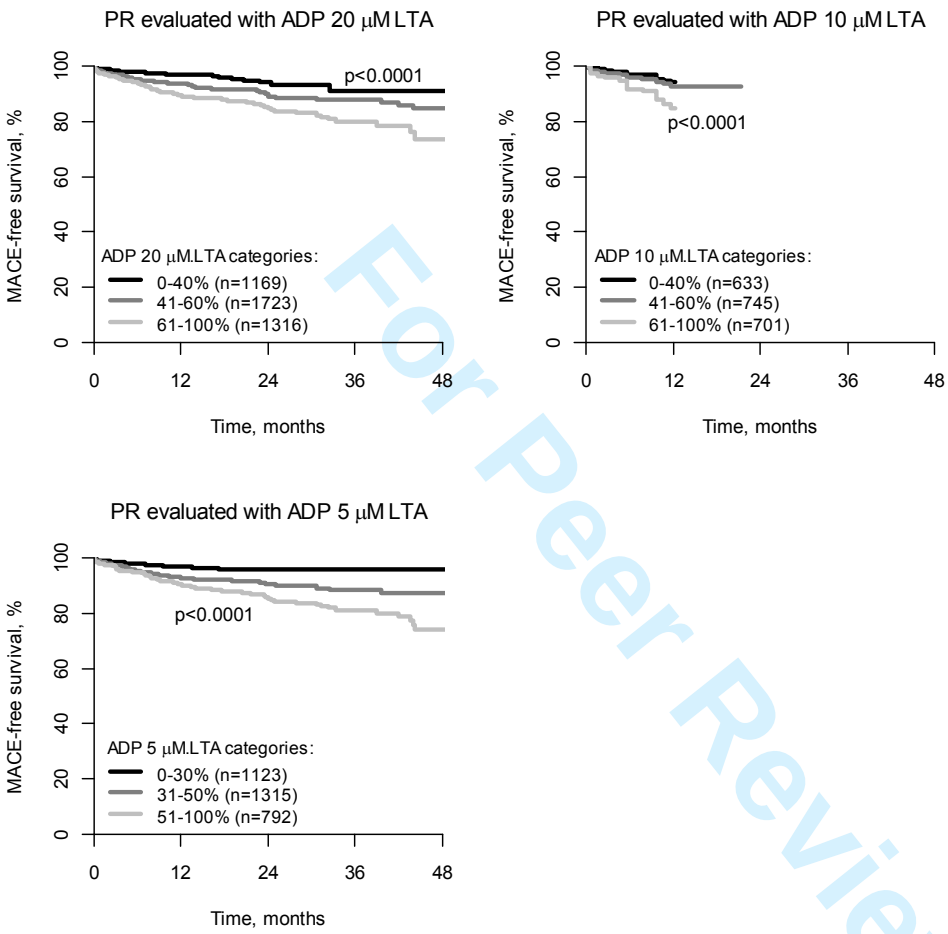


Figure 3: Association between platelet reactivity and the occurrence of MACE according to the level of risk. Low-risk patients have none of the risk factors (among age > 75 years, acute coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have one risk factor and high-risk patients have two or more risk factors. PR was assessed with 20 µM ADP LTA.

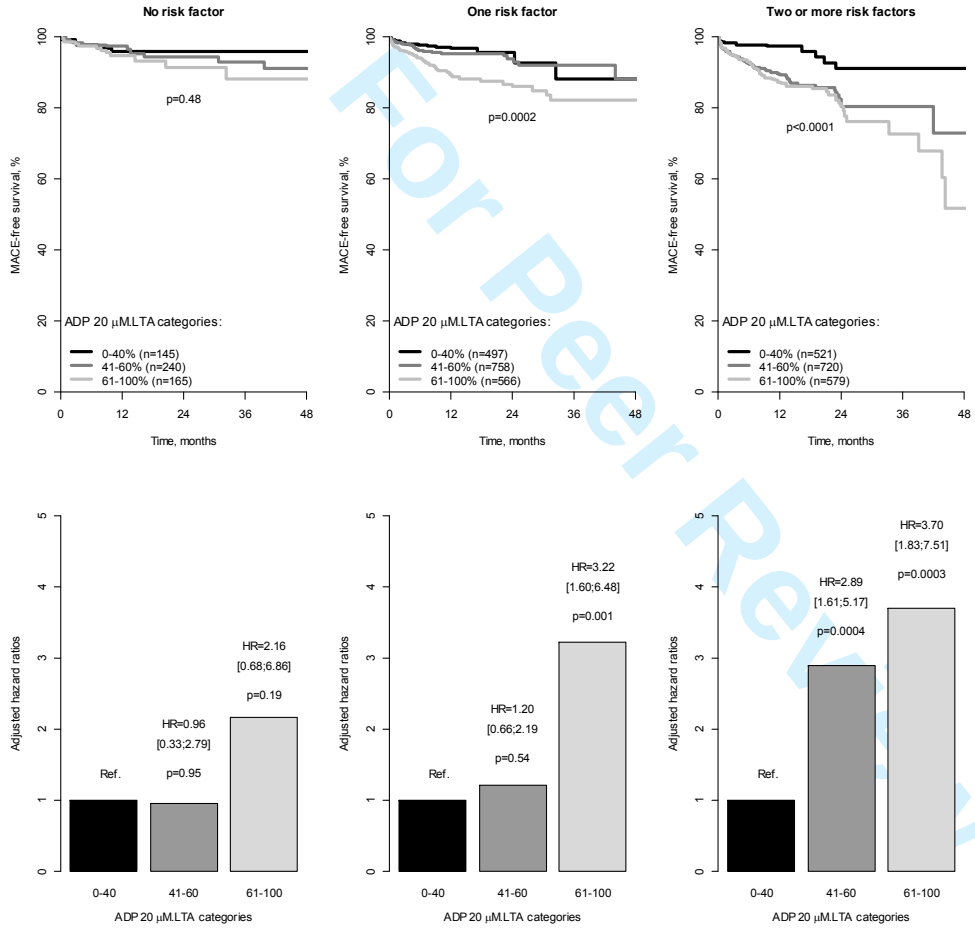
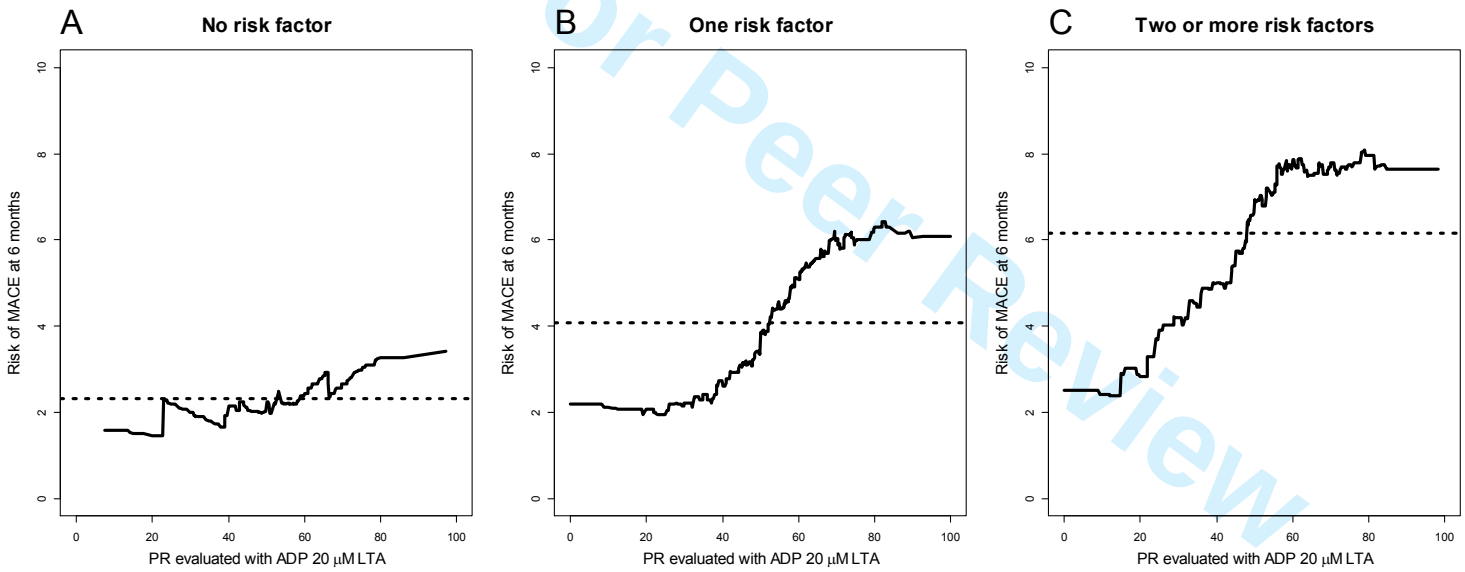


Figure 4: 6-month risk of MACE according to platelet reactivity in the different risk groups. The dashed line represents the overall risk, ignoring platelet reactivity and the black line shows the risk according to the platelet reactivity assessed with 20 µM ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).



Rebuttal to BMJ reviewers' comments

Reviewer: 1

Comments:
I think this is a fascinating study performed by experts in the field of platelet function analysis. There is a clear demonstration of high residual platelet reactivity in treated patients with intermediate and high levels of vascular risk factors and its contribution to further adverse events. This paper will help redirect further research in this area and could like to significant health benefits at if it can be demonstrated that changes in therapy provide better healthcare.

Response : NA

Reviewer: 2

Comments:
In the present paper, the relation between platelet reactivity testing and number of vascular risk factors is studied affecting major adverse cardiovascular events. The study may add to existing literature as intervention studies so far did show the expected benefit in patients with low reactivity.

1. The background of the studies included in this meta analysis should be better clarified: The differential between purely observational cohorts versus studies in patients undergoing revascularization studies. In patients undergoing revascularization the risk for periprocedural adverse outcome is considered especially high in patients with low reactivity but this category is not clearly differentiated from the patients receiving secundairy preventive medical treatment only. Please make this more clear to the reader.

Response : we fully agree with this comment and purposely provided the information in table 1 on patients with ACS and/or PCI at inclusion (a majority of patients). It was likely that in patients with ACS, platelet reactivity would play a more important role but it was, up to now, not clearly established with strong data. The fact that ACS is a risk factor showing an interaction with platelet reactivity toward the outcome of MACE was identified in the present meta-analysis, within the set of risk factors associated with the outcome.

2. The endpoints are ACS, ischemic stroke and vascular death. Why not including any death ?

Response : these endpoints are consensual and the widely used so-called MACE or MACCE endpoints. While we agree that it is important to monitor « all deaths » when performing a clinical trial, it may not be relevant to include non-vascular deaths in a composite outcome when a study is interested in platelet reactivity and the risk of recurrent thrombotic events. One can speculate that platelet reactivity could play a role in cancer-related deaths thereby having an impact on total deaths but we were interested in a potential interaction between platelet reactivity and vascular risk factors. In order to avoid any diluting or noise effect due to the inclusion of total deaths we restricted our composite outcome to ischemic events only. Finally, as the outcome items were pre-specified and did not include non-vascular deaths we did not request individual patient data on this latter item and cannot perform this analysis now.

3. Did the authors look at separate outcome parameters within this dataset; especially on ACS versus stroke leaving vascular death out of the perspective ?

Response : we did not look at each outcome separately or leaving vascular death aside. As mentioned in the results section, > 90% of the events were ACS (383 ACS out of 421 MACE), thereby precluding a reliable analysis on separate outcomes. Of note, vascular deaths were adjudicated by an independent adjudicating committee in some of the included studies.

4. Results page 10 line 47: reporting of stent thrombosis as outcome. This was not indicated as outcome parameter or it should be that ALL thrombosis led to ACS ? please explain.

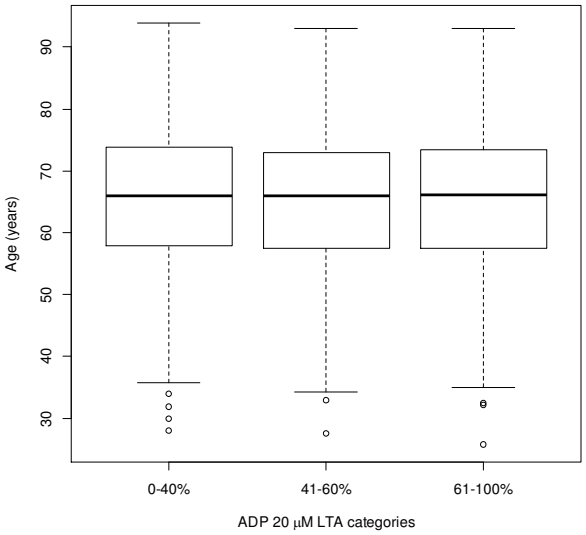
Response : the information on stent thrombosis is provided as a descriptive statistics. All stent thrombosis indeed led to an ACS as this was mentioned in the manuscript ("There were 83 stent thromboses, including 79 definite or probable and four possible ones, all included in the composite outcome of MACE")

5. A serious limitation is the use of LTA for PR only. Especially as the authors state in the introduction that verify now was never tested likewise it would have seemed easy to also look for outcome of verify Now testing. Please explain. Also, make clear in the conclusion section that observed results account for LTA only !

Response: we acknowledge that the conclusion is supported by LTA data only and this was indeed the design of this IPD meta-analysis. We extensively discussed this in the manuscript. The definition of PR is given in the abstract, in the introduction, in the methods, in the results and in the discussion. We feel that this is clearly stated throughout the manuscript and that the conclusion should remain concise.
As mentioned in the Discussion section, LTA is considered the gold against which all other point of care assays were developed. Finally, a meta-analysis on 3 059 individual patient data using the VerifyNow assay has been performed, but its power was lower than the present work and the interaction with vascular risk was not investigated (Brar SS, ten Berg J, Marcucci R, Price MJ, Valgimigli M, Kim HS, Patti G, Breet NJ, DiSciascio G, Cuisset T, Dangas G. Impact of platelet reactivity on clinical outcomes after percutaneous coronary intervention. A collaborative meta-analysis of individual participant data. J Am Coll Cardiol. 2011;58:1945-1954)

6. In all risk prediction models in patients with cardiovascular patients age shows the largets effect. please extend more on the relation ship between age as a single risk factor and platelet reactivity.

Response: for reviewing purposes we performed the suggested analysis. The category of platelets reactivity (measured with ADP 20 μM LTA) was not associated with age (Kruskal-Wallis test: p=0.44): the median (IQR) values were 66.0 years (58.0 to 73.9) in the ADP aggregation category 0-40%, 66.0 years (57.5 to 73.0) in the 41-60% category and 66.2 years (57.5 to 73.3) in the 41-100% category. Distributions of age are shown in the Figure below:



In a univariate Cox regression model with a mixed effect to take the clustering into account, age was significantly associated with the risk of MACE (HR=1.67 for patients above 75 years versus patients below 75 years old, 95%CI 1.35 to 2.07, p<0.0001).

In a univariate Cox regression model with a mixed effect to take the clustering into account, the category of platelet reactivity was significantly associated with the risk of MACE. The hazard ratios compared with the reference 0-40% category were:

- *HR=1.90 (95%CI 1.29 to 2.80, p=0.001) for the 41-60% category*
- *HR=3.11 (95%CI 2.12 to 4.57, p<0.0001) for the 61-100% category*

When the association between MACE and platelet reactivity measured by 20 μM ADP aggregation was adjusted for age, the hazards ratios for platelet reactivity categories and age of patients were similar:

- *HR=1.88 (95%CI 1.28 to 2.77, p=0.001) for the 41-60% category*
- *HR=3.01 (95%CI 2.05 to 4.43, p<0.0001) for the 61-100% category*
- *HR=1.56 (95%CI 1.20 to 2.02, p=0.001) for age (patients older than 75 years versus patients less than 75 years old)*

In patients younger than 75 years, platelet reactivity was significantly associated with the risk of MACE (p<0.0001) and the hazard ratios were:

- *HR=1.55 (95%CI 1.01 to 2.40), p=0.046) for the category 41-60%*
- *HR=2.76 (95%CI 1.80 to 4.23), p<0.0001) for the category 61-100%*

In patients older than 75 years old, platelet reactivity was also associated with risk of MACE (p=0.0004) and the hazard ratios were greater than for patients younger than 75 years old:

- *HR=3.56 (95%CI 1.48 to 8.56), p=0.005) for the category 41-60%*
- *HR=4.15 (95%CI 1.71 to 10.08), p=0.002) for the category 61-100%*

However, the interaction between platelet reactivity and age for prediction of MACE was not statistically significant (p=0.12).

These results confirm the overall robustness of the main interaction finding but we believe that this type of sub-analysis should not be provided as it complicates the message and is not statistically significant.

7. Conclusion; "suggesting that PR tailored strategies may be most effective in higher risk patients" is not warranted based on the data presented in this study. Therefore this statement does not belong to the conclusion.

Response: We agree that this may sound too speculative and this was stated only as a suggestion. In order to remain conservative and avoid any overstatement we deleted this part of the conclusion

Reviewer: 3

Comments:
This manuscript reports the results of a meta-analysis of individual patient data on the relationship of the risk of major adverse cardiovascular events (MACE) to platelet reactivity and clinical risk factors in patients treated with clopidogrel prior to percutaneous coronary interventions.The study was performed by a team of multinational European investigators with experience in epidemiology and clinical cardiology. The data base analyzed was large (13 studies including 6478 patients), and the methods used were appropriate. The paper is well organized and clearly written. The conclusions are supported by the data.
The results of the study are important. They make a major contribution to a field of investigation that has high clinical relevance, but one that is burdened by methological disputes and conflicting outcomes. The results described provide new understanding of how platelet function data might be more usefully interpreted. A strong virtue of the study is that the results are consistent with clinical data. The paper will be of significant interest to clinicians.

Critique:
1)The number of authors is excessive. Major contributors should be selected or the work presented on behalf of a coalition consistent with BMJ editorial policy.

Response : we very respectfully disagree as all authors had an active participation in the management of their own studies, provided their own individual patient data and actively participated to this meta-

analysis and this manuscript. We kindly ask the editors to allow the inclusion of 20 authors, all complying with authorship rules, for this manuscript

2)Page 8, paragrapgh 3: the statement that 20 micromolar ADP is better requires some qualification and references. It is reasonable to think that a maximal agonist stimulus would be the most useful, but it is apparent from the data presented that there is no standard that has been uniformly applied.

Response : We fully agree with this comment. Indeed, it was previously shown that ADP-induced platelet aggregation at low concentration (up to 10 microM) in citrated plasma was dependant of the artefactual generation of TxA2 that is sensitive to aspirin (Cattaneo M. Aspirin and clopidogrel: Efficacy, safety, and the issue of drug resistance. Arterioscler Thromb Vasc Biol. 2004;24:1980-1987). This may be associated with an additional background noise in which the interaction between the identified risk factors and PR to predict MACE is blurred, as seen with the lowest concentrations of ADP This was stated in the discussion and the above reference was cited. We have now added a clear mention of the "lowest concentration" as 5 micromolar and the intermediate 10 micromolar.

3)Data presented in Table 3 and in Figure 2 could be interpreted to indicate that the concentration of ADP used is of limited significance since the influence of risk factors appears in all three ADP concentration groups. this should be discussed by the authors

Response : we fully agree and we have added a sentence with this interpretation. The consistency of the interaction independently of ADP concentration further support the main findings of this meta-analysis.

4)Since the bulk of the work is statistical , the paper should receive careful statistical review.

Response : this was done within the BMJ editorial committee including a statistician, Rafael Perera, who did not have any methodological or statistical issues related to this meta-analysis. In addition we provide a detailed description of the methods used and several sensitivity analyses that support the robustness of the findings (supplemental online material). Finely, we included a quality assessment tool, PROBAST, that was recently and specifically designed for meta-analysis or prognostic studies. The PROBAST tool was kindly made available to us by Dr Penny Whitting as acknowledged in the manuscript.

MOOSE checklist designed for meta-analyses of observational studies (1) in lieu of the PRISMA checklist(2) focused on meta-analyses of randomized and intervention trials.

Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data. Reny JL et al.

Reporting of background should include

Problem definition: lines 127-128 (Introduction)

Hypothesis statement: lines 132-136 (Introduction)

Description of study outcome(s): line 136 (Introduction), lines 192-207 (Methods)

Type of exposure or intervention used: lines 136-137 (Introduction), lines 192-207 (Methods)

Type of study designs used: line 137 (meta-analysis design, Introduction), lines 162-164 (designs od studies included in the meta-analysis, Methods)

Study population: lines 154-155 (Methods)

Reporting of search strategy should include:

Qualifications of searchers (eg, librarians and investigators): investigators, methods p 6 line 149

Search strategy, including time period included in the synthesis and keywords: lines 143-151 (Methods)

Effort to include all available studies, including contact with authors: lines 171-173

Databases and registries searched: lines 143-151 (Methods)

Search software used, name and version, including special features used (eg, explosion): databases cited in the section ”Methods” only lines 143-145

Use of hand searching (eg, reference lists of obtained articles): lines 148-149 (Methods)

List of citations located and those excluded, including justification: Figure 1 (flow-chart)

Method of addressing articles published in languages other than English: Not applicable.

Method of handling abstracts and unpublished studies: no congress abstract retrieved

Description of any contact with authors: lines 170-181 (Methods)

Reporting of methods should include:

Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested: The relevance of studies was guaranteed by the inclusion criteria. Lines 154-165

Rationale for the selection and coding of data (eg, sound clinical principles or convenience): Individual patient’s data. The format of data provided by authors was harmonized (standardization of units) – lines 178-181 (Methods)

Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability): Data were not extracted from published papers but provided by authors. The list of required variables was provided to authors as well as the definition of the biological and clinical outcomes. Data were checked for completeness and consistency with published reports. Any discrepancies were resolved with the corresponding authors – lines 178-181 (Methods)

Assessment of confounding (eg, comparability of cases and controls in studies where appropriate): the quality of studies, assessed with the PROBAST tool (methods and acknowledgements), included the risk of bias related to the outcome measurement, follow-up of patients and measure of exposure. Lines 185-189.

Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results: assessment of the quality with the PROBAST tool – lines 185-189 (Methods) and supplement Appendix Table 1.

Assessment of heterogeneity: lines 212-215 (Methods) and Supplement Appendix ("Detailed statistical analysis")

Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated : lines 209-228 (Methods) and Supplement Appendix (("Detailed statistical analysis")

Provision of appropriate tables and graphics: No tables, no graphics in the "Methods" section

Reporting of results should include:

Graphic summarizing individual study estimates and overall estimate: Individual study estimates are not reported because studies were not powered to test the interaction term between the platelet reactivity and the level of risk. Only overall estimates are reported. Tables 2, 3 and section "Results".

Table giving descriptive information for each study included: Table 1

Results of sensitivity testing (eg, subgroup analysis): Sensitivity are summarized lines 353-365 (Results) and detailed further in Supplement Appendix

Indication of statistical uncertainty of findings: p values for testing the interaction term, 95% confidence interval for all estimates, publication and availability biases assessed in results (lines 367-380, Results)

Reporting of discussion should include:

Quantitative assessment of bias (eg, publication bias): publication and availability biases assessed in results (lines 367-380, Results and Supplement Appendix Figure 4)

Justification for exclusion (eg, exclusion of non–English-language citations): Line 143 and reference Moher D, Pham B, Klassen TP, et al. What contributions do languages other than English make on the results of meta-analyses? J Clin Epidemiol. 2000;53(9):964-972.

Assessment of quality of included studies: Lines 241-242 (Results) and Supplement appendix Figure 2), lines 459-460 (discussion)

Reporting of conclusions should include:

Consideration of alternative explanations for observed results: Lines 466-474

Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review) : abstract and discussion lines 383-384, 463-466

Guidelines for future research: trials on PR tailored strategy warranted in HPR patients, lines 477-479

Disclosure of funding source : Lines 481-486.

1. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283(15):2008-12. Epub 2000/05/02.
2. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009;6(7):e1000097. Epub 2009/07/22.

Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data

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Keywords:	clopidogrel, drug response, platelets, cardiovascular diseases, ischemic events

Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data

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Abstract

Objective: Prior studies have shown an association between high on-clopidogrel platelet reactivity (PR) and the risk of major adverse cardiovascular events (MACE). However, large intervention trials on PR-tailored treatments have been neutral, possibly owing to the inclusion of patients at low cardiovascular risk. The role and usefulness of PR with regard to levels of cardiovascular risk are unclear. We assessed the clinical relevance of PR in predicting MACE according to patients' cardiovascular risk levels.

Design: Systematic review and meta-analysis of individual patient data on MACE outcomes (acute coronary syndromes, ischemic strokes, and vascular deaths) in relation to PR and its interaction with cardiovascular risk levels. PR was determined using ADP-induced light transmission aggregometry (LTA) with a primary concentration of 20μM ADP and defined as high (>60% aggregation), medium (41-60%) or low (<41%). A surrogate for the level of cardiovascular risk was defined as the number of conventional vascular risk factors with homogeneous definitions across studies and identified as predictors of MACE in the meta-analysis. Associations between the number of risk factors, PR strata, and risk of MACE were analysed using multivariate, mixed-effect Cox models. The net reclassification index (NRI) for survival data and the % of patients reclassified to a different risk level were computed to quantify the contribution of PR testing for the prediction of the 6-month risk of MACE in patients with increasing numbers of traditional risk factors.

Data sources: Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC). Free-text search conducted using an 'ADP' and 'aggregation' and 'clopidogrel' key-word combination. Inclusion criteria: (a) patients treated with clopidogrel and with symptomatic atherothrombosis; (b) response to clopidogrel evaluated using the maximal aggregation value from LTA with 20, 10, or 5 μM; (c) LTA performed remote from platelet function interfering drugs other than aspirin or clopidogrel; (d) prospective follow-up for MACE for at least 30 days; (e) prospective cohort or a randomised therapeutic trial.

Corresponding authors of selected studies were contacted to collaborate to the meta-analysis and to provide their individual patient's data.

Results: Thirteen prospective studies totalled 6,478 clopidogrel-treated patients who experienced 421 MACE (6.5%) during a median follow-up of 12 months. The risk of MACE associated with PR increased differentially according to the number of risk factors present (age>75 years, ACS at inclusion, diabetes, and hypertension; interaction p=0.04): no association to PR in low-risk patients (no risk factor) (p=0.48); 3.2 (1.6 to 6.5, p=0.001) times greater risk of MACE in high PR intermediate-risk patients (one risk factor); 2.9 (1.6 to 5.2, p=0.0004) and 3.7 (1.8 to 7, p=0.0003) times greater risk of MACE in medium PR and high PR high-risk patients (≥2 risk factors). PR allowed the reclassification of 44% (1837/4193 patients) of the total population to a different risk level for the outcome of MACE, mostly in patients originally identified as intermediate or high risk.

Conclusion: The magnitude of the association between PR and MACE risk is strongly dependant on the level of cardiovascular risk faced by patients on clopidogrel suggesting that PR-tailored strategies may be most effective in higher-risk patients.

Keywords: clopidogrel, drug response, platelets, cardiovascular diseases, ischemic events.

Atherosclerotic diseases account for more than 40% of deaths in Western countries, and antiplatelet therapy is a major preventive strategy in this setting.[1] Clopidogrel, a $P2Y_{12}$ receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is widely prescribed for secondary prevention in patients with atherosclerotic diseases. When combined with aspirin, clopidogrel is particularly effective in patients with acute coronary syndromes (ACS),[2] and has proved superior to aspirin alone in several other large randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide inter-individual variability.[3 4] Numerous cohort studies, often performed on patients with acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions (PCI), have shown an association between high on-treatment platelet reactivity (PR) and the risk of recurrent major adverse cardiovascular events (MACE).[5-7] However, recent studies in cohorts of stable cardiovascular outpatients[8 9] or in medically managed ACS patients[10] failed to confirm these results. Several randomised trials aimed at reducing the recurrence of ischemic events have compared standard clopidogrel treatment to a $P2Y_{12}$-inhibitor strategy tailored according to the presence of high PR. Although initial small trials were promising,[11 12] more recent larger trials showed no benefit from adjusting clopidogrel doses or switching to prasugrel based on PR testing in low-risk coronary patients undergoing PCI.[13 14] These contrasting results, both from observational studies and randomised intervention trials, may be explained by different patient characteristics including the level of risk, but to date few data substantiate these hypotheses. We previously showed, in a study-level meta-analysis, that the risk of recurrent MACE associated with high PR was greater in studies using GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did not.[7] Another meta-regression from a study-level meta-analysis of randomised trials suggested that the higher the incidence of coronary stent thrombosis in a given study, the larger the net clinical benefit from a PR-tailored strategy.[15] Finally, the ADAPT-DES registry of patients undergoing PCI showed that high PR was predictive of stent thrombosis mostly in ACS patients, but there was no interaction reported between PR and the presence of an ACS at inclusion.[16]

This information suggests the hypothesis that high PR might be more relevant in high-risk populations, but convincing data at the individual level are lacking. To date, the only meta-analysis on individual patient data performed on 6 studies totalling 3,059 patients assessed with the VerifyNow P2Y12 assay did not explore this hypothesis.[17] Similarly, one of the largest and more recent meta-analysis on 8 studies and 4817 patients did not explore this interaction due to the lack of individual data.[18] To further investigate this interaction on a larger population we performed a collaborative meta-analysis of individual patient data and focused on the interaction between relevant vascular risk factors and PR, assessed with ADP induced light transmission aggregometry (LTA), in order to better define the risk of MACE. ADP-induced LTA is the assay upon which all $P2Y_{12}$ receptor inhibitors have been developed, thus supporting its use in the present meta-analysis. In addition, among several available assays to evaluate PR, LTA is the historical gold standard with which most platelet function assays were compared.

Methods

Data sources

Literature review, confined to articles in English,[19] was based on electronic databases (Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC). A free-text search was conducted using an 'ADP' and 'aggregation' and 'clopidogrel' key-word combination. Articles were selected on the basis of abstracts, before examination of the full text. Reference lists of selected articles were also hand-searched to identify additional relevant reports. Reviewers (JLR and PF) were not blinded to the journal, authors or institutions in the publications as this has been shown to be unnecessary.[20] The electronic database search was last updated on 31 July, 2013.

Study selection

Selected studies met the following criteria: (a) patients were treated with clopidogrel and had symptomatic atherothrombosis (clinical signs related to vascular atherothrombotic lesions); (b) pharmacodynamic response to clopidogrel was evaluated using the maximal aggregation value from LTA on platelet-rich plasma with 20, 10, or 5 µM ADP as an agonist; (c) LTA was performed remote from platelet function interfering drugs such as GpIIb/IIIa inhibitors; (d) patients were prospectively monitored for MACE for at least 30 days, defined using at least one of the following items: acute coronary syndrome (unstable angina, myocardial infarction with/without ST segment elevation), ischemic stroke (acute neurological deficit due to a cerebral infarction), and vascular death; (e) studies involved either a prospective cohort or a randomised therapeutic trial, but one in which treatment was allocated independently of the response to clopidogrel. When studies were suspected of including the same patients, the authors were asked to provide data from the largest possible number of independent patients. The flow of references through the review process is shown in Figure 1.

Data extraction

The corresponding authors or principal investigators of eligible studies were contacted and asked to participate in the CLOpidogrel and Vascular ISchemic events – Individual Patient Data (CLOVIS-IPD) meta-analysis group. Investigators provided individual data on: the qualifying cardiovascular condition and clinical setting at inclusion (ACS or stable disease); MACE and date of occurrence during follow-up; platelet reactivity (PR) with ADP 20, 10, and/or 5 μM and its timing relative to loading dose of clopidogrel; age, gender, height, and weight; current smoking status, diabetes, hypercholesterolemia, and hypertension; left ventricular ejection fraction; platelet count; PCI; use of GpIIb/IIIa inhibitors and timing; concomitant medications; and bleeding events and timing during follow-up. Data were checked for completeness and consistency with published reports. Any discrepancies were resolved with the corresponding authors. After format harmonisation, data were compiled for statistical analysis. All studies were approved by their respective institutional review boards.

Quality assessment of studies

A new quality assessment tool for prognostic studies called PROBAST (see Acknowledgements) was used to estimate risks of bias and concerns about applicability. As PROBAST is not customised for meta-analyses of individual patient data, items were adapted accordingly. Based on the present study's list of relevant criteria, risks of bias, and concerns about applicability are rated as low, unclear, or high. Supplemental Figure 1 shows the list of criteria.

Primary outcomes and measures

The primary clinical outcome was the occurrence of MACE, as defined above (see Study selection (d)). The primary biological outcome was maximal aggregation with 20 μM ADP, as it is a better concentration for analysing the effects of clopidogrel than lower ones. PR was categorised in three strata. The higher cut-offs were selected on the basis of previously

published cut-offs (59% to 64% for 20 µM ADP, and 43% to 46% for 5 µM ADP),[21] and to keep relatively balanced numbers of patients in each PR categories. Three pre-specified categories allowed a better description of the dose-dependent effects of PR on the risk of MACE compared to the usual dichotomic high and low PR categorization. Three categories were also chosen to better parallel the analysis with a therapeutic PR window that has been associated with optimal net clinical benefit.[22] A surrogate for the level of cardiovascular risk was defined as the number of factors with homogeneous definitions across studies, and these were markers of MACE in the meta-analysis. The factors were selected from among age, diabetes, hypertension, smoking, hypercholesterolemia, and the presence of an ACS at inclusion (as defined in study selection (d)), and were all provided at the time of inclusion and PR testing.

Statistical analysis

MACE-free survival curves were derived from individual patient data using the Kaplan-Meier estimator; curves were compared using log-rank tests stratified by study. Associations between conventional risk factors, PR strata, and risk of MACE were analysed using multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the size of the random effects (Tau^2) which is an estimate of the between study variability.[23] The presence of heterogeneity was tested by comparing models with and without random effects (likelihood ratio test). The interactions between the level of risk and PR strata were tested. MACE-free survival according to PR, as a continuous variable, was assessed using the R package prodlim using the symmetrical nearest neighbourhoods method.[24] Sensitivity analyses were conducted to check the robustness of the findings with respect to: the risks of bias and concerns about the applicability of studies; the definition of MACE, including target vessel revascularisation or PCI at inclusion, and; the influence of a given specific study. The net reclassification index (NRI) for survival data[25] was computed to quantify the contribution of PR testing for the prediction of the 6-month risk of MACE in patients with increasing numbers of traditional risk factors. The event and non-event continuous NRIs were reported.

Potential publication bias was checked for. P-values below 0.05 were considered significant and all tests were two-sided. Published guidelines for meta-analysis of observational studies in epidemiology (MOOSE) and their reporting[26] were followed. Details on statistical methods are given in the online data supplement.

Results

Characteristics of included studies

The Figure 1 flow-chart details how 13 of 20 qualifying studies were included, totalling 6,478 patients.[8 27-38] Table 1 shows their characteristics. Data on body mass index, concomitant medications, left ventricular ejection fraction, or the occurrence of target and non-target vessel revascularisation during follow-up were only available in some studies. All studies provided individual data allowing a homogeneous definition of MACE, current smoking status, ACS, diabetes (fasting plasma glucose ≥ 7.0 mmol/l, 2-h plasma glucose ≥ 11.1 mmol/l after 75g oral glucose load or background therapy for diabetes), and hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or a documented history of hypertension). Hypercholesterolemia was not defined in a homogeneous fashion across studies and plasma LDL-cholesterol levels were not available for more than 2,000 patients. Overall, risks of bias and concerns about applicability were low (online data supplement further details study characteristics, bias, and applicability). Information on bleeding was limited to five studies, with only 67 major and 20 moderate/minor bleedings.

MACE and level of risk

Overall, 421 MACE occurred in 6,478 patients (6.5%), the majority being ACS (n = 383). There were 83 stent thromboses, including 79 definite or probable and four possible ones, all included in the composite outcome of MACE. The MACE-free survival rate across the different studies at the end of follow-up ranged from 77.4% to 97.3%. In a multivariate analysis, four factors were found relevant to determining patients' levels of risk: age greater than 75 years, diabetes, ACS at inclusion, and hypertension (Table 2). The number of these

factors was used as a surrogate for the individual risk of MACE. Patients with none of these factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with two or more factors 'high-risk' (global p-value <0.0001 for the trend).

MACE and PR

Nine studies (n = 4,438 patients) performed LTA using 20 µM ADP, four studies (n = 2,144 patients) used 10 µM ADP, and eight studies (n = 3,317 patients) used 5 µM ADP. Figure 2 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE increased significantly with PR with 20 µM ADP, 10 µM ADP, and 5 µM ADP.

With adjustment, high PR was still significantly associated with an increased risk of MACE (Table 3). However, for PR evaluated using 10 µM ADP, risk only increased for the highest PR category, corresponding to LTA values greater than 60%.

Interaction between risk level and PR for the outcome of MACE

Platelet reactivity assessed with 20 µM ADP

Patients with none of the four risk factors showed no significantly increased risk associated with PR, while for patients with one risk factor only, the higher strata of PR was associated with an increased risk of MACE. Patients with two or more risk factors showed an increased risk of MACE for both the medium and higher strata of PR. (Figure 3). In a Cox model, the interaction between PR strata and the risk level was statistically significant (p=0.04). The corresponding hazard ratios (HRs) are shown in Figure 3. Heterogeneity was not detected for the overall interaction (p=0.81), as well as when it was restricted to each risk level category (intermediate versus low risk level, p=0.45, and high versus low risk level, p=0.90). Additional results on heterogeneity are provided in the supplemental material. Figure 4A shows that PR, when analysed in a continuous fashion, barely affects the risk of MACE at 6 months in patients with no risk factors: the risk is close to 2% at six months, irrespective of the level of platelet reactivity. Conversely, patients with one risk factor and an overall 4.1% risk of MACE at six months have in fact a 2% risk of MACE when they have a low PR, or a

6% risk of MACE when they have a high PR (Figure 4B). Similarly, patients with two or more risk factors and an overall 6% risk of MACE at six months can indeed have a 2% risk of MACE when they have a low PR (Figure 4C). The reclassification of the 6-month risk of MACE, according to the three categories of platelet reactivity, in patients with no, one and two or more risk factors, is shown in Table 4. Overall, PR allowed the reclassification of 44% of the total population (1837/4193 patients) included in a 6-month follow-up to a different level, mostly in patients originally identified as intermediate or high risk on the basis of the number of risk factors only. In patients experiencing MACE in the first 6 months of follow-up, the risk predicted by the combination of PR and risk factors was on average increased compared with the risk predicted from risk factors only: the continuous event net reclassification index (NRI) was 0.39 (95%CI 0.23 to 0.62). Conversely, in patients free of MACE at 6 months, the measure of PR did not modify the predicted risk: the continuous non-event NRI was 0.01 (95%CI -0.16 to 0.09). The overall NRI was 0.39 (95%CI 0.22 to 0.57).

Platelet reactivity assessed with 10 µM ADP

A total of only five low-risk patients in four studies performing 10 µM ADP LTA to assess PR precluded an analysis of this low-risk group. Furthermore, the surrogate for risk level failed to demonstrate an association with the observed risk of MACE in these studies. Figure 4B shows that the risk of MACE increased in both intermediate- and high-risk patients for PR values above 40%, without any obvious relation with the level of risk.

Platelet reactivity assessed with 5 µM ADP

The direction of interaction between PR using 5 µM ADP and the risk level was similar to that observed for PR using 20 µM ADP, even though overall interaction did not reach the significance level (p=0.17). Of note there were 980 fewer patients in the studies performing 5 µM ADP than in those using 20 µM ADP. The increased risk of MACE as PR increases is indeed similar for intermediate- and high-risk patients; for low-risk patients PR is not associated with a MACE outcome (online data supplement). Heterogeneity was not detected

for the overall interaction (p=0.19). Figure 4C shows that the risk of MACE was unaffected by PR in low-risk patients while it increased for PR values above 30% in intermediate-risk patients and for PR values above 10%–20% in high-risk patients.

Sensitivity analyses

Sensitivity analyses were performed for PR using 20 μM ADP to assess: the robustness of the association between PR and risk of MACE and its interaction with the level of cardiovascular risk; the robustness of the results in the population of PCI patients and when target vessel revascularisation is added to the composite outcome. All analyses showed that the sizes of the effects remained similar, and whilst in some instances the statistical significance of the interactions could be lost, there was no impact on their magnitudes (supplemental Tables 1 and 2). Notably, when PR was categorised in quartiles (20 μM ADP maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%. 51.4%–63.0%, 63.1%–100%) the interaction between PR and the number of risk factors remained significant (p=0.01). When restricted to the population of 3,564 patients treated with PCI and tested using 20 μM ADP the interaction was of similar magnitude but no longer significant (supplemental Table3).

Publication and availability biases

A check for potential publication bias was made for PR using 20 μM ADP, on which the main analyses were performed. A funnel plot was obtained by representing the HR of PR using 20 μM ADP and the standard error, assessed in each separate study (supplemental Figure 4). Two studies with a negative association between PR using 20 μM ADP and the risk of MACE (with small sample sizes) were detected as missing using the 'trim and fill' method for making the funnel plot symmetrical. When these missing studies were added, the pooled HR was not significantly modified. These findings suggested that the publication bias in our meta-analysis was minor.

Seven qualifying studies could not provide individual patient data. It is of note that in five of these, the relation between clopidogrel non-response and ischemic events was not a study

objective (pharmacokinetic-pharmacodynamic studies or randomised trials of different clopidogrel loading doses). The two remaining studies (n = 101 and 111 patients) were specifically interested in the prognostic value of PR for MACE.

Discussion

In the present meta-analysis of individual patient data conducted in clopidogrel-treated patients we demonstrated that the association between PR and the risk of MACE depended strongly on the level of cardiovascular risk. When using 20 µM ADP, the most commonly used concentration in LTA, the risk of MACE associated with PR increased with the level of cardiovascular risk. Indeed, PR did not affect the risk of MACE in patients presenting no risk factors, however it gradually increased the risk of MACE as the number of cardiovascular risk factors increased, reaching a 3.7 times greater risk in high-risk patients with a high PR. The measure of PR with 20 µM ADP, in addition to risk factors, modified the interpretation of the 6-month risk of MACE in 44% of patients, mainly in patients with at least one risk factor. Interestingly, smoking and hypercholesterolemia were not associated with the outcome of MACE and were not included in the analysis of the interaction between PR and risk factors. In randomised controlled trials, the benefit of clopidogrel in reducing the incidence of MACE is primarily seen in smokers, with little benefit to non-smokers.[39] With regard to the cohort studies of clopidogrel-treated patients included in this meta-analysis, this differential effect suggests that the increased risk of MACE related to smoking is offset by the benefit clopidogrel provides to smokers; it thereby weakens any possible analysis of the interaction between smoking and PR for outcomes of MACE. Regarding hypercholesterolemia, this conventional risk factor is likely to be confounded by indications for statin treatment. Indeed, in the ADAPT-DES registry[16] hyperlipidemia was protective against mortality with a HR=0.60 (0.41–0.86) and was not prognostic of MACE in post-ACS patients with optimal medical therapy.[40] In addition, hypercholesterolemia was not homogeneously defined across the studies in the present meta-analysis and other markers, such as plasma LDL-cholesterol levels, were not widely available.

When PR was evaluated using 5 µM ADP, its interaction with the level of cardiovascular risk for the prediction of MACE was of a similar magnitude, although non-significant. These findings may reflect the lower number of patients available in studies using 5 µM ADP, and a

corresponding loss of power. Moreover, it was previously shown that ADP-induced platelet aggregation in citrated plasma was dependent on the artifactual generation of TxA2 that was modulated by aspirin, at least at lower ADP concentrations.[41] This may be associated with an additional background noise in which the interaction between the identified risk factors and PR to predict MACE is blurred, as seen with the lowest concentrations of ADP. Only four of the studies analysed used 10 µM ADP, and two of these had a follow-up limited to 30 days; with only 124 MACEs during follow-up, this accounts for a limitation in power to reliably study interactions. Which laboratory assay and which platelet agonist concentration are best suited for the clinical evaluation of platelet function is the matter of some debate. ADP-induced LTA is highly reproducible within a given laboratory, but its lack of standardisation across studies may have slightly weakened the positive findings or lower the level of significance for the interactions found in the present meta-analysis. Of note, the present meta-analysis does not aim to promote the use of LTA to tailor antiplatelet therapy but it rather relied on a historical gold standard in platelet function testing to evidence an interaction with patients'characteristics that should be considered for a tailored approach. The point-of care VerifyNow $P2Y_{12}$ assay, used in several intervention trials, correlates well with ADP-induced LTA[42 43] and we speculate that the main findings of the present meta-analysis would have been similar, had PR been evaluated using the VerifyNow $P2Y_{12}$ assay.

Several intervention trials have compared conventional clopidogrel treatment to an antiplatelet strategy tailored according to PR. Early, small randomised trials[11 12] that utilised vasodilator-stimulated phosphoprotein phosphorylation level measurement to indicate $P2Y_{12}$ receptor reactivity, showed a protective effect for repeat 600 mg clopidogrel loading doses in ACS patients prior to PCI. However, recent larger trials utilising the VerifyNow $P2Y_{12}$ assay were negative. Indeed, the GRAVITAS[13] and ARCTIC[14] studies failed to show the benefit of a PR-tailored antiplatelet strategy after PCI. Various limitations of these trials were addressed in a recent consensus publication.[22] The event rate of the GRAVITAS study was low compared to the one used for power calculation, and the antiplatelet effect of the high-dose regimen may have been suboptimal as it reduced the prevalence of high PR by only 22%.

Similarly, the ARTIC study population was also at a low absolute risk of subsequent cardiovascular events because the prevalence of ACS patients was low, and the composite endpoint also included other events that may not be related to platelet function. The interaction of PR and the number of risk factors, as identified in the present meta-analysis, substantiates the hypothesis that the risk associated with high PR was not clinically relevant in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a beneficial reduction of MACE for these low-risk patients. Based on these observations we speculate that higher risk patients are more likely to benefit from a therapy tailored to their initial PR. This may explain why early interventions designed to efficiently blunt high PR in ACS patients with multiple conventional risk factors translated into a reduction of MACE.[11 12 22].

In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk. However, a recent cost-effectiveness analysis for six European perspectives showed that the universal use of newer $P2Y_{12}$ inhibitors for ACS patients is probably not as cost-effective as strategies based on PR.[44] It should also be kept in mind that ticagrelor and prasugrel increase the risk of bleeding and that a therapeutic medium-PR window is associated with optimal net clinical benefit.[22] The net benefits of newer $P2Y_{12}$ inhibitors could also probably be improved not only by testing for PR, but also by incorporating patient risk levels in the decision-making process. Although ongoing trials on tailored $P2Y_{12}$ strategies, including TROPICAL-ACS (ClinicalTrials.gov identifier: NCT01959451) and ANTARCTIC[45] partly include this concept of risk levels, further efforts in this direction are needed.

This meta-analysis has several strengths, such as the good overall quality of the studies included, as assessed using a quality tool specifically adapted to prognostic studies. The availability of individual patient data allowed a reliable evaluation of the risk associated with PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a secondary prevention population were thus identified. The consistency of results across the

different ADP concentrations used in the different studies to assess PR, as well as the sensitivity analyses, indicated that the results were robust.

Despite the advantages related to the availability of individual patient data, this meta-analysis also had some limitations, including a low proportion of women (25%). This did not allow a stratification of the analyses by gender, as is usually the case in risk assessment tools such the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is not considered as one of traditional risk factors, but is rather presented in separate charts for women and men. There were incomplete data on concomitant medications or other relevant risk factors such as the left ventricular ejection fraction, cholesterol levels or renal insufficiency. Finally, information on bleeding was limited to five studies and a low number of events, thus precluding a reliable analysis of bleeding events and their relation to PR.

In conclusion, high PR in patients on clopidogrel is associated with an increased risk of MACE in patients with vascular risk factors, but not in low-risk patients. These findings suggest that trials on tailored PR treatment strategies should be primarily stratified on the individual vascular risk factors in order to assess a truly personalized approach.

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Contributors:

Reny JL, Fontana P, and Combescure C are guarantors for the study, had full access to the data and take responsibility for the integrity of the data and the accuracy of its analysis

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All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data.

Conflicts of interest:

Reny JL: payment for lectures by Merck Sharp and Dohme

Fontana P: consultancy for Evolva; grants from Evolva and Astra Zeneca, payment for lectures by Bayer, AstraZeneca.

Hochholzer W: no conflicts of interest.

Neumann FJ: no conflicts of interest

Ten Berg J: consultancy for Astra Zeneca, Eli Lilly, Merck, and Daiichi Sankyo

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Geisler T: consultancy for Bayer, Medicines company, Eli Lilly, Pfizer, BMS, and Daiichi Sankyo; payments for lectures by Bayer, Medicines company, Eli Lilly, Pfizer, BMS, and Daiichi Sankyo; MSD, Boehringer, Astra Zeneca.

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Marcucci R: no conflicts of interest

Gori AM: no conflicts of interest

Cuisset T: no conflicts of interest

Alessi MC: Board membership for Astra Zeneca and Lilly; lectures for Roche;

Berdagué P: no conflicts of interest

Gurbel P: Served as a consultant for Daiichi Sankyo, Sankyo, Lilly, Bayer, AstraZeneca, Accumetrics, Merck, Medtronic, CSL, and Haemonetics; receiving grants from the National Institutes of Health, Daiichi Sankyo, Lilly, CSL, AstraZeneca, Harvard Clinical Research Institute, Haemonetics, and Duke Clinical Research Institute; receiving payment for lectures, including service on speakers' bureaus, from Lilly, Daiichi Sankyo, and Merck; receiving payment for development of educational presentations from Merck, the Discovery Channel, and Pri-Med; Dr. Gurbel holds stock or stock options in Merck, Medtronic, and Pfizer; and holds patents in the area of personalised antiplatelet therapy and interventional cardiology.

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Beigel R: no conflicts of interest

Campo G: no conflicts of interest

Combescure C: no conflicts of interest

What this paper adds

What is already known on this subject

Prior meta-analyses have shown an association between high on-clopidogrel platelet reactivity (PR) and the risk of major adverse cardiovascular events (MACE). However, data are heterogeneous and large intervention trials on PR-tailored treatments have been neutral, possibly owing to the inclusion of patients at low cardiovascular risk. The role and usefulness of PR with regard to levels of cardiovascular risk are unclear and may explain these discrepancies.

What this study adds

- The magnitude of the association between PR and MACE risk is strongly dependant on the level of cardiovascular risk faced by patients suggesting that trials on tailored PR treatment strategies should be primarily stratified on the individual vascular risk and clinical setting.
- This study suggests that medical policies edicted around the concept of personalized medicine should not be restricted to a single biological phenotype or single nucleotide variant but should also emphasize the role of individual clinical risk factors.

Table 1. Main characteristics of published studies

Study	Years of publication	Patients (n)	Age (y)	Male (%)	Diabetics (%)	Smokers (%)	Hyper-tension (%)	Hypercholes-terolemia (%)	ACS at inclusion (%)	PCI (%)	GpIIb/IIIa inhibitor (%)	Follow-up (months)*	ADP (μM)
Campo et al.[27]	2006	70	64±13	69	19	37	63	34	100	100	100	10 (15)	5, 20
Hochholzer et al.[28]	2006	765	66±9	78	24	11	82	92	0	100	0	12 (12)	5, 20
Angiolillo et al.[29]	2007	173	67±9	65	100	13	65	68	0	0	0	24 (36)	20
Cuisset et al.[30]	2007	190	65±12	76	33	48	58	53	87.4	100	14.7	1 (1)	10, 20
Geisler et al.[31]	2008	1,092	67±11	74	33	39	80	59	51.7	100	7.7	1 (1)	20
Gurbel et al.[32]	2008	297	65±12	65	41	55	74	82	0	100	42	24 (24)	5, 20
Cuisset et al.[33]	2009	598	65±12	78	35	39	56	55	100	100	9.9	1 (1)	10
Yong et al.[34]	2009	248	63±12	71	22	27	53	52	100	55	39.7	6 (21)	5, 10, 20
Breet et al.[35]	2010	1,069	64±11	75	81	11	77	80	0	100	7.0	12 (12)	5, 20
Marcucci et al.[36]	2010	1,108	69±10	75	24	23	66	55	100	100	26.0	12 (12)	10
Beigel et al.[37]	2011	174	59±12	83	27	41	51	45	100	100	-	6 (6)	5
Aradi et al.[38]	2012	160	62±9	63	38	36	84	50	0	100	0	12 (12)	5
Reny et al.[8]	2012	534	62±12	82	21	20	56	63	0	0	0	32 (50)	5, 20

Age, mean ± standard deviation; CAD, coronary artery disease; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP, adenosine diphospate concentration used for the evaluation of platelet reactivity
* Median (maximum)

Table 2: Multivariate analysis to assess the associations between the risk factors and the composite outcome of MACE. This analysis was conducted on the patients of the 13 studies of the meta-analysis (n=6,256 after exclusion of missing data). MACE were observed in 412 patients. Hazard ratios (HR) greater than one show an increased risk of MACE in patients having the corresponding risk factor.

Factors collected in studies	Adjusted HR [95% CI]	p	*Level of risk of MACE **	HR [95% CI]	p
Current smoking status	0.92 [0.71;1.18]	0.50	***Low risk (n=579)***	1	
Age (> 75)	1.56 [1.25;1.95]	<0.0001	***Intermediate risk (n=2444)***	1.61 [1.05;2.45]	0.03
Diabetes	1.58 [1.27;1.96]	<0.0001	***High risk (n=3435)***	2.58 [1.69;3.94]	<0.0001
Hypercholesterolemia	0.86 [0.69;1.06]	0.15			
Hypertension	1.23 [0.98;1.54]	0.07			
ACS at inclusion	2.00 [1.27;3.16]	0.003			
Gender (Male)	1.11 [0.89;1.40]	0.35			

*: a surrogate for the level of risk was defined as the number of risk factors (among age, diabetes, hypertension, and ACS at inclusion): low risk for no risk factor, intermediate risk for one risk factor and high risk for two or more risk factors).

Table 3: Associations between the ADP categories and the composite outcome of MACE with adjustment on the factors collected in the studies of the meta-analysis (factors shown in Table 2).

	ADP 20 µM		ADP 10 µM		ADP 5 µM	
	N		N		N	
Studies	9		4		8	
Events	287		124		229	
Patients (after exclusion of missing data)	4,140		2,077		3,160	
	HR [95% CI]	**p**	**HR [95% CI]**	**p**	**HR [95% CI]**	**p**
ADP		0.0003		0.03		0.02
Lower category *	1		1		1	
Intermediate category *	1.85 [1.26;2.73]	0.002	1.31 [0.79;2.17]	0.30	1.79 [1.02;3.14]	0.04
Higher category *	2.91 [1.78;4.74]	<0.0001	2.61 [1.64;4.16]	<0.0001	2.79 [1.50;5.22]	0.001

* Categories for ADP 20 and 10 µM are 0%-40%, 41%-60%, 61%-100%,

and for ADP 5 µM are 0%-30%, 31%-50%, 51%-100%

HR, Hazard Ratio; CI, Confidence Interval

Table 4: Reclassification of the 6-month risk of MACE when the individual risk was predicted from platelet reactivity measured by 20μM ADP in addition to risk factors. The predicted risk was stratified in three levels (low: ≤3%, intermediate: >3% and ≤5%, high: >5%) in agreement with the 6-month risk observed in patients with none, one and two or more risk factors (2.3%, 4.1% and 6.2% respectively). Patients were stratified according to their number of risk factors and to the level of the predicted risk. The numbers of patients and, in brackets, the corresponding observed 6-month risk of MACE in each stratum.

		Risk predicted by the combination of risk factors and platelet reactivity measured by 20μM ADP			
		Low risk (≤3%)	**Intermediate risk (>3% and ≤5%)**	**High risk (>5%)**	Total
Risk predicted by the number of risk factors only	Low risk - no risk factor	**524 * (2.4% **)**	**26 ***	**0 ***	550 * (2.3% **)
	Intermediate risk - one risk factor	**625 * (2.1% **)**	**576 * (3.7% **)**	**622 * (6.3% **)**	1823 * (4.1% **)
	High risk - two or more risk factors	**102 * (0.0% **)**	**462 * (3.0% **)**	**1256 * (7.6% **)**	1820 * (6.2% **)
	Total	**1251 * (2.1% **)**	**1064 * (3.4% **)**	**1878 * (7.1% **)**	4193 * (4.7% **)

*: number of patients

**: observed 6-month risk of MACE

Figure Legends

Figure 1: Flow chart of the meta-analysis

Figure 2: Kaplan-Meier survival curve for the occurrence of MACE

Figure 3: Association between platelet reactivity and the occurrence of MACE according to the level of risk

Low-risk patients have none of the risk factors (among age > 75 years, acute coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have one risk factor and high-risk patients have two or more risk factors. PR was assessed with 20 µM ADP LTA.

Figure 4: 6-month risk of MACE according to platelet reactivity in the different risk groups. The dashed line represents the overall risk, ignoring platelet reactivity and the black line shows the risk according to the platelet reactivity assessed with 20 µM ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).

Figure 1

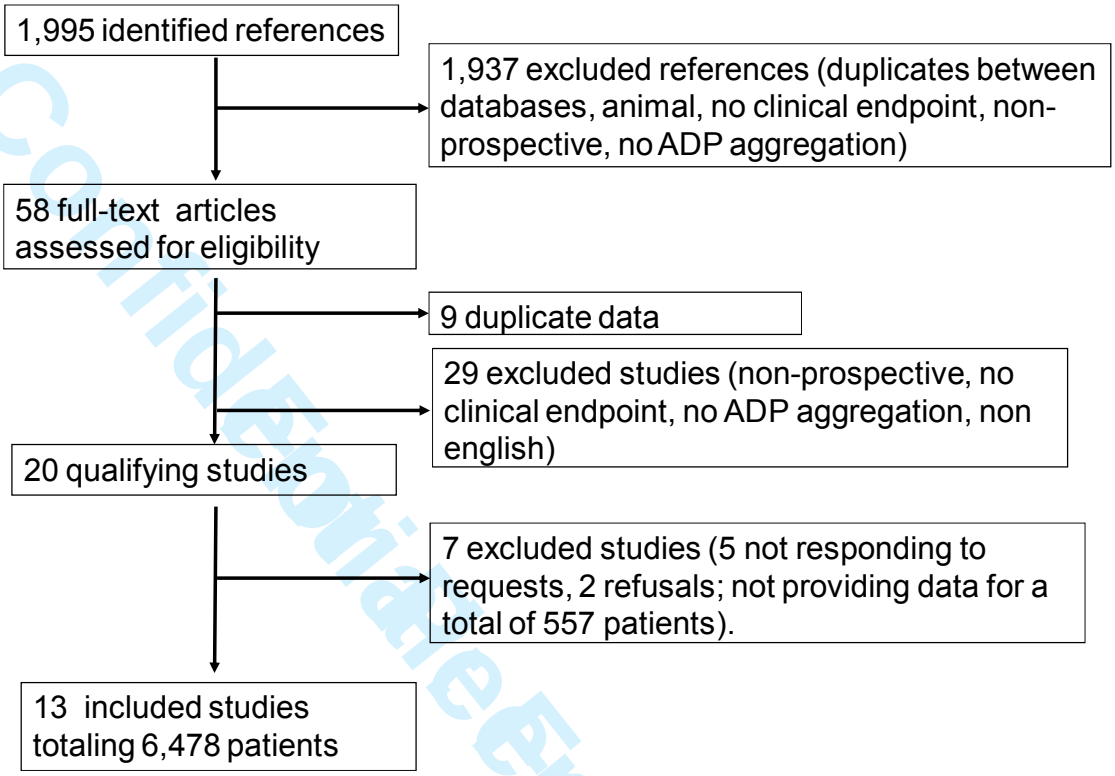


Figure 2

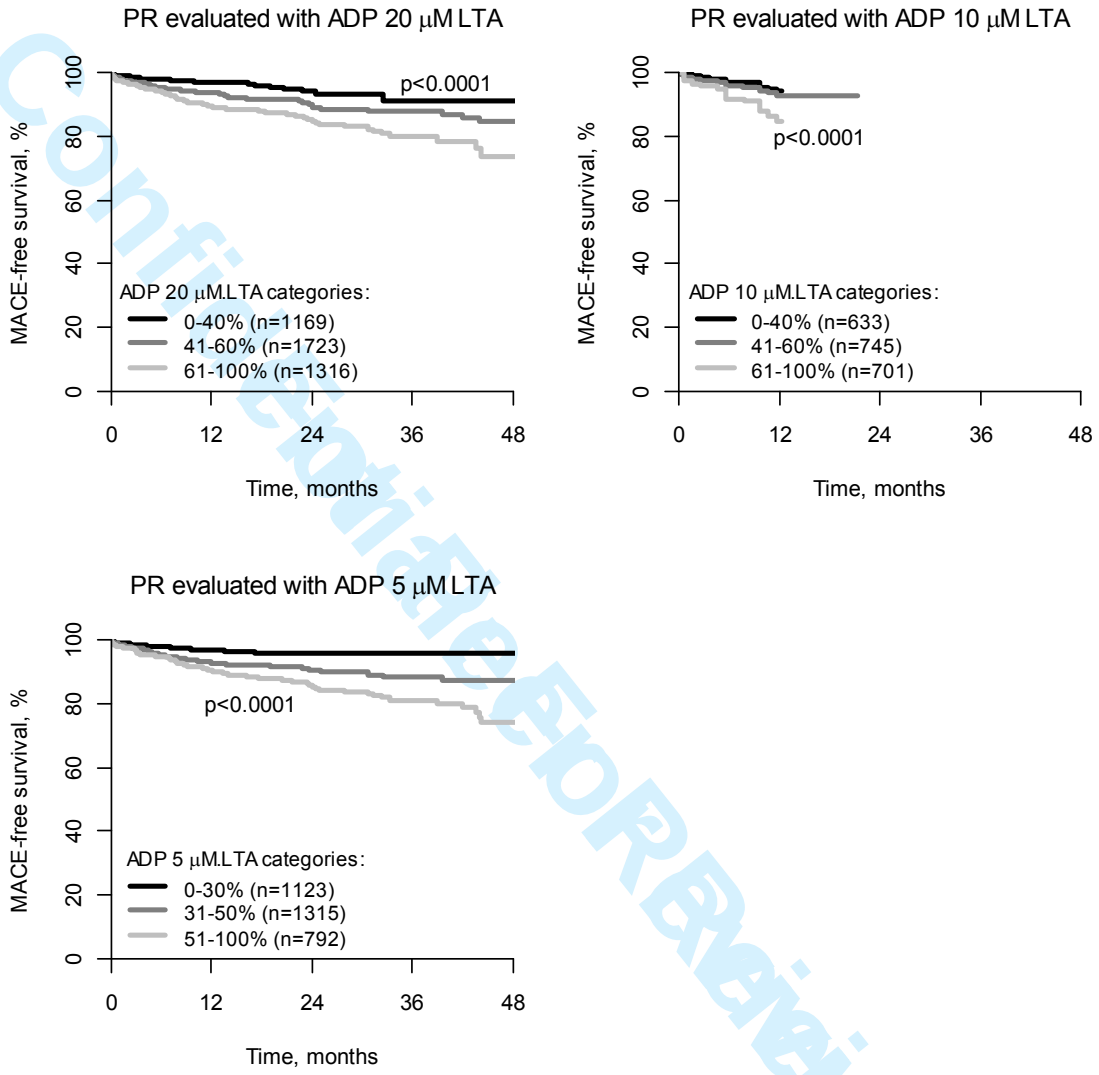


Figure 3

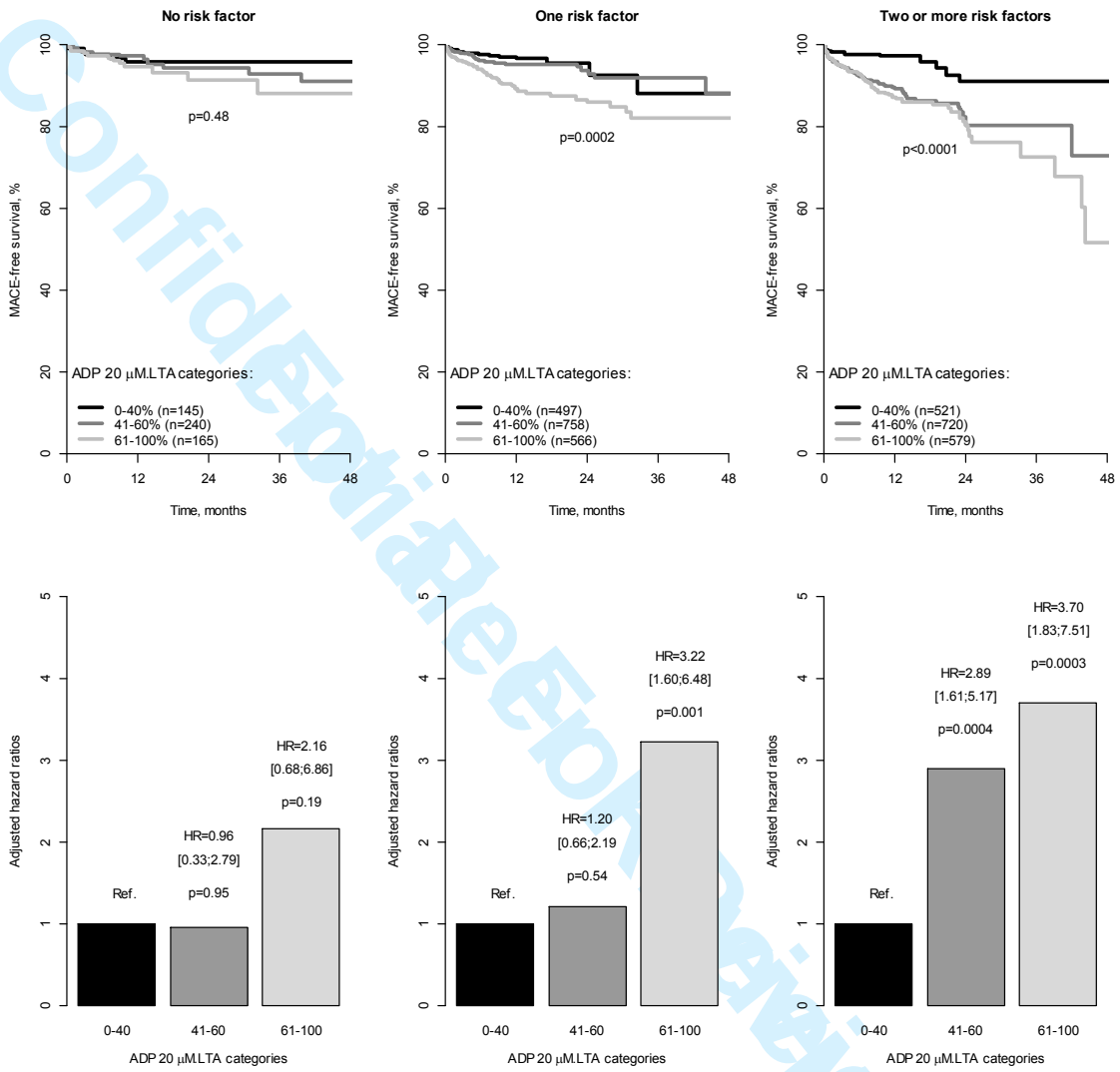
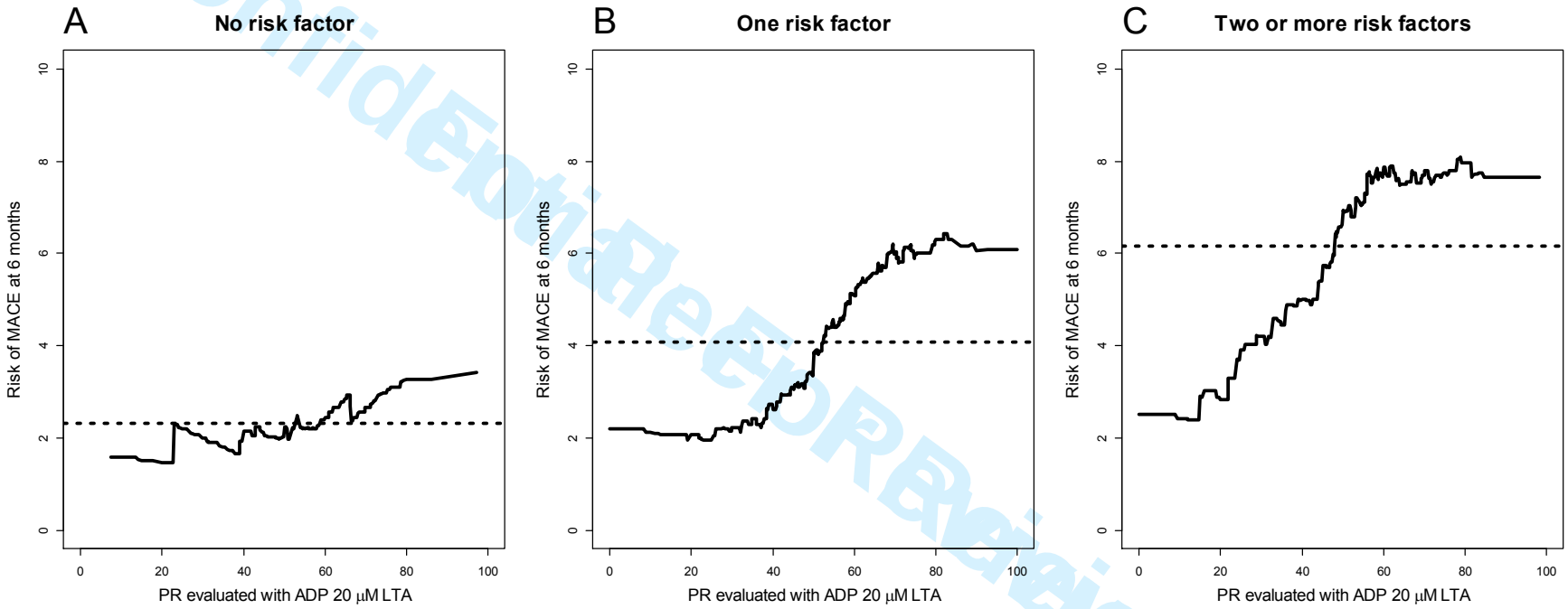


Figure 4



Supplemental material

Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of major adverse cardiovascular events in patients on clopidogrel: Systematic review and collaborative meta-analysis of individual patient data

Content

Detailed statistical analysis

MACE-free survival curves were obtained using the Kaplan-Meier estimator and by pooling data from studies. Comparisons between subgroups of PR with ADP 20, 10, and 5 µM were performed using log-rank tests stratified on the studies. A surrogate of the individual level of risk of MACE was obtained by identifying the factors associated with the MACE outcome in a multivariate mixed-effect Cox model and by counting the number of these factors. The tested factors were the traditional risk factors (age, hypercholesterolemia, diabetes, hypertension, smoking, and acute ischemic event at inclusion). Other risk factors, such as body mass index or family history, were available in only a limited number of studies and were not included as covariates. The between-study variability in the baseline hazard was accounted for by a random coefficient. This analysis was conducted on the whole sample with the R package '*coxme*'.[46 47]

In subsets of studies reporting PR evaluated using 20 µM, 10 µM, and 5 µM ADP, associations between PR, expressed in categories (low, intermediate, high PR) and the risk of MACE, was analysed using mixed-effect Cox models with adjustment for traditional risk factors. The between-study variability was accounted for by a random coefficient for the baseline hazard and for each category of PR. The surrogate for the level of MACE risk (number of risk factors) was explored as a modifier of the association between PR and the risk of MACE: the interaction term was tested in a mixed-effect Cox model. The HRs were reported for intermediate and high PR categories, taking the category low as the reference and according it to the number of risk factors. To better describe the modification of the associations between PR and the risk of MACE, the MACE-free survival rates in patients at low-, intermediate-, and high-risk were assessed according to PR, as continuous variables. This analysis was conducted using the R package *prodlim*, using the symmetrical nearest neighbourhoods method[24]. The assumption of the proportionality of hazards was tested for all models using Cox models,[48] since this procedure was not available for mixed-effect Cox models and by plotting the complementary log-log survival against the logarithm of time. Sensitivity analyses were conducted to check the robustness of the findings with respect to the risks of bias and concerns for applicability of studies, as well as the definition of MACE including target vessel revascularisation and the influence of a given specific study (leave-one-out analysis). A potential publication bias was visually inspected on a funnel plot. The 'trim and fill' method was also applied to detect missing studies (for the funnel plot to be symmetric) and to test the sensitivity of the estimate to these missing studies.[49] The improvement in the assessment of 6-month risk of MACE related to the measure of platelet

reactivity was evaluated by using the net reclassification index for survival data [REF PENCINA].The event and non-event continuous NRIs were reported. When the continuous event NRI is positive, the predicted risk in patients experiencing MACE is more often increased than decreased, following the addition of PR to risk factors than when it involves risk factors only. Similarly, when the continuous non-event NRI, the predicted risk in patients experiencing MACE is more often decreased than increased when the prediction involves PR in addition to risk factors than when it involves risk factors only. To assess the event and non-event NRIs, we used the risk assessed according to PR as a continuous variable (previously described).

All analyses were conducted using R version 3.0.1 (R Development Core Team. *R: A Language and Environment for Statistical Computing*, Vienna, Austria: R Foundation for Statistical Computing; 2010) and Comprehensive Meta-Analysis Version 2 (Biostat, Engelwood, NJ, USA). P-values less than 0.05 were considered significant and all tests were two-sided.

Complementary characteristics of studies, risks of bias, and concerns regarding applicability

In several instances, data on covariates were not available. For example, data on the use of proton pump inhibitors and statins were not available from eight studies. Aspirin was part of the treatment for all patients in ten studies, and was given to 79%, 90%, and 95% of the patients in the three remaining studies. All but one study included coronary artery disease (CAD) patients exclusively; this last study[8] included 85% CAD patients, 10% peripheral arterial disease patients, and 5% ischemic stroke patients. PCI was performed on 87% of patients. The studies differed markedly with respect to the frequency of diabetes (19%–100%), smoking (11%–55%), the use of GpIIb/IIIa inhibitors (0%–100%), the ADP concentration used for aggregation tests (5–20 μM) to assess PR, and the presence of acute ischemia at inclusion (0%–100%). The median follow-up was 12 months, and in all the studies the modalities of the follow-up were identical for clopidogrel responders and non-responders. LTA was most frequently performed using ADP 20 μM.

Overall risks of bias and concerns about applicability of the studies were low (Figures 1 and 2). In one study using LTA with ADP 10 μM, MACE was defined as stent thrombosis during a 30-day follow-up period;[33] however, with no specific information on myocardial infarction or stroke (as was the case in most of the studies), this lead to a potential high risk of outcome bias. In three studies,[27 36 38] the risk of outcome bias was unclear because either they did not include stroke in the composite outcome of MACE or they did not mention whether adjudication of the outcome was performed blinded to PR test results. In another study, using LTA using ADP 20 μM, the risk of bias with respect to flow and timing was unclear as 13% of

patients had been lost to follow-up.[31] The characteristics, PR test results, and treatments of these patients were similar to the rest of the cohort. Regarding their applicability, only two studies caused concern, because of an exclusively diabetic population in one,[29] and because of the absence of upper and lower limits to the platelet counts of the patients included in the second.[38]

Supplemental Figure 1 Criteria to assess risks of bias and concerns about applicability. Derived from the PROBAST tool available at www.systematic-reviews.com/probast

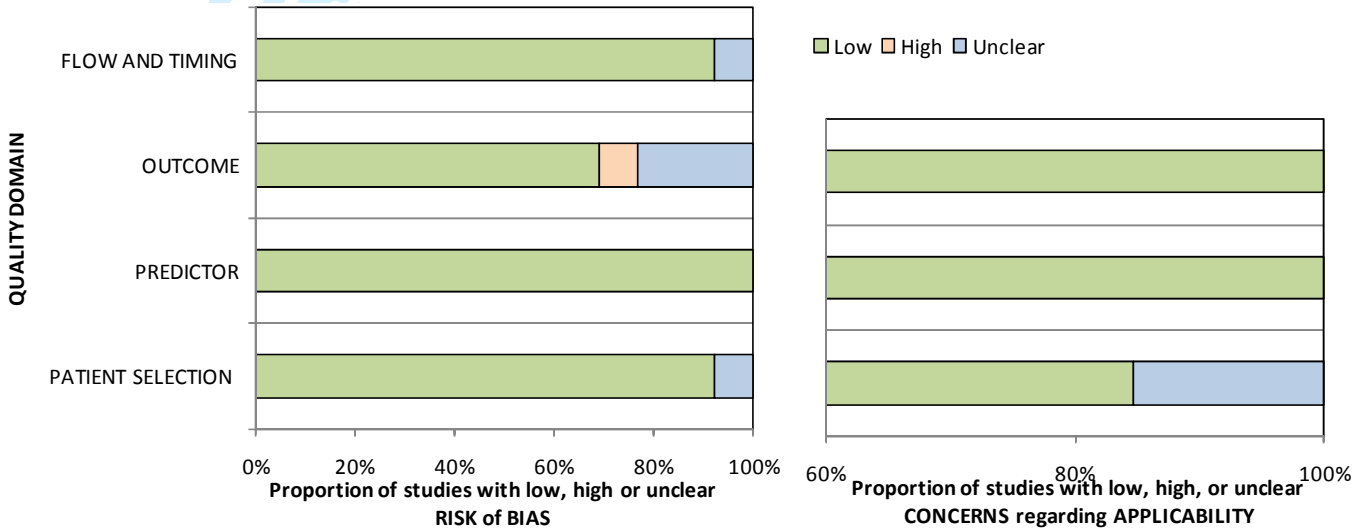
Risk of bias

Domains	Criteria
Patient selection	Consecutive
	Inappropriate inclusion or exclusion criteria
	Disease at similar stage (inclusion)
Predictor ADP LTA and covariates	Pre-specified or standard technique used
	ADP LTA done at the same time for all participants
	Vascular risk factors available
Outcome	All items defining MACE available
	MACE diagnosed blinded to ADP test results
	Adjudicating committee blinded to ADP test results
	Same MACE definition for all participants
	ADP test results did not form part of MACE outcome
Flow and timing	All patients included in the analysis
	Lost to follow up
	Clopidogrel was not stopped during follow-up
	All the patients benefited from the same MACE assessment
	Exact date of MACE known

Concerns about applicability

Domains	Criteria
Patient selection	Sample representative of review's target population
Predictors	Standard definition for covariates
Outcome	Unexpected relative frequency of one or more MACE items
	Differences in the quality of assessment of each MACE item
	The time of assessment of MACE is relevant to the clinical situation

Supplemental Figure 2: Result of the assessment by domains for risks of bias and concerns about applicability



Cox models and assumption of proportionality of hazards

For the multivariate Cox model stratified on studies and conducted on the whole sample to identify risk factors, the hazards were found to be approximately proportional. The p-value for the test on residuals was greater than 0.10 for any factor. A visual inspection of the log minus log survival plots did not reveal any major deviation from the proportionality of hazards. When PR evaluated using ADP 20 μM was added in the model, the p-values were 0.33 for the 41%–60% PR category and 0.28 for the 61%–100% category. When PR evaluated using ADP 10 μM was added in the model, the p-values were 0.89 for the 41%–60% PR category and 0.63 for the 61%–100% category. When PR evaluated using ADP 5 μM was added in the model, the p-values were 0.72 for the 31%–50% PR category and 0.19 for the 51%–100% category. A visual inspection of the log minus log survival plots revealed that when using 10 μM ADP, the survival curves crossed in the first 20 days of follow-up. However, the survival in this period was close to one and the cross was not meaningful. In models testing the interaction between PR (evaluated using 20 μM ADP and 5 μM ADP) and the number of risk factors, p-values for all coefficients were greater than 0.20.

Complementary results on heterogeneity

The amount of heterogeneity is represented by the variance of the random effects[23] corresponding to the between-study variance (Tau^2). Theses variances are reported in the following table for the mixed-effects Cox model when PR is evaluated using ADP 20 μM.

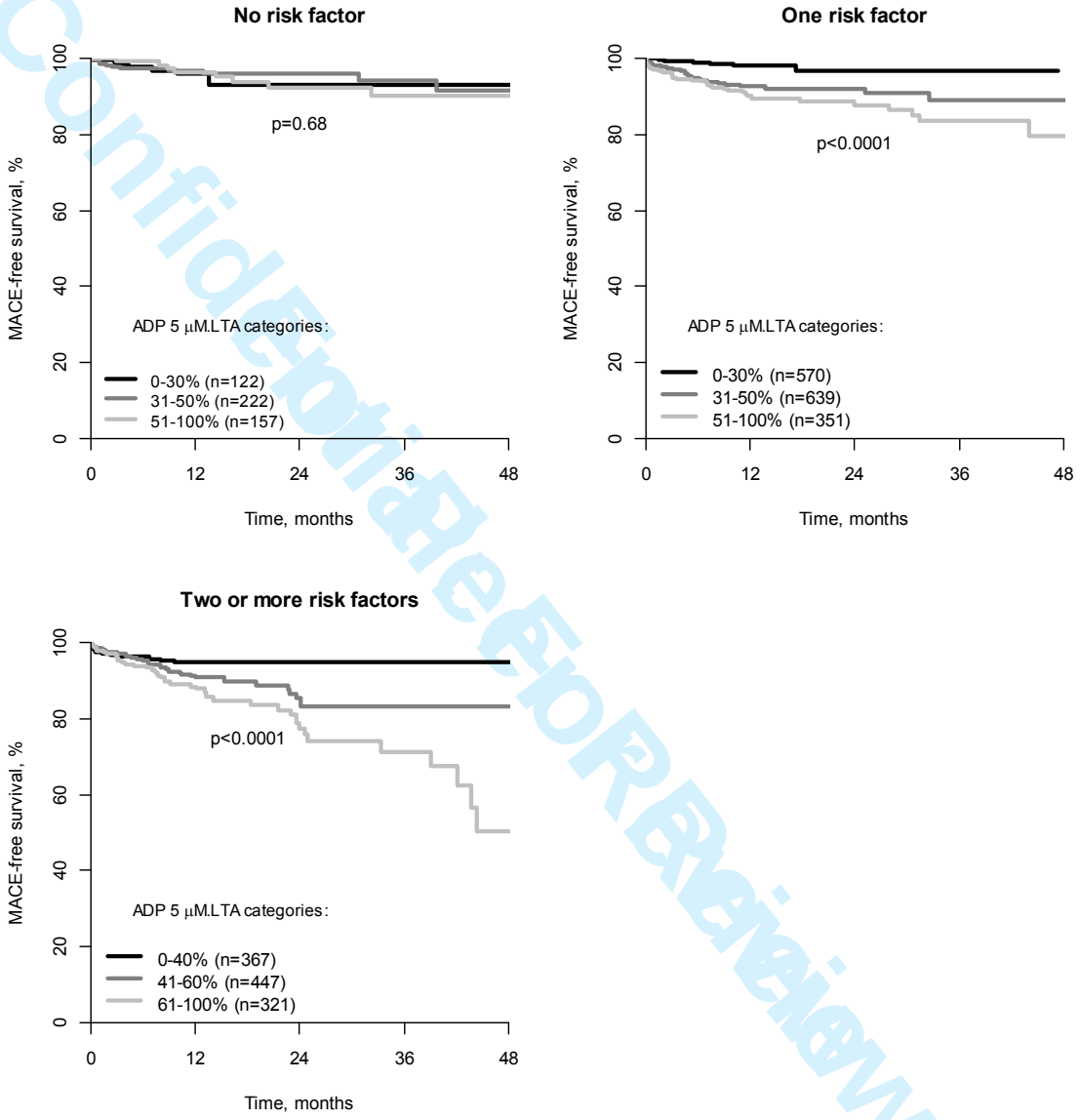
Random effects	Tau^2
Intercept	0.112
PR categories	
41%–60%	0.000
61%–100%	0.324
Interaction terms	
*ADP category 2 * Intermediate risk level*	0.134
*ADP category 3 * Intermediate risk level*	0.086
*ADP category 2 * High risk level*	0.043
*ADP category 3 * High risk level*	0.002

Detailed results on studies using ADP 5 μM to assess platelet reactivity

Similarly to results found using 20 μM ADP, for patients with none of the four risk factors described above, there was no increased risk for any of the PR strata (HR=0.74 [0.27;2.01], p=0.56 for PR=31%–50%; HR=1.20 [0.42;3.47], p=0.73 for PR=51%–100%). In intermediate risk patients (one risk factor), there was an increased risk for both the medium and high strata of PR (HR=2.87 [1.40;5.90] and p=0.004 for ADP 5 μM 31%–50%; HR=4.81

[2.29;10.10] and p<0.0001 for ADP 5 µM 51%–100%), while in high risk patients (two or more risk factors), the direction of the effect was the same but only significant for the high PR category (HR=1.73 [0.91;3.28] and p=0.10 for ADP 5 µM 31%–50%; HR=2.84 [1.49;5.43] and p=0.002 for ADP 5 µM 51%–100%). Figure 4C, in the main text, describes the influence of PR, analysed as a continuous variable, on the two-year risk of MACE for the different patient risk levels. In low-risk patients, the MACE-free survival fluctuates between 90% and 95% with no pattern of decreased survival with higher PR. Intermediate risk patients have a reduced MACE-free survival corresponding to an increased risk of MACE for PR values above 30%, while the risk of MACE in high-risk patients increases earlier (for PR values above 20%).

Supplemental Figure 3. Interaction between 5 µM ADP LTA values and the level of risk
Low-risk patients have none of the risk factors (among age > 75 years, acute coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have one risk factor and high-risk patients have two or more risk factors.



Sensitivity analyses

Sensitivity analyses were performed for PR using ADP 20 µM. A leave-one-out approach was applied to check the robustness of the association between PR using ADP 20 µM and risk of MACE. Whichever study was removed, the association remained significant. The HR for the intermediate category of maximal aggregation using ADP 20 µM (41%–60%) ranged from 1.64 (when Gurbel et al.'s study was removed) to 2.04 (when Angiolillo et al.'s study was removed). The HR for the higher category of maximal aggregation values (61%–100%) ranged from 2.34 (when Gurbel et al.'s study was removed) to 3.29 (when Hochholzer et al.'s study was removed). Thus the association detected in the meta-analyses was not caused by any single study (Table1).

The leave-one-out approach was also applied to evaluate the robustness of the interaction between the number of risk factors and the level of PR in predicting MACE outcomes. The interaction remained at the same magnitude, but depending on which study was removed it was sometimes no longer significant (Table 1). This was most marked when Reny et al.'s study was removed, leading to a p-value of 0.22 for that interaction. In this particular case the magnitude of the non-significant interaction remained the same (Table 2).

In the studies evaluating PR using ADP 20 µM, two studies had an unclear risk of bias: Geisler et al.'s study, in the domain of 'flow and timing' due to patients lost to follow-up;[31] and Campo et al.'s study, because adjudication of the outcome was not stated as blinded to the PR test results, and because stroke and vascular death were not part of the composite MACE outcome.[27] When these two studies were removed, the results on the interaction with risk factors were similar (Table 2). There were unclear concerns about applicability of Angiolillo et al.'s study as it included diabetic patients exclusively.[29] Removing this study did not affect the interaction with risk factors.

For the main analysis, target vessel revascularisation (TVR) was not included in the composite MACE outcome; however, four studies had TVR information available. A re-analysis of data, restricted to these four studies and comprising 1,066 patients, was performed with a definition of MACE including TVR (n=160). The results were similar to those obtained when TVR was not included in the composite MACE outcome: the adjusted HRs were 2.92 [1.55;5.51] (p=0.0009) and 4.98 [1.72;14.43] (p=0.003) for the intermediate and high categories of PR, respectively. When restricted to the population of 3,564 patients treated with PCI and tested using 20 µM ADP, the interaction was of similar magnitude but no longer significant (Table 3). Similarly, the interactions with the number of risk factors

(Table 3) remained of the same magnitude (compared to the main analysis of nine studies on 4,438 patients), but were no longer significant (p=0.25).

The robustness of the findings with regard of the choice of cut-offs for PR evaluated using ADP 20 µM was checked. Categories of PR were determined by the quartile of PR: 0%–38.1%, 38.2%–51.3%, 51.4%–63.0%, 63.1–100%. In low-risk patients (no risk factors), the HRs for categories 38.2%–51.3%, 51.4%–63.0%, and 63.1–100% were respectively 0.45 [0.12;1.68] (p=0.23), 0.88 [0.29;2.71] (p=0.82), and 1.98 [0.61;6.48] (p=0.24). In intermediate-risk patients (one risk factor), the HRs were 1.33 [0.64;2.72] (p=0.44), 1.54 [0.77;3.09] (p=0.22), and 4.73 [2.17;10.31] (p<0.0001). In high-risk patients (two or more risk factors), the HRs were 2.64 [1.35;5.17] (p=0.005), 3.58 [1.90;6.75] (p<0.0001), and 4.21 [1.96;9.05] (p=0.0002). The interaction between the level of cardiovascular risk and PR was statistically significant (p=0.01).

An additional sensitivity analysis was carried out to check the robustness of the main findings with a different categorization of the level of risk. Alternate choices were restricted for different reasons : i) the increase in the risk of MACE is aleardy present in patients with one risk factor compared to those with none (HR=1.61 [1.05;2.45], p=0.03, as shown in table 2 of the manuscript) thus precluding the grouping of patients with 0 or 1 risk factor; ii) patients with 4 risk factors had the highest risk of MACE. However, the size of this sub-group (n=173) was much too small to analyze it as a single category of risk level. We therefore performed a sensitivity analysis with four risk levels : first level = 0 risk factor, second level = 1 risk factor, third level = 2 risk factors, fourth levels = 3 or 4 risk factors. The magnitude of the interaction between risk level and PR level was similar between this categorization with four risk levels and the categorization with three risk levels shown in the manuscript. However the interaction term was not significant with this new categorization (p=0.11 from the mixed effect Cox model). This can logically be explained by the loss of power due to the higher number of parameters involved with the additional risk category. Detailed results are shown in the tables below

Number of patients according to the number of risk factors in studies with PR measured by 20µM ADP, 10µM ADP and 5µM ADP (after exclusion of patients with missing data for PR).

	20µM ADP	10µM ADP	5µM ADP
No RF	554	5	519
1 RF	1874	520	1564
2 RFs	1371	856	934
3 RFs	455	604	189
4 RFs	85	97	13

HRs for MACE accrording to PR within each risk level.

	20μM ADP				
	0 - 40	41 - 60		61 - 100	
Risk level	HR	HR [95%CI]	p value	HR [95%CI]	p value
No RF	1 (ref)	0.96 [0.33;2.79]	0.95	2.15 [0.68;6.82]	0.19
1 RF	1 (ref)	1.20 [0.66;2.18]	0.55	3.19 [1.59;6.41]	0.001
2 RFs	1 (ref)	2.91 [1.42;5.97]	0.004	3.87 [1.70;8.81]	0.001
3, 4 RFs	1 (ref)	2.99 [1.12;7.97]	0.03	3.31 [1.15;9.54]	0.03

Supplemental Table 1. Sensitivity analysis for the association between PR using 20 µM ADP and risk of MACE (leave-one-out procedure)

		20 µM ADP LTA 41%–60%		20 µM ADP LTA 61%–100%		Interaction between PR and risk level
Removed study	N analysed / N events	HR [95% CI]	p	HR [95% CI]	p	p
Hochholzer et al.	3375/267	1.98 [1.29;3.04]	0.002	3.29 [2.00;5.43]	<0.0001	0.08
Reny et al.	3637/235	1.91 [1.25;2.92]	0.003	3.19 [1.86;5.47]	<0.0001	0.22
Angiolillo et al.	3967/251	2.04 [1.35;3.10]	0.0008	2.93 [1.74;4.94]	<0.0001	0.02
Campo et al.	4075/283	1.77 [1.20;2.62]	0.004	2.57 [1.60;4.12]	<0.0001	0.04
Cuisset et al. 2007	3951/279	1.81 [1.23;2.67]	0.003	2.81 [1.67;4.73]	<0.0001	0.08
Geisler et al.	3191/259	1.97 [1.28;3.05]	0.002	3.20 [1.81;5.68]	<0.0001	0.04
Gurbel et al.	3882/252	1.61 [1.07;2.41]	0.02	2.31 [1.46;3.66]	0.0004	0.08
Breet et al.	3089/197	1.75 [1.15;2.67]	0.009	2.89 [1.61;5.18]	0.0004	0.03
Yong et al.	3953/273	1.84 [1.24;2.75]	0.003	3.06 [1.85;5.06]	<0.0001	0.07

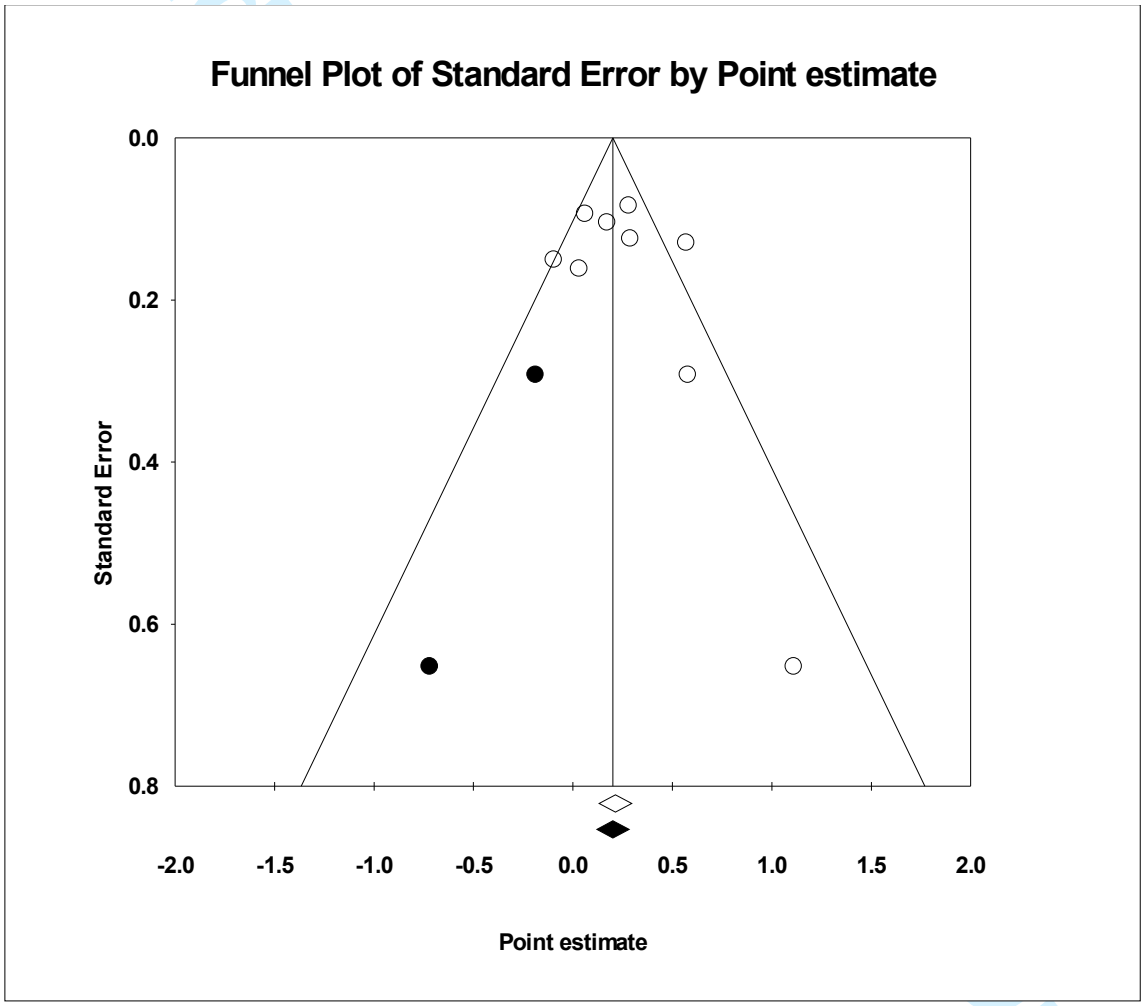
Supplemental Table 2. Results of the sensitivity analysis for the modification of the association between PR, assessed with 20 µM ADP and risk of MACE

	Reny study removed		Geisler study removed		Campo study removed		Geisler & Campo studies removed		Angiolillo study removed	
	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p
Interaction with Nb Risk factors										
No risk factors										
ADP 20 0%-40%	Ref		Ref		Ref		Ref		Ref	
ADP 20 41%-60%	1.26 [0.31;5.03]	0.75	0.94 [0.32;2.71]	0.90	0.96 [0.33;2.78]	0.95	0.93 [0.32;2.69]	0.90	0.96 [0.33;2..78]	0.94
ADP 20 61%-100%	2.23 [0.50;9.72]	0.29	2.05 [0.60;6.93]	0.25	1.88 [0.61;5.77]	0.27	1.68 [0.52;5.36]	0.38	1.98 [0.62;6.35]	0.25
One risk factor										
ADP 20 0%-40%	Ref		Ref		Ref		Ref		Ref	
ADP 20 41%-60%	1.43 [0.74;2.77]	0.28	1.45 [0.75;2.83]	0.27	1.16 [0.64;2.11]	0.62	1.40 [0.72;2.72]	0.33	1.47 [1.01;2.14]	0.54
ADP 20 61%-100%	4.03 [1.89;8.63]	0.0003	4.24 [1.85;9.69]	0.0006	2.77 [1..44;5.31]	0.002	3.43 [1.63;7.24]	0.001	3.02 [1.45;6.26]	0.003
Two or more risk factors										
ADP 20 0%-40%	Ref		Ref		Ref		Ref		Ref	
ADP 20 41%-60%	2.47 [1.36;4.49]	0.003	3.04 [1.54;6.00]	0.001	2.82 [1.55;5.13]	0.0007	2.96 [1.46;6.00]	0.003	3.85 [1.95;7.58]	<0.0001
ADP 20 61%-100%	3.54 [1.71;7.31]	0.0006	4.01 [1.71;9.38]	0.001	3.15 [1.60;6.19]	0.0009	3.20 [1.43;7.14]	0.005	4.47 [1.97;10.16]	0.0003
Interaction (p)		0.22		0.04		0.04		0.04		0.02

Supplemental Table 3. Results of the sensitivity analysis for the modification of the association between PR using 20 µM ADP and risk of MACE in patients with PCI at inclusion and when target vessel revascularisation (TVR) is included in the composite outcome of MACE.

	Patients with PCI		MACE event definition incl. TVR	
N patients	3,406		1,066	
N events	198		160	
	HR [95% CI]	p	HR [95% CI]	p
Interaction with				
Nb Risk factors				
No risk factors				
ADP 20 0%-40%	Ref		Ref	
ADP 20 41%-60%	1.26 [0.31;5.04]	0.75	1.43 [0.69;5.04]	0.58
ADP 20 61%-100%	2.32 [0.51;10.49]	0.27	3.41 [0.67;17.41]	0.14
One risk factor				
ADP 20 0%-40%	Ref		Ref	
ADP 20 41%-60%	1.44 [0.72;2.89]	0.3	2.24 [0.73;6.89]	0.16
ADP 20 61%-100%	4.16 [1.78;9.72]	0.001	7.52 [1.86;30.37]	0.005
Two or more risk factors				
ADP 20 0%-40%	Ref		Ref	
ADP 20 41%-60%	3.36 [1.61;6.8]	0.001	4.07 [1.79;9.28]	0.0008
ADP 20 61%-100%	4.97 [2.03;12.16]	0.0004	6.14 [1.82;20.65]	0.003
Interaction (p)		0.21		0.25

Supplemental Figure 4. Funnel plot for detection of a potential publication bias.
The hazard ratio for the association between PR using 20 µM ADP (per 10%) and the risk of MACE was assessed in each study with adjustment on risk factors. The logarithm of the hazard ratios and their standard errors were represented in the funnel plot (white circles). The white diamond shows the pooled hazard ratio (1.25, 95% CI 1.08 to 1.44). Two studies were detected as missing using the 'trim and fill' method for the funnel plot to be symmetrical (black circles). However, when these missing studies were added, the pooled hazard ratio was not significantly modified (1.21, 95% CI 1.05 to 1.40).



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