



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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Keywords:	Antiplatelet agents, Metaanalysis, Ischaemic heart disease

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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

Running head: Vascular risk, platelet reactivity, and prognosis

Authors:

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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 20 μ M 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.

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73 **Introduction**

74 Atherosclerotic diseases account for more than 40% of deaths in Western countries, and
75 antiplatelet therapy is a major preventive strategy in this setting(1). Clopidogrel, a P2Y₁₂
76 receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is
77 widely prescribed for secondary prevention in patients with atherosclerotic diseases. When
78 combined with aspirin, clopidogrel is particularly effective in patients with acute coronary
79 syndromes (ACS)(2), and has proved superior to aspirin alone in several other large
80 randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide
81 inter-individual variability(3, 4). Numerous cohort studies, often performed on patients with
82 acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions
83 (PCI), have shown an association between high on-treatment platelet reactivity (PR) and the
84 risk of recurrent major adverse cardiovascular events (MACE)(5-7). However, recent studies
85 in cohorts of stable cardiovascular outpatients(8, 9) or in medically managed ACS
86 patients(10) failed to confirm these results. Several randomized trials aimed at reducing the
87 recurrence of ischemic events have compared standard clopidogrel treatment to a P2Y₁₂-
88 inhibitor strategy tailored according to the presence of high PR. Although initial small trials
89 were promising(11, 12) more recent larger trials showed no benefit from adjusting clopidogrel
90 doses or switching to prasugrel based on PR testing in low-risk coronary patients undergoing
91 PCI(13, 14). These contrasting results, both from observational studies and randomized
92 intervention trials, may be explained by different patient characteristics including the level of
93 risk, but to date few data substantiate these hypotheses. We previously showed, in a study-
94 level meta-analysis, that the risk of recurrent MACE associated with high PR was greater in
95 studies using GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did
96 not(7). Another meta-regression from a study-level meta-analysis of randomized trials
97 suggested that the higher the incidence of coronary stent thrombosis in a given study, the
98 larger the net clinical benefit from a PR-tailored strategy(15). Finally, the ADAPT-DES
99 registry of patients undergoing PCI showed that high PR was predictive of stent thrombosis
100 mostly in ACS patients, but there was no interaction reported between PR and the presence

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2 101 of an ACS at inclusion(16). This information suggests the hypothesis that high PR might be
3
4 102 more relevant in high-risk populations, but convincing data at the individual level are lacking.
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6 103 To date, the only meta-analysis on individual patient data performed on 6 studies totaling
7
8 104 3,059 patients assessed with the VerifyNow P2Y₁₂ assay did not explore this hypothesis(17).
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10 105 Similarly, one of the largest and more recent meta-analysis on 8 studies and 4817 patients
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12 106 did not explore this interaction due to the lack of individual data(18). To further investigate
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14 107 this interaction on a larger population we performed a collaborative meta-analysis of
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16 108 individual patient data and focused on the interaction between relevant vascular risk factors
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18 109 and PR, assessed with ADP induced light transmission aggregometry (LTA), in order to
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20 110 better define the risk of MACE. ADP-induced LTA is the assay upon which all P2Y₁₂ receptor
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22 111 inhibitors have been developed, thus supporting its use in the present meta-analysis. In
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24 112 addition, among several available assays to evaluate PR, LTA is the historical gold standard
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26 113 with which most platelet function assays were compared.
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115 **Methods**

116 *Data sources*

117 Literature review, confined to articles in English(19), was based on electronic databases
118 (Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and
119 abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC).
120 A free-text search was conducted using an ‘ADP’ and ‘aggregation’ and ‘clopidogrel’ key-
121 word combination. Articles were selected on the basis of abstracts, before examination of the
122 full text. Reference lists of selected articles were also hand-searched to identify additional
123 relevant reports. Reviewers (JLR and PF) were not blinded to the journal, authors or
124 institutions in the publications as this has been shown to be unnecessary(20). The electronic
125 database search was last updated on 31 July, 2013. The objective of this individual patients’
126 data meta-analysis was described in a project that was part of French ministry of health’s
127 initiative to encourage meta-analyses (PHRC 15-07 to JL Reny “*Etudes prospectives sur la*
128 *réponse biologique au clopidogrel et événements ischémiques chez les patients*
129 *athérombotiques : Métaanalyse sur données individuelles et résumées*” [http://www.plan-](http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf)
130 [alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus - 2 mai 2008.pdf](http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf)). Protocol in
131 French available upon request.

132
133 *Study selection*

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

1
2 143 randomised therapeutic trial, but one in which treatment was allocated independently of the
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4 144 response to clopidogrel. When studies were suspected of including the same patients, the
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6 145 authors were asked to provide data from the largest possible number of independent patients.
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10 147 *Data extraction*

11 148 The corresponding authors or principal investigators of eligible studies were contacted and
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13 149 asked to participate in the CLOpidogrel and Vascular ISchemic events – Individual Patient
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15 150 Data (CLOVIS-IPD) meta-analysis group. Investigators provided individual data on: the
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17 151 qualifying cardiovascular condition and clinical setting at inclusion (ACS or stable disease);
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19 152 MACE and date of occurrence during follow-up; platelet reactivity (PR) with ADP 20, 10,
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21 153 and/or 5 µM and its timing relative to loading dose of clopidogrel; age, gender, height, and
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23 154 weight; current smoking status, diabetes, hypercholesterolemia, and hypertension; left
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25 155 ventricular ejection fraction; platelet count; PCI; use of GpIIb/IIIa inhibitors and timing;
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27 156 concomitant medications; and bleeding events and timing during follow-up. Data were
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29 157 checked for completeness and consistency with published reports. Any discrepancies were
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31 158 resolved with the corresponding authors. After format harmonization, data were compiled for
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33 159 statistical analysis. All studies were approved by their respective institutional review boards.
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38 161 *Quality assessment of studies*

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40 162 A new quality assessment tool for prognostic studies called PROBAST (see
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42 163 Acknowledgements) was used to estimate risks of bias and concerns about applicability. As
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44 164 PROBAST is not customized for meta-analyses of individual patient data, items were
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46 165 adapted accordingly. Based on the present study's list of relevant criteria, risks of bias, and
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48 166 concerns about applicability are rated as low, unclear, or high. Supplemental Figure 1 shows
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50 167 the list of criteria.
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55 169 *Primary outcomes and measures*
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2 170 The primary clinical outcome was the occurrence of MACE, as defined above (see Study
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4 171 selection (d)). The primary biological outcome was maximal aggregation with 20 μ M ADP, as
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6 172 it is a better concentration for analyzing the effects of clopidogrel than lower ones. PR was
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8 173 categorized in three strata. The higher cut-offs were selected on the basis of previously
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10 174 published cut-offs (59% to 64% for 20 μ M ADP, and 43% to 46% for 5 μ M ADP)(21), and to
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12 175 keep relatively balanced numbers of patients in each PR categories. Three pre-specified
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14 176 categories allowed a better description of the dose-dependent effects of PR on the risk of
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16 177 MACE compared to the usual dichotomous high and low PR categorization. Three categories
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18 178 were also chosen to better parallel the analysis with a therapeutic PR window that has been
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20 179 associated with optimal net clinical benefit(22). A surrogate for the level of cardiovascular
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22 180 risk was defined as the number of factors with homogeneous definitions across studies, and
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24 181 these were markers of MACE in the meta-analysis. The factors were selected from among
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26 182 age, diabetes, hypertension, smoking, hypercholesterolemia, and the presence of an ACS at
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28 183 inclusion (as defined in study selection (d)), and were all provided at the time of inclusion and
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30 184 PR testing.

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33 34 186 *Statistical analysis*

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36 187 MACE-free survival curves were derived from individual patient data using the Kaplan-Meier
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38 188 estimator; curves were compared using log-rank tests stratified by study. Associations
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40 189 between conventional risk factors, PR strata, and risk of MACE were analyzed using
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42 190 multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the
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44 191 size of the random effects (Tau^2) which is an estimate of the between study variability(23).
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46 192 The presence of heterogeneity was tested by comparing models with and without random
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48 193 effects (likelihood ratio test). The interactions between the level of risk and PR strata were
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50 194 tested. MACE-free survival according to PR, as a continuous variable, was assessed using
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52 195 the R package prodlm using the symmetrical nearest neighborhoods method.(24) Sensitivity
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54 196 analyses were conducted to check the robustness of the findings with respect to: the risks of
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56 197 bias and concerns about the applicability of studies; the definition of MACE, including target

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2 198 vessel revascularization or PCI at inclusion, and; the influence of a given specific study. The
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4 199 net reclassification index (NRI) for survival data(25) was computed to quantify the
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6 200 contribution of PR testing for the prediction of the 6-month risk of MACE in patients with
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8 201 increasing numbers of traditional risk factors. The event and non-event continuous NRIs
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10 202 were reported. Potential publication bias was checked for. P-values below 0.05 were
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12 203 considered significant and all tests were two-sided. Published guidelines for meta-analysis of
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14 204 observational studies in epidemiology (MOOSE) and their reporting(26) were followed.
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16 205 Details on statistical methods are given in the online data supplement.
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207 **Results**

208 *Characteristics of included studies*

209 The Figure 1 flow-chart details how 13 of 20 qualifying studies were included, totaling 6,478
210 patients(8, 27-38). Table 1 shows their characteristics. Data on body mass index,
211 concomitant medications, left ventricular ejection fraction, or the occurrence of target and
212 non-target vessel revascularization during follow-up were only available in some studies. All
213 studies provided individual data allowing a homogeneous definition of MACE, current
214 smoking status, ACS, diabetes (fasting plasma glucose ≥ 7.0 mmol/l, 2-h plasma glucose \geq
215 11.1 mmol/l after 75g oral glucose load or background therapy for diabetes), and
216 hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or
217 a documented history of hypertension). Hypercholesterolemia was not defined in a
218 homogeneous fashion across studies and plasma LDL-cholesterol levels were not available
219 for more than 2,000 patients. Overall, risks of bias and concerns about applicability were low
220 (online data supplement further details study characteristics, bias, and applicability).
221 Information on bleeding was limited to five studies, with only 67 major and 20
222 moderate/minor bleedings.

224 *MACE and level of risk*

1
2 225 Overall, 421 MACE occurred in 6,478 patients (6.5%), the majority being ACS (n = 383).
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4 226 There were 83 stent thromboses, including 79 definite or probable and four possible ones, all
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6 227 included in the composite outcome of MACE. The MACE-free survival rate across the
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8 228 different studies at the end of follow-up ranged from 77.4% to 97.3%. In a multivariate
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10 229 analysis, four factors were found relevant to determining patients' levels of risk: age greater
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12 230 than 75 years, diabetes, ACS at inclusion, and hypertension (Table 2). The number of these
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14 231 factors was used as a surrogate for the individual risk of MACE. Patients with none of these
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16 232 factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with
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18 233 two or more factors 'high-risk' (global p-value <0.0001 for the trend).
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21 235 *MACE and PR*

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24 236 Nine studies (n = 4,438 patients) performed LTA using 20 μ M ADP, four studies (n = 2,144
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26 237 patients) used 10 μ M ADP, and eight studies (n = 3,317 patients) used 5 μ M ADP. Figure 2
27
28 238 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE
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30 239 increased significantly with PR with 20 μ M ADP, 10 μ M ADP, and 5 μ M ADP.

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32 240 With adjustment, high PR was still significantly associated with an increased risk of MACE
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34 241 (Table 3). However, for PR evaluated using 10 μ M ADP, risk only increased for the highest
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36 242 PR category, corresponding to LTA values greater than 60%.
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39 244 *Interaction between risk level and PR for the outcome of MACE*

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44 246 Platelet reactivity assessed with 20 μ M ADP. Patients with none of the four risk factors
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46 247 showed no significantly increased risk associated with PR, while for patients with one risk
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48 248 factor only, the higher strata of PR was associated with an increased risk of MACE. Patients
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50 249 with two or more risk factors showed an increased risk of MACE for both the medium and
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52 250 higher strata of PR. (Figure 3). In a Cox model, the interaction between PR strata and the
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54 251 risk level was statistically significant (p=0.04). The corresponding hazard ratios (HRs) are
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56 252 shown in Figure 3. Heterogeneity was not detected for the overall interaction (p=0.81), as
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2 253 well as when it was restricted to each risk level category (intermediate versus low risk level,
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4 254 $p=0.45$, and high versus low risk level, $p=0.90$). Additional results on heterogeneity are
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6 255 provided in the supplemental material. Figure 4A shows that PR, when analyzed in a
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8 256 continuous fashion, barely affects the risk of MACE at 6 months in patients with no risk
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10 257 factors: the risk is close to 2% at six months, irrespective of the level of platelet reactivity.
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12 258 Conversely, patients with one risk factor and an overall 4.1% risk of MACE at six months
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14 259 have in fact a 2% risk of MACE when they have a low PR, or a 6% risk of MACE when they
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16 260 have a high PR (Figure 4B). Similarly, patients with two or more risk factors and an overall
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18 261 6% risk of MACE at six months can indeed have a 2% risk of MACE when they have a low
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20 262 PR (Figure 4C). The reclassification of the 6-month risk of MACE, according to the three
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22 263 categories of platelet reactivity, in patients with no, one and two or more risk factors, is
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24 264 shown in Table 4. Overall, PR allowed the reclassification of 44% of the total population
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26 265 (1837/4193 patients) included in a 6-month follow-up to a different level, mostly in patients
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28 266 originally identified as intermediate or high risk on the basis of the number of risk factors only.
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30 267 In patients experiencing MACE in the first 6 months of follow-up, the risk predicted by the
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32 268 combination of PR and risk factors was on average increased compared with the risk
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34 269 predicted from risk factors only: the continuous event net reclassification index (NRI) was
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36 270 0.39 (95%CI 0.23 to 0.62). Conversely, in patients free of MACE at 6 months, the measure of
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38 271 PR did not modify the predicted risk: the continuous non-event NRI was 0.01 (95%CI -0.16 to
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40 272 0.09). The overall NRI was 0.39 (95%CI 0.22 to 0.57).

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44 274 Platelet reactivity assessed with 10 μ M ADP. A total of only five low-risk patients in four
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46 275 studies performing 10 μ M ADP LTA to assess PR precluded an analysis of this low-risk
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48 276 group. Furthermore, the surrogate for risk level failed to demonstrate an association with the
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50 277 observed risk of MACE in these studies. Figure 4B shows that the risk of MACE increased in
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52 278 both intermediate- and high-risk patients for PR values above 40%, without any obvious
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54 279 relation with the level of risk.

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2 281 Platelet reactivity assessed with 5 μ M ADP. The direction of interaction between PR using 5
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4 282 μ M ADP and the risk level was similar to that observed for PR using 20 μ M ADP, even
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6 283 though overall interaction did not reach the significance level ($p=0.17$). Of note there were
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8 284 980 fewer patients in the studies performing 5 μ M ADP than in those using 20 μ M ADP. The
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10 285 increased risk of MACE as PR increases is indeed similar for intermediate- and high-risk
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12 286 patients; for low-risk patients PR is not associated with a MACE outcome (online data
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14 287 supplement). Heterogeneity was not detected for the overall interaction ($p=0.19$). Figure 4C
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16 288 shows that the risk of MACE was unaffected by PR in low-risk patients while it increased for
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18 289 PR values above 30% in intermediate-risk patients and for PR values above 10%–20% in
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20 290 high-risk patients.

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24 292 *Sensitivity analyses*

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26 293 Sensitivity analyses were performed for PR using 20 μ M ADP to assess: the robustness of
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28 294 the association between PR and risk of MACE and its interaction with the level of
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30 295 cardiovascular risk; the robustness of the results in the population of PCI patients and when
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32 296 target vessel revascularization is added to the composite outcome. All analyses showed that
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34 297 the sizes of the effects remained similar, and whilst in some instances the statistical
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36 298 significance of the interactions could be lost, there was no impact on their magnitudes
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38 299 (supplemental Tables 1 and 2). Notably, when PR was categorized in quartiles (20 μ M ADP
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40 300 maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%, 51.4%–63.0%, 63.1%–100%)
41
42 301 the interaction between PR and the number of risk factors remained significant ($p=0.01$).

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44 302 When restricted to the population of 3,564 patients treated with PCI and tested using 20 μ M
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46 303 ADP the interaction was of similar magnitude but no longer significant (supplemental Table
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48 304 3).

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52 306 *Publication and availability biases*

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54 307 A check for potential publication bias was made for PR using 20 μ M ADP, on which the main
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56 308 analyses were performed. A funnel plot was obtained by representing the HR of PR using 20
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2 309 μM ADP and the standard error, assessed in each separate study (supplemental Figure 4).
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4 310 Two studies with a negative association between PR using 20 μM ADP and the risk of MACE
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6 311 (with small sample sizes) were detected as missing using the 'trim and fill' method for making
7
8 312 the funnel plot symmetrical. When these missing studies were added, the pooled HR was not
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10 313 significantly modified. These findings suggested that the publication bias in our meta-analysis
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12 314 was minor.
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14 315 Seven qualifying studies could not provide individual patient data. It is of note that in five of
15
16 316 these, the relation between clopidogrel non-response and ischemic events was not a study
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18 317 objective (pharmacokinetic-pharmacodynamic studies or randomized trials of different
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20 318 clopidogrel loading doses). The two remaining studies ($n = 101$ and 111 patients) were
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22 319 specifically interested in the prognostic value of PR for MACE.
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321 **Discussion**

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

332 Interestingly, smoking and hypercholesterolemia were not associated with the outcome of
333 MACE and were not included in the analysis of the interaction between PR and risk factors.

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

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

1
2 349 corresponding loss of power. Moreover, it was previously shown that ADP-induced platelet
3
4 350 aggregation in citrated plasma was dependent on the artifactual generation of TxA₂ that was
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6 351 modulated by aspirin, at least at lower ADP concentrations(41). This may be associated with
7
8 352 an additional background noise in which the interaction between the identified risk factors
9
10 353 and PR to predict MACE is blurred, as seen with the lowest 5 µM ADP concentrations and
11
12 354 partially also with the intermediate 10 µM ADP concentrations. Only four of the studies
13
14 355 analysed used 10 µM ADP, and two of these had a follow-up limited to 30 days; with only
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16 356 124 MACEs during follow-up, this accounts for a limitation in power to reliably study
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18 357 interactions. Overall, the concentration of ADP used is of limited significance since the
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20 358 influence of risk factors appears in all three ADP concentration groups (table 3 and figure 2).
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22 359 Which laboratory assay and which platelet agonist concentration are best suited for the
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24 360 clinical evaluation of platelet function is the matter of some debate. ADP-induced LTA is
25
26 361 highly reproducible within a given laboratory, but its lack of standardization across studies
27
28 362 may have slightly weakened the positive findings or lower the level of significance for the
29
30 363 interactions found in the present meta-analysis. Of note, the present meta-analysis does not
31
32 364 aim to promote the use of LTA to tailor antiplatelet therapy but it rather relied on a historical
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34 365 gold standard in platelet function testing to evidence an interaction with patients'
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36 366 characteristics that should be considered for a tailored approach. The point-of care
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38 367 VerifyNow P2Y₁₂ assay, used in several intervention trials, correlates well with ADP-induced
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40 368 LTA(42, 43) and we speculate that the main findings of the present meta-analysis would
41
42 369 have been similar, had PR been evaluated using the VerifyNow P2Y₁₂ assay.
43
44 370 Several intervention trials have compared conventional clopidogrel treatment to an
45
46 371 antiplatelet strategy tailored according to PR. Early, small randomized trials(11, 12) that
47
48 372 utilized vasodilator-stimulated phosphoprotein phosphorylation level measurement to indicate
49
50 373 P2Y₁₂ receptor reactivity, showed a protective effect for repeat 600 mg clopidogrel loading
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52 374 doses in ACS patients prior to PCI. However, recent larger trials utilizing the VerifyNow P2Y₁₂
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54 375 assay were negative. Indeed, the GRAVITAS(13) and ARCTIC(14) studies failed to show the
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56 376 benefit of a PR-tailored antiplatelet strategy after PCI. Various limitations of these trials were
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2 377 addressed in a recent consensus publication(22). The event rate of the GRAVITAS study
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4 378 was low compared to the one used for power calculation, and the antiplatelet effect of the
5
6 379 high-dose regimen may have been suboptimal as it reduced the prevalence of high PR by
7
8 380 only 22%. Similarly, the ARTIC study population was also at a low absolute risk of
9
10 381 subsequent cardiovascular events because the prevalence of ACS patients was low, and the
11
12 382 composite endpoint also included other events that may not be related to platelet function.
13
14 383 The interaction of PR and the number of risk factors, as identified in the present meta-
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16 384 analysis, substantiates the hypothesis that the risk associated with high PR was not clinically
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18 385 relevant in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a
19
20 386 beneficial reduction of MACE for these low-risk patients. Based on these observations we
21
22 387 speculate that higher risk patients are more likely to benefit from a therapy tailored to their
23
24 388 initial PR. This may explain why early interventions designed to efficiently blunt high PR in
25
26 389 ACS patients with multiple conventional risk factors translated into a reduction of MACE(11,
27
28 390 12, 22).
29
30 391 In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the
31
32 392 management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk.
33
34 393 However, a recent cost-effectiveness analysis for six European perspectives showed that the
35
36 394 universal use of newer P2Y₁₂ inhibitors for ACS patients is probably not as cost-effective as
37
38 395 strategies based on PR(44). It should also be kept in mind that ticagrelor and prasugrel
39
40 396 increase the risk of bleeding and that a therapeutic medium-PR window is associated with
41
42 397 optimal net clinical benefit(22). The net benefits of newer P2Y₁₂ inhibitors could also probably
43
44 398 be improved not only by testing for PR, but also by incorporating patient risk levels in the
45
46 399 decision-making process. Although ongoing trials on tailored P2Y₁₂ strategies, including
47
48 400 TROPICAL-ACS ([ClinicalTrials.gov identifier: NCT01959451](https://clinicaltrials.gov/ct2/show/study/NCT01959451)) and ANTARCTIC(45) partly
49
50 401 include this concept of risk levels, further efforts in this direction are needed.
51
52 402 This meta-analysis has several strengths, such as the good overall quality of the studies
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54 403 included, as assessed using a quality tool specifically adapted to prognostic studies. The
55
56 404 availability of individual patient data allowed a reliable evaluation of the risk associated with
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1
2 405 PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a
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4 406 secondary prevention population were thus identified. The consistency of results across the
5
6 407 different ADP concentrations used in the different studies to assess PR, as well as the
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8 408 sensitivity analyses, indicated that the results were robust.

9
10 409 Despite the advantages related to the availability of individual patient data, this meta-analysis
11
12 410 also had some limitations, including a low proportion of women (25%). This did not allow a
13
14 411 stratification of the analyses by gender, as is usually the case in risk assessment tools such
15
16 412 the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is
17
18 413 not considered as one of traditional risk factors, but is rather presented in separate charts for
19
20 414 women and men. There were incomplete data on concomitant medications or other relevant
21
22 415 risk factors such as the left ventricular ejection fraction, cholesterol levels or renal
23
24 416 insufficiency. Finally, information on bleeding was limited to five studies and a low number of
25
26 417 events, thus precluding a reliable analysis of bleeding events and their relation to PR.

27
28 418 In conclusion, high PR in patients on clopidogrel is associated with an increased risk of
29
30 419 MACE in patients with vascular risk factors, but not in low-risk patients. These findings
31
32 420 suggest that trials on tailored PR treatment strategies should be primarily stratified on the
33
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43
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13
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35
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For Peer Review

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For Peer Review

631 **Table 1.** Main characteristics of published studies

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Study	Years of publication	Patients (n)	Age (y)	Male (%)	Diabetics (%)	Smokers (%)	Hypertension (%)	Hypercholesterolemia (%)	ACS at inclusion (%)	PCI (%)	GpIIb/IIIa inhibitor (%)	Follow-up (months)*	ADP (μ M)
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

633 Age, mean \pm standard deviation; CAD, coronary artery disease; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP,

634 adenosine diphosphate concentration used for the evaluation of platelet reactivity

635 * Median (maximum)

636

637 **Table 2:** Multivariate analysis to assess the associations between the risk factors and the composite outcome of MACE. This analysis was
 638 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.
 639 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
<i>Current smoking status</i>	0.92 [0.71;1.18]	0.50	<i>Low risk (n=579)</i>	1	
<i>Age (> 75)</i>	1.56 [1.25;1.95]	<0.0001	<i>Intermediate risk (n=2444)</i>	1.61 [1.05;2.45]	0.03
<i>Diabetes</i>	1.58 [1.27;1.96]	<0.0001	<i>High risk (n=3435)</i>	2.58 [1.69;3.94]	<0.0001
<i>Hypercholesterolemia</i>	0.86 [0.69;1.06]	0.15			
<i>Hypertension</i>	1.23 [0.98;1.54]	0.07			
<i>ACS at inclusion</i>	2.00 [1.27;3.16]	0.003			
<i>Gender (Male)</i>	1.11 [0.89;1.40]	0.35			

640 *: 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
 641 no risk factor, intermediate risk for one risk factor and high risk for two or more risk factors).

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

	ADP 20 μ M		ADP 10 μ M		ADP 5 μ M	
	N		N		N	
<i>Studies</i>	9		4		8	
<i>Events</i>	287		124		229	
<i>Patients (after exclusion of missing data)</i>	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**
<i>Lower category *</i>	1		1		1	
<i>Intermediate category *</i>	1.85 [1.26;2.73]	0.002	1.31 [0.79;2.17]	0.30	1.79 [1.02;3.14]	0.04
<i>Higher category *</i>	2.91 [1.78;4.74]	<0.0001	2.61 [1.64;4.16]	<0.0001	2.79 [1.50;5.22]	0.001

646 HR, Hazard Ratio; CI, Confidence Interval

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

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

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5 650 **Table 4:** Reclassification of the 6-month risk of MACE when the individual risk was predicted from platelet reactivity measured by 20 μ M ADP in
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7 651 addition to risk factors. The predicted risk was stratified in three levels (low: $\leq 3\%$, intermediate: $>3\%$ and $\leq 5\%$, high: $>5\%$) in agreement with the 6-
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9 652 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
10
11 653 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
12
13 654 risk of MACE in each stratum.

	Risk predicted by the combination of risk factors and platelet reactivity measured by 20 μ M ADP			
Risk predicted by the number of risk factors only	Low risk ($\leq 3\%$)	Intermediate risk ($>3\%$ and $\leq 5\%$)	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% **)

27 655 *: number of patients

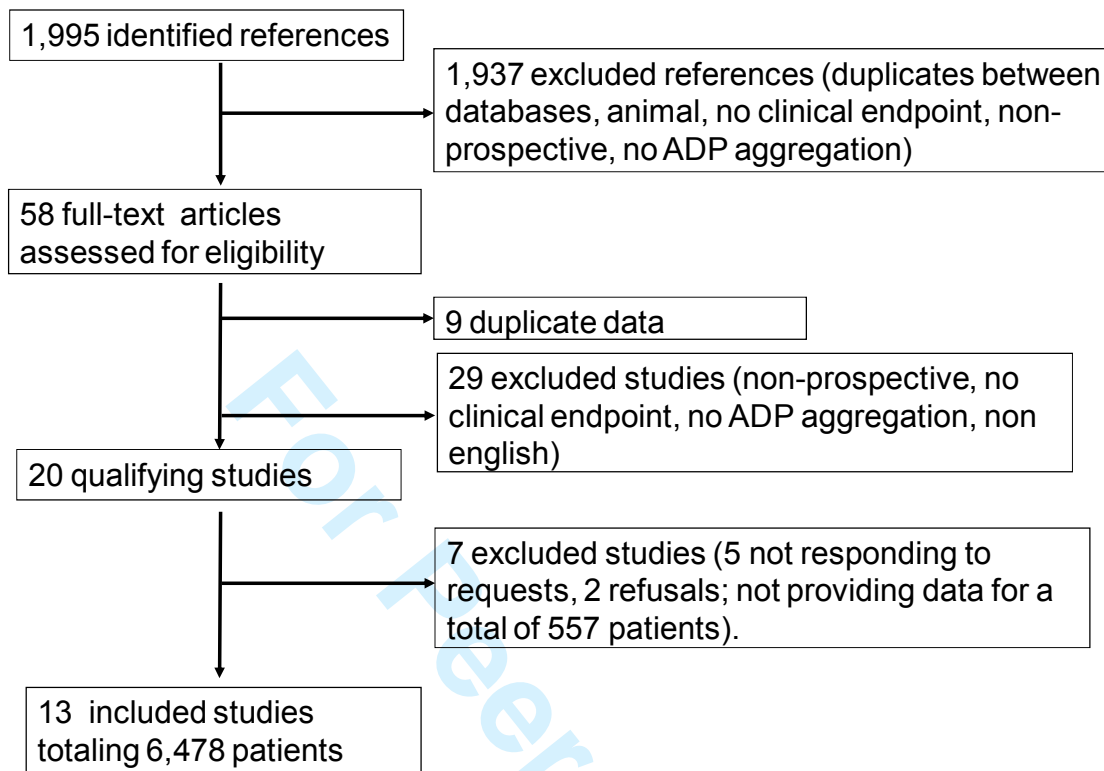
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29 656 **: observed 6-month risk of MACE

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659 **Figure 1:** Flow chart of the meta-analysis

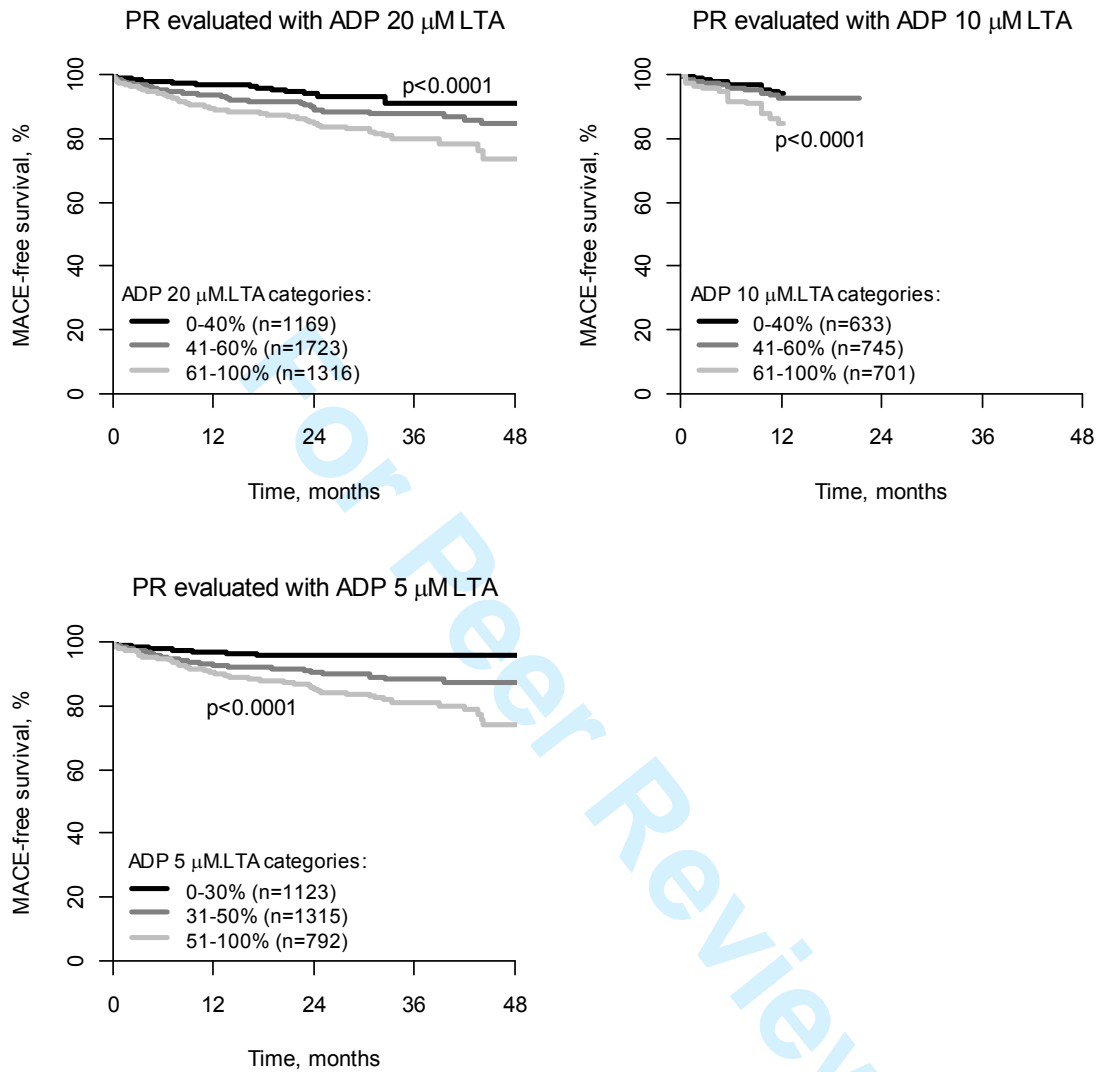
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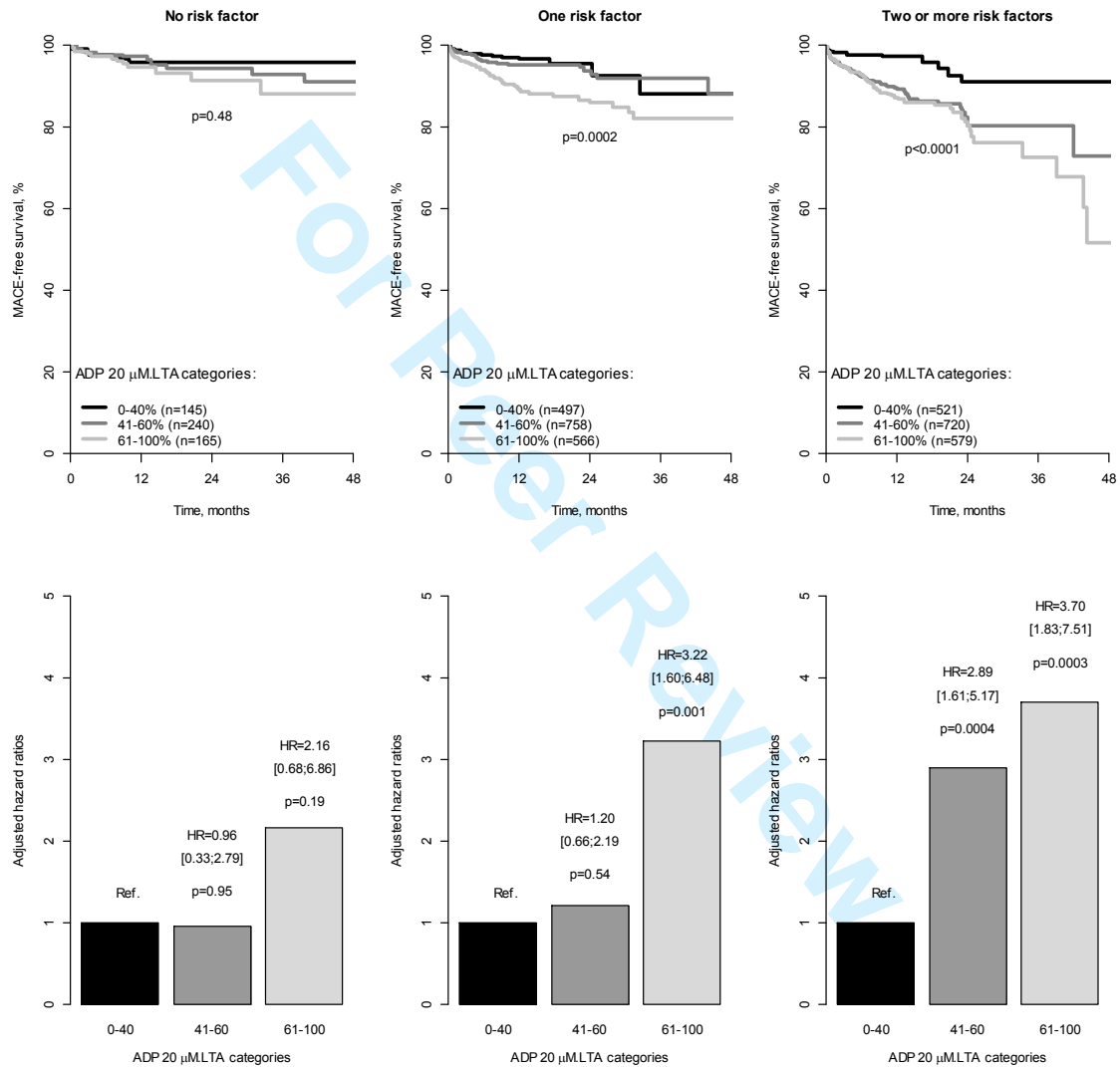
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662 **Figure 2:** Kaplan-Meier survival curve for the occurrence of MACE

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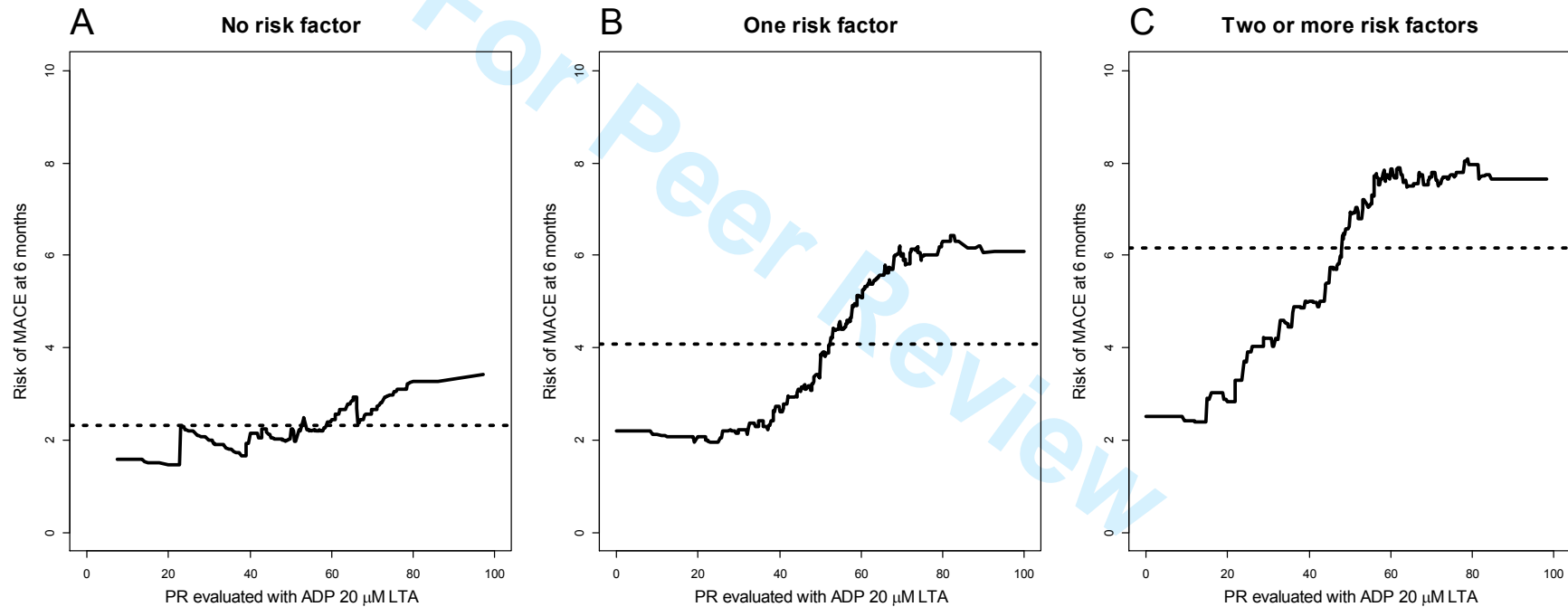


665 **Figure 3:** Association between platelet reactivity and the occurrence of MACE according to
 666 the level of risk. Low-risk patients have none of the risk factors (among age > 75 years, acute
 667 coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have
 668 one risk factor and high-risk patients have two or more risk factors. PR was assessed with 20
 669 μ M ADP LTA.
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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 μ M ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).



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6 1 **Title:** Vascular risk levels affect the predictive value of platelet reactivity for the occurrence of
7 2 major adverse cardiovascular events in patients on clopidogrel: Systematic review and
8 3 collaborative meta-analysis of individual patient data
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12 5 **Running head:** Vascular risk, platelet reactivity, and prognosis
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15
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57 48

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

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5 73 **Introduction**
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7 74 Atherosclerotic diseases account for more than 40% of deaths in Western countries, and
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9 75 antiplatelet therapy is a major preventive strategy in this setting(1). Clopidogrel, a P2Y₁₂
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11 76 receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is
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13 77 widely prescribed for secondary prevention in patients with atherosclerotic diseases. When
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15 78 combined with aspirin, clopidogrel is particularly effective in patients with acute coronary
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17 79 syndromes (ACS)(2), and has proved superior to aspirin alone in several other large
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19 80 randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide
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21 81 inter-individual variability(3, 4). Numerous cohort studies, often performed on patients with
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23 82 acute coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions
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25 83 (PCI), have shown an association between high on-treatment platelet reactivity (PR) and the
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27 84 risk of recurrent major adverse cardiovascular events (MACE)(5-7). However, recent studies
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29 85 in cohorts of stable cardiovascular outpatients(8, 9) or in medically managed ACS
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31 86 patients(10) failed to confirm these results. Several randomized trials aimed at reducing the
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33 87 recurrence of ischemic events have compared standard clopidogrel treatment to a P2Y₁₂-
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35 88 inhibitor strategy tailored according to the presence of high PR. Although initial small trials
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37 89 were promising(11, 12) more recent larger trials showed no benefit from adjusting clopidogrel
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39 90 doses or switching to prasugrel based on PR testing in low-risk coronary patients undergoing
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41 91 PCI(13, 14). These contrasting results, both from observational studies and randomized
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43 92 intervention trials, may be explained by different patient characteristics including the level of
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45 93 risk, but to date few data substantiate these hypotheses. We previously showed, in a study-
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47 94 level meta-analysis, that the risk of recurrent MACE associated with high PR was greater in
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49 95 studies using GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did
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51 96 not(7). Another meta-regression from a study-level meta-analysis of randomized trials
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53 97 suggested that the higher the incidence of coronary stent thrombosis in a given study, the
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55 98 larger the net clinical benefit from a PR-tailored strategy(15). Finally, the ADAPT-DES
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57 99 registry of patients undergoing PCI showed that high PR was predictive of stent thrombosis
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59 100 mostly in ACS patients, but there was no interaction reported between PR and the presence
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6 101 of an ACS at inclusion(16). This information suggests the hypothesis that high PR might be
7 102 more relevant in high-risk populations, but convincing data at the individual level are lacking.
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9 103 To date, the only meta-analysis on individual patient data performed on 6 studies totaling
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11 104 3,059 patients assessed with the VerifyNow P2Y₁₂ assay did not explore this hypothesis(17).
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13 105 Similarly, one of the largest and more recent meta-analysis on 8 studies and 4817 patients
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15 106 did not explore this interaction due to the lack of individual data(18). To further investigate
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17 107 this interaction on a larger population we performed a collaborative meta-analysis of
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19 108 individual patient data and focused on the interaction between relevant vascular risk factors
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21 109 and PR, assessed with ADP induced light transmission aggregometry (LTA), in order to
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23 110 better define the risk of MACE. ADP-induced LTA is the assay upon which all P2Y₁₂ receptor
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25 111 inhibitors have been developed, thus supporting its use in the present meta-analysis. In
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27 112 addition, among several available assays to evaluate PR, LTA is the historical gold standard
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29 113 with which most platelet function assays were compared.
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5 115 **Methods**

6 116 *Data sources*

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9 117 Literature review, confined to articles in English(19), was based on electronic databases
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11 118 (Medline, Embase, Web of Science, Cochrane Central Register of Controlled Trials) and
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13 119 abstracts from major international meetings held from 2010–2013 (ISTH, AHA, ACC, ESC).
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15 120 A free-text search was conducted using an ‘ADP’ and ‘aggregation’ and ‘clopidogrel’ key-
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17 121 word combination. Articles were selected on the basis of abstracts, before examination of the
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19 122 full text. Reference lists of selected articles were also hand-searched to identify additional
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21 123 relevant reports. Reviewers (JLR and PF) were not blinded to the journal, authors or
22
23 124 institutions in the publications as this has been shown to be unnecessary(20). The electronic
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25 125 database search was last updated on 31 July, 2013. The objective of this individual patients’
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27 126 data meta-analysis was described in a project that was part of French ministry of health’s
28
29 127 initiative to encourage meta-analyses (PHRC 15-07 to JL Reny “*Etudes prospectives sur la*
30
31 128 *réponse biologique au clopidogrel et évènements ischémiques chez les patients*
32
33 129 *athérotrombotiques : Métaanalyse sur données individuelles et résumées*” [http://www.plan-](http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf)
34
35 130 [alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf](http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf)). Protocol in
36
37 131 French available upon request.

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41 133 *Study selection*

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43 134 Selected studies met the following criteria: (a) patients were treated with clopidogrel and had
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45 135 symptomatic atherothrombosis (clinical signs related to vascular atherothrombotic lesions);
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47 136 (b) pharmacodynamic response to clopidogrel was evaluated using the maximal aggregation
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49 137 value from LTA on platelet-rich plasma with 20, 10, or 5 μ M ADP as an agonist; (c) LTA was
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51 138 performed remote from platelet function interfering drugs such as GpIIb/IIIa inhibitors; (d)
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53 139 patients were prospectively monitored for MACE for at least 30 days, defined using at least
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55 140 one of the following items: acute coronary syndrome (unstable angina, myocardial infarction
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57 141 with/without ST segment elevation), ischemic stroke (acute neurological deficit due to a
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59 142 cerebral infarction), and vascular death; (e) studies involved either a prospective cohort or a
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6 143 randomised therapeutic trial, but one in which treatment was allocated independently of the
7 144 response to clopidogrel. When studies were suspected of including the same patients, the
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9 145 authors were asked to provide data from the largest possible number of independent patients.
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13 147 *Data extraction*

14 148 The corresponding authors or principal investigators of eligible studies were contacted and
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16 149 asked to participate in the CLOpidogrel and Vascular ISchemic events – Individual Patient
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18 150 Data (CLOVIS-IPD) meta-analysis group. Investigators provided individual data on: the
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20 151 qualifying cardiovascular condition and clinical setting at inclusion (ACS or stable disease);
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22 152 MACE and date of occurrence during follow-up; platelet reactivity (PR) with ADP 20, 10,
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24 153 and/or 5 μ M and its timing relative to loading dose of clopidogrel; age, gender, height, and
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26 154 weight; current smoking status, diabetes, hypercholesterolemia, and hypertension; left
27
28 155 ventricular ejection fraction; platelet count; PCI; use of GpIIb/IIIa inhibitors and timing;
29
30 156 concomitant medications; and bleeding events and timing during follow-up. Data were
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32 157 checked for completeness and consistency with published reports. Any discrepancies were
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34 158 resolved with the corresponding authors. After format harmonization, data were compiled for
35
36 159 statistical analysis. All studies were approved by their respective institutional review boards.

37 160

38 161 *Quality assessment of studies*

39 162 A new quality assessment tool for prognostic studies called PROBAST (see
40
41 163 Acknowledgements) was used to estimate risks of bias and concerns about applicability. As
42
43 164 PROBAST is not customized for meta-analyses of individual patient data, items were
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45 165 adapted accordingly. Based on the present study's list of relevant criteria, risks of bias, and
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47 166 concerns about applicability are rated as low, unclear, or high. Supplemental Figure 1 shows
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49 167 the list of criteria.

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51 169 *Primary outcomes and measures*

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6 170 The primary clinical outcome was the occurrence of MACE, as defined above (see Study
7 171 selection (d)). The primary biological outcome was maximal aggregation with 20 μM ADP, as
8
9 172 it is a better concentration for analyzing the effects of clopidogrel than lower ones. PR was
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11 173 categorized in three strata. The higher cut-offs were selected on the basis of previously
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13 174 published cut-offs (59% to 64% for 20 μM ADP, and 43% to 46% for 5 μM ADP)(21), and to
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15 175 keep relatively balanced numbers of patients in each PR categories. Three pre-specified
16 176 categories allowed a better description of the dose-dependent effects of PR on the risk of
17
18 177 MACE compared to the usual dichotomous high and low PR categorization. Three categories
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20 178 were also chosen to better parallel the analysis with a therapeutic PR window that has been
21
22 179 associated with optimal net clinical benefit(22). A surrogate for the level of cardiovascular
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24 180 risk was defined as the number of factors with homogeneous definitions across studies, and
25 181 these were markers of MACE in the meta-analysis. The factors were selected from among
26
27 182 age, diabetes, hypertension, smoking, hypercholesterolemia, and the presence of an ACS at
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29 183 inclusion (as defined in study selection (d)), and were all provided at the time of inclusion and
30 184 PR testing.

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34 186 *Statistical analysis*

35 187 MACE-free survival curves were derived from individual patient data using the Kaplan-Meier
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37 188 estimator; curves were compared using log-rank tests stratified by study. Associations
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39 189 between conventional risk factors, PR strata, and risk of MACE were analyzed using
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41 190 multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the
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43 191 size of the random effects (Tau^2) which is an estimate of the between study variability(23).
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45 192 The presence of heterogeneity was tested by comparing models with and without random
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47 193 effects (likelihood ratio test). The interactions between the level of risk and PR strata were
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49 194 tested. MACE-free survival according to PR, as a continuous variable, was assessed using
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51 195 the R package prodlm using the symmetrical nearest neighborhoods method.(24) Sensitivity
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53 196 analyses were conducted to check the robustness of the findings with respect to: the risks of
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55 197 bias and concerns about the applicability of studies; the definition of MACE, including target

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6 198 vessel revascularization or PCI at inclusion, and; the influence of a given specific study. The
7 199 net reclassification index (NRI) for survival data(25) was computed to quantify the
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9 200 contribution of PR testing for the prediction of the 6-month risk of MACE in patients with
10 201 increasing numbers of traditional risk factors. The event and non-event continuous NRIs
11 202 were reported. Potential publication bias was checked for. P-values below 0.05 were
12 203 considered significant and all tests were two-sided. Published guidelines for meta-analysis of
13 204 observational studies in epidemiology (MOOSE) and their reporting(26) were followed.
14
15 205 Details on statistical methods are given in the online data supplement.
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21 207 **Results**

23 208 *Characteristics of included studies*

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25 209 The Figure 1 flow-chart details how 13 of 20 qualifying studies were included, totaling 6,478
26 210 patients(8, 27-38). Table 1 shows their characteristics. Data on body mass index,
27 211 concomitant medications, left ventricular ejection fraction, or the occurrence of target and
28 212 non-target vessel revascularization during follow-up were only available in some studies. All
29 213 studies provided individual data allowing a homogeneous definition of MACE, current
30 214 smoking status, ACS, diabetes (fasting plasma glucose ≥ 7.0 mmol/l, 2-h plasma glucose \geq
31 215 11.1 mmol/l after 75g oral glucose load or background therapy for diabetes), and
32 216 hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or
33 217 a documented history of hypertension). Hypercholesterolemia was not defined in a
34 218 homogeneous fashion across studies and plasma LDL-cholesterol levels were not available
35 219 for more than 2,000 patients. Overall, risks of bias and concerns about applicability were low
36 220 (online data supplement further details study characteristics, bias, and applicability).
37 221 Information on bleeding was limited to five studies, with only 67 major and 20
38 222 moderate/minor bleedings.
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51 224 *MACE and level of risk*

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5 225 Overall, 421 MACE occurred in 6,478 patients (6.5%), the majority being ACS (n = 383).
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7 226 There were 83 stent thromboses, including 79 definite or probable and four possible ones, all
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9 227 included in the composite outcome of MACE. The MACE-free survival rate across the
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11 228 different studies at the end of follow-up ranged from 77.4% to 97.3%. In a multivariate
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13 229 analysis, four factors were found relevant to determining patients' levels of risk: age greater
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15 230 than 75 years, diabetes, ACS at inclusion, and hypertension (Table 2). The number of these
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17 231 factors was used as a surrogate for the individual risk of MACE. Patients with none of these
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19 232 factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with
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21 233 two or more factors 'high-risk' (global p-value <0.0001 for the trend).
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23 235 *MACE and PR*

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25 236 Nine studies (n = 4,438 patients) performed LTA using 20 µM ADP, four studies (n = 2,144
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27 237 patients) used 10 µM ADP, and eight studies (n = 3,317 patients) used 5 µM ADP. Figure 2
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29 238 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE
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31 239 increased significantly with PR with 20 µM ADP, 10 µM ADP, and 5 µM ADP.
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33 240 With adjustment, high PR was still significantly associated with an increased risk of MACE
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35 241 (Table 3). However, for PR evaluated using 10 µM ADP, risk only increased for the highest
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37 242 PR category, corresponding to LTA values greater than 60%.
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39 244 *Interaction between risk level and PR for the outcome of MACE*

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43 246 Platelet reactivity assessed with 20 µM ADP. Patients with none of the four risk factors
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45 247 showed no significantly increased risk associated with PR, while for patients with one risk
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47 248 factor only, the higher strata of PR was associated with an increased risk of MACE. Patients
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49 249 with two or more risk factors showed an increased risk of MACE for both the medium and
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51 250 higher strata of PR. (Figure 3). In a Cox model, the interaction between PR strata and the
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53 251 risk level was statistically significant (p=0.04). The corresponding hazard ratios (HRs) are
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55 252 shown in Figure 3. Heterogeneity was not detected for the overall interaction (p=0.81), as
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5 253 well as when it was restricted to each risk level category (intermediate versus low risk level,
6 254 $p=0.45$, and high versus low risk level, $p=0.90$). Additional results on heterogeneity are
7 255 provided in the supplemental material. Figure 4A shows that PR, when analyzed in a
8 256 continuous fashion, barely affects the risk of MACE at 6 months in patients with no risk
9 257 factors: the risk is close to 2% at six months, irrespective of the level of platelet reactivity.
10 258 Conversely, patients with one risk factor and an overall 4.1% risk of MACE at six months
11 259 have in fact a 2% risk of MACE when they have a low PR, or a 6% risk of MACE when they
12 260 have a high PR (Figure 4B). Similarly, patients with two or more risk factors and an overall
13 261 6% risk of MACE at six months can indeed have a 2% risk of MACE when they have a low
14 262 PR (Figure 4C). The reclassification of the 6-month risk of MACE, according to the three
15 263 categories of platelet reactivity, in patients with no, one and two or more risk factors, is
16 264 shown in Table 4. Overall, PR allowed the reclassification of 44% of the total population
17 265 (1837/4193 patients) included in a 6-month follow-up to a different level, mostly in patients
18 266 originally identified as intermediate or high risk on the basis of the number of risk factors only.
19 267 In patients experiencing MACE in the first 6 months of follow-up, the risk predicted by the
20 268 combination of PR and risk factors was on average increased compared with the risk
21 269 predicted from risk factors only: the continuous event net reclassification index (NRI) was
22 270 0.39 (95%CI 0.23 to 0.62). Conversely, in patients free of MACE at 6 months, the measure of
23 271 PR did not modify the predicted risk: the continuous non-event NRI was 0.01 (95%CI -0.16 to
24 272 0.09). The overall NRI was 0.39 (95%CI 0.22 to 0.57).

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42 274 Platelet reactivity assessed with 10 μ M ADP. A total of only five low-risk patients in four
43 275 studies performing 10 μ M ADP LTA to assess PR precluded an analysis of this low-risk
44 276 group. Furthermore, the surrogate for risk level failed to demonstrate an association with the
45 277 observed risk of MACE in these studies. Figure 4B shows that the risk of MACE increased in
46 278 both intermediate- and high-risk patients for PR values above 40%, without any obvious
47 279 relation with the level of risk.

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5 281 Platelet reactivity assessed with 5 μ M ADP. The direction of interaction between PR using 5
6 282 μ M ADP and the risk level was similar to that observed for PR using 20 μ M ADP, even
7 283 though overall interaction did not reach the significance level ($p=0.17$). Of note there were
8 284 980 fewer patients in the studies performing 5 μ M ADP than in those using 20 μ M ADP. The
9 285 increased risk of MACE as PR increases is indeed similar for intermediate- and high-risk
10 286 patients; for low-risk patients PR is not associated with a MACE outcome (online data
11 287 supplement). Heterogeneity was not detected for the overall interaction ($p=0.19$). Figure 4C
12 288 shows that the risk of MACE was unaffected by PR in low-risk patients while it increased for
13 289 PR values above 30% in intermediate-risk patients and for PR values above 10%–20% in
14 290 high-risk patients.
15 291

25 292 *Sensitivity analyses*

26 293 Sensitivity analyses were performed for PR using 20 μ M ADP to assess: the robustness of
27 294 the association between PR and risk of MACE and its interaction with the level of
28 295 cardiovascular risk; the robustness of the results in the population of PCI patients and when
29 296 target vessel revascularization is added to the composite outcome. All analyses showed that
30 297 the sizes of the effects remained similar, and whilst in some instances the statistical
31 298 significance of the interactions could be lost, there was no impact on their magnitudes
32 299 (supplemental Tables 1 and 2). Notably, when PR was categorized in quartiles (20 μ M ADP
33 300 maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%, 51.4%–63.0%, 63.1%–100%)
34 301 the interaction between PR and the number of risk factors remained significant ($p=0.01$).
35 302 When restricted to the population of 3,564 patients treated with PCI and tested using 20 μ M
36 303 ADP the interaction was of similar magnitude but no longer significant (supplemental Table
37 304 3).
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50 306 *Publication and availability biases*

51 307 A check for potential publication bias was made for PR using 20 μ M ADP, on which the main
52 308 analyses were performed. A funnel plot was obtained by representing the HR of PR using 20
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5 309 μM ADP and the standard error, assessed in each separate study (supplemental Figure 4).
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7 310 Two studies with a negative association between PR using 20 μM ADP and the risk of MACE
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9 311 (with small sample sizes) were detected as missing using the 'trim and fill' method for making
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11 312 the funnel plot symmetrical. When these missing studies were added, the pooled HR was not
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13 313 significantly modified. These findings suggested that the publication bias in our meta-analysis
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15 314 was minor.
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17 315 Seven qualifying studies could not provide individual patient data. It is of note that in five of
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19 316 these, the relation between clopidogrel non-response and ischemic events was not a study
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21 317 objective (pharmacokinetic-pharmacodynamic studies or randomized trials of different
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23 318 clopidogrel loading doses). The two remaining studies (n = 101 and 111 patients) were
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25 319 specifically interested in the prognostic value of PR for MACE.
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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 μ M 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 μ M 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 μ M 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 μ M ADP, and a

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6 349 corresponding loss of power. Moreover, it was previously shown that ADP-induced platelet
7 350 aggregation in citrated plasma was dependent on the artifactual generation of TxA₂ that was
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9 351 modulated by aspirin, at least at lower ADP concentrations(41). This may be associated with
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11 352 an additional background noise in which the interaction between the identified risk factors
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13 353 and PR to predict MACE is blurred, as seen with the lowest 5 μ M ADP concentrations and
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15 354 partially also with the intermediate 10 μ M ADP concentrations. Only four of the studies
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17 355 analysed used 10 μ M ADP, and two of these had a follow-up limited to 30 days; with only
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19 356 124 MACEs during follow-up, this accounts for a limitation in power to reliably study
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21 357 interactions. Overall, the concentration of ADP used is of limited significance since the
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23 358 influence of risk factors appears in all three ADP concentration groups (table 3 and figure 2).
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25 359 Which laboratory assay and which platelet agonist concentration are best suited for the
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27 360 clinical evaluation of platelet function is the matter of some debate. ADP-induced LTA is
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29 361 highly reproducible within a given laboratory, but its lack of standardization across studies
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31 362 may have slightly weakened the positive findings or lower the level of significance for the
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33 363 interactions found in the present meta-analysis. Of note, the present meta-analysis does not
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35 364 aim to promote the use of LTA to tailor antiplatelet therapy but it rather relied on a historical
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37 365 gold standard in platelet function testing to evidence an interaction with patients'
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39 366 characteristics that should be considered for a tailored approach. The point-of care
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41 367 VerifyNow P2Y₁₂ assay, used in several intervention trials, correlates well with ADP-induced
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43 368 LTA(42, 43) and we speculate that the main findings of the present meta-analysis would
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45 369 have been similar, had PR been evaluated using the VerifyNow P2Y₁₂ assay.
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47 370 Several intervention trials have compared conventional clopidogrel treatment to an
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49 371 antiplatelet strategy tailored according to PR. Early, small randomized trials(11, 12) that
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51 372 utilized vasodilator-stimulated phosphoprotein phosphorylation level measurement to indicate
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53 373 P2Y₁₂ receptor reactivity, showed a protective effect for repeat 600 mg clopidogrel loading
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55 374 doses in ACS patients prior to PCI. However, recent larger trials utilizing the VerifyNow P2Y₁₂
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57 375 assay were negative. Indeed, the GRAVITAS(13) and ARCTIC(14) studies failed to show the
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59 376 benefit of a PR-tailored antiplatelet strategy after PCI. Various limitations of these trials were
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6 377 addressed in a recent consensus publication(22). The event rate of the GRAVITAS study
7 378 was low compared to the one used for power calculation, and the antiplatelet effect of the
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9 379 high-dose regimen may have been suboptimal as it reduced the prevalence of high PR by
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11 380 only 22%. Similarly, the ARTIC study population was also at a low absolute risk of
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13 381 subsequent cardiovascular events because the prevalence of ACS patients was low, and the
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15 382 composite endpoint also included other events that may not be related to platelet function.
16 383 The interaction of PR and the number of risk factors, as identified in the present meta-
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18 384 analysis, substantiates the hypothesis that the risk associated with high PR was not clinically
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20 385 relevant in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a
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22 386 beneficial reduction of MACE for these low-risk patients. Based on these observations we
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24 387 speculate that higher risk patients are more likely to benefit from a therapy tailored to their
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26 388 initial PR. This may explain why early interventions designed to efficiently blunt high PR in
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28 389 ACS patients with multiple conventional risk factors translated into a reduction of MACE(11,
29 390 12, 22).

30 391 In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the
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32 392 management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk.
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34 393 However, a recent cost-effectiveness analysis for six European perspectives showed that the
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36 394 universal use of newer P2Y₁₂ inhibitors for ACS patients is probably not as cost-effective as
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38 395 strategies based on PR(44). It should also be kept in mind that ticagrelor and prasugrel
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40 396 increase the risk of bleeding and that a therapeutic medium-PR window is associated with
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42 397 optimal net clinical benefit(22). The net benefits of newer P2Y₁₂ inhibitors could also probably
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44 398 be improved not only by testing for PR, but also by incorporating patient risk levels in the
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46 400 TROPICAL-ACS ([ClinicalTrials.gov identifier: NCT01959451](https://clinicaltrials.gov/ct2/show/study/NCT01959451)) and ANTARCTIC(45) partly
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48 401 include this concept of risk levels, further efforts in this direction are needed.

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50 402 This meta-analysis has several strengths, such as the good overall quality of the studies
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52 403 included, as assessed using a quality tool specifically adapted to prognostic studies. The
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54 404 availability of individual patient data allowed a reliable evaluation of the risk associated with

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5 405 PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a
6 406 secondary prevention population were thus identified. The consistency of results across the
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8 407 different ADP concentrations used in the different studies to assess PR, as well as the
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10 408 sensitivity analyses, indicated that the results were robust.

11 409 Despite the advantages related to the availability of individual patient data, this meta-analysis
12 410 also had some limitations, including a low proportion of women (25%). This did not allow a
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14 411 stratification of the analyses by gender, as is usually the case in risk assessment tools such
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16 412 the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is
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18 413 not considered as one of traditional risk factors, but is rather presented in separate charts for
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20 414 women and men. There were incomplete data on concomitant medications or other relevant
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22 415 risk factors such as the left ventricular ejection fraction, cholesterol levels or renal
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24 416 insufficiency. Finally, information on bleeding was limited to five studies and a low number of
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26 417 events, thus precluding a reliable analysis of bleeding events and their relation to PR.

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28 418 In conclusion, high PR in patients on clopidogrel is associated with an increased risk of
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30 419 MACE in patients with vascular risk factors, but not in low-risk patients. These findings
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32 420 suggest that trials on tailored PR treatment strategies should be primarily stratified on the
33
34 421 individual vascular risk factors in order to assess a truly personalized approach.

35 422
36
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43 426 [alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf](http://www.plan-alzheimer.gouv.fr/IMG/pdf/Liste_des_dossiers_retenus_-_2_mai_2008.pdf)) and the Geneva
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12

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15
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17
18 439 data and take responsibility for the integrity of the data and the accuracy of its analysis
19

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21 441 Acquisition of data: Reny JL, Fontana P, Hochholzer W, Neumann FJ, Ten Berg J, Janssen

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27 444 Data management and statistical analysis: Combescure C and Reny JL

28 445 Drafting and critical revision of the manuscript for important intellectual content:: Reny JL,
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30 446 Fontana P, Hochholzer W, Neumann FJ, Ten Berg J, Janssen P, Geisler T, Gawaz M,
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32 447 Marcucci R, Gori AM, Cuisset T, Alessi MC, Berdagué P, Gurbel P, Yong G, Angiolillo D,
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34 448 Aradi D, Beigel R, Campo G, Combescure C.
35

36 449 All authors had full access to all of the data (including statistical reports and tables) in the
37

38 450 study and can take responsibility for the integrity of the data.
39

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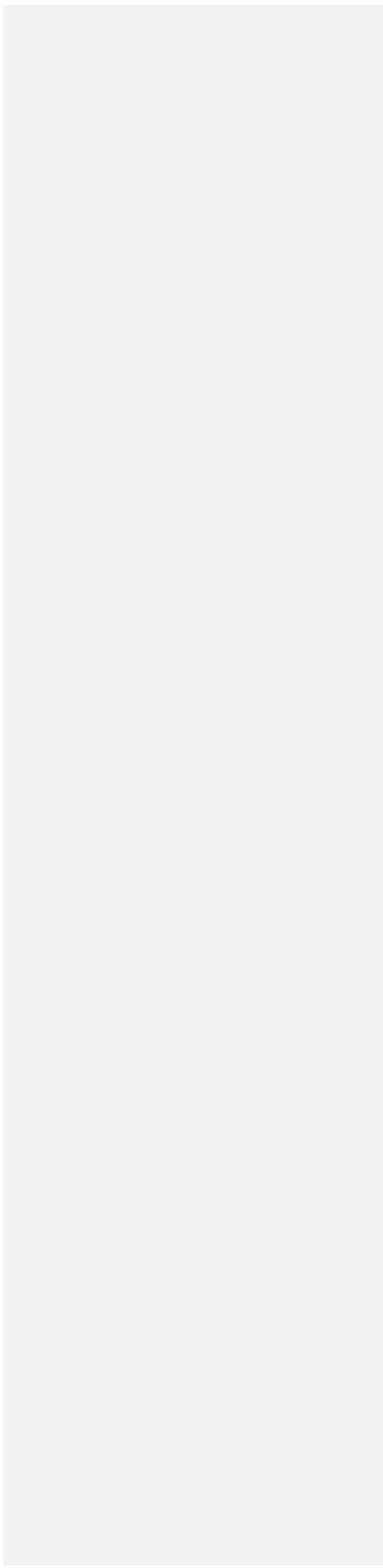
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For Peer Review



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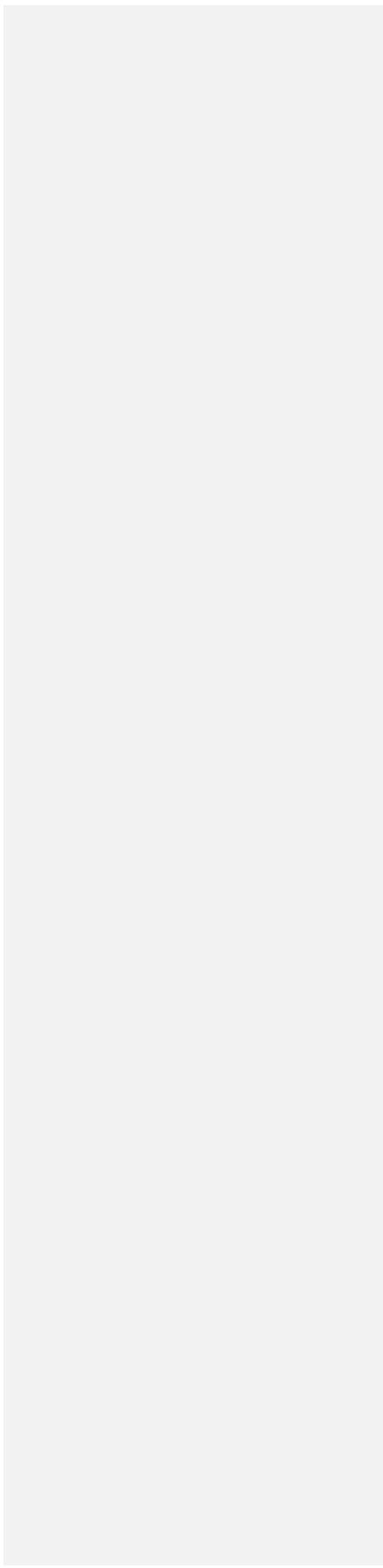
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For Peer Review



631 **Table 1.** Main characteristics of published studies

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

633 Age, mean \pm standard deviation; CAD, coronary artery disease; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; ADP,

634 adenosine diphosphate concentration used for the evaluation of platelet reactivity

635 * Median (maximum)

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637 **Table 2:** Multivariate analysis to assess the associations between the risk factors and the composite outcome of MACE. This analysis was
638 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.
639 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
<i>Current smoking status</i>	0.92 [0.71;1.18]	0.50	<i>Low risk (n=579)</i>	1	
<i>Age (> 75)</i>	1.56 [1.25;1.95]	<0.0001	<i>Intermediate risk (n=2444)</i>	1.61 [1.05;2.45]	0.03
<i>Diabetes</i>	1.58 [1.27;1.96]	<0.0001	<i>High risk (n=3435)</i>	2.58 [1.69;3.94]	<0.0001
<i>Hypercholesterolemia</i>	0.86 [0.69;1.06]	0.15			
<i>Hypertension</i>	1.23 [0.98;1.54]	0.07			
<i>ACS at inclusion</i>	2.00 [1.27;3.16]	0.003			
<i>Gender (Male)</i>	1.11 [0.89;1.40]	0.35			

640 *: 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
641 no risk factor, intermediate risk for one risk factor and high risk for two or more risk factors).

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

	ADP 20 μ M		ADP 10 μ M		ADP 5 μ M	
	N		N		N	
<i>Studies</i>	9		4		8	
<i>Events</i>	287		124		229	
<i>Patients (after exclusion of missing data)</i>	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**
<i>Lower category *</i>	1		1		1	
<i>Intermediate category *</i>	1.85 [1.26;2.73]	0.002	1.31 [0.79;2.17]	0.30	1.79 [1.02;3.14]	0.04
<i>Higher category *</i>	2.91 [1.78;4.74]	<0.0001	2.61 [1.64;4.16]	<0.0001	2.79 [1.50;5.22]	0.001

646 HR, Hazard Ratio; CI, Confidence Interval

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

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

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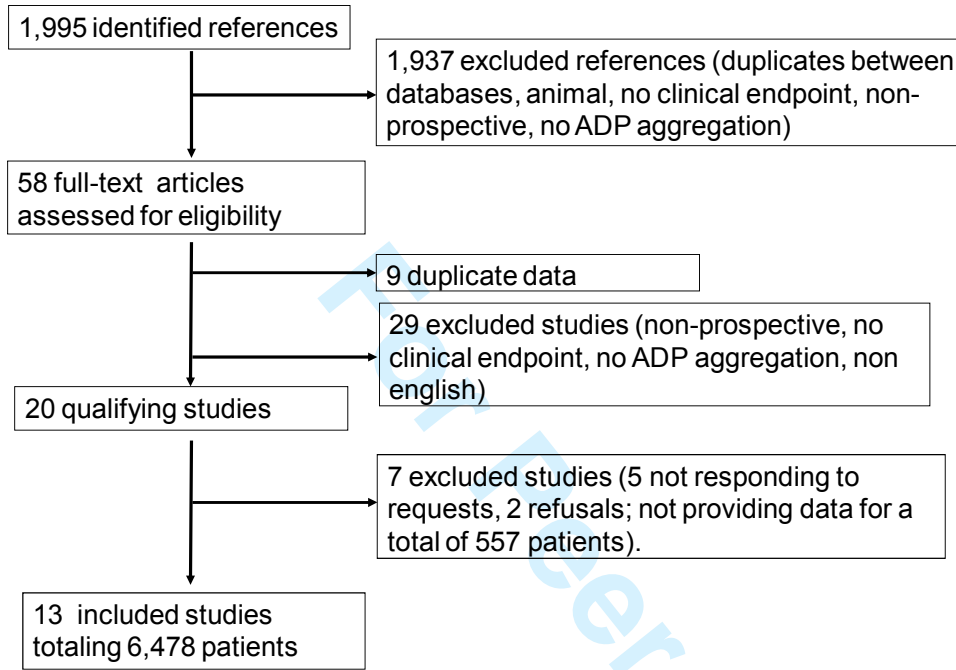
Table 4: Reclassification of the 6-month risk of MACE when the individual risk was predicted from platelet reactivity measured by 20µM ADP in addition to risk factors. The predicted risk was stratified in three levels (low: ≤3%, intermediate: >3% and ≤5%, high: >5%) in agreement with the 6-month risk 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 number of risk factors only	Risk predicted by the combination of risk factors and platelet reactivity measured by 20µM ADP			Total
	Low risk (≤3%)	Intermediate risk (>3% and ≤5%)	High risk (>5%)	
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

659 **Figure 1:** Flow chart of the meta-analysis

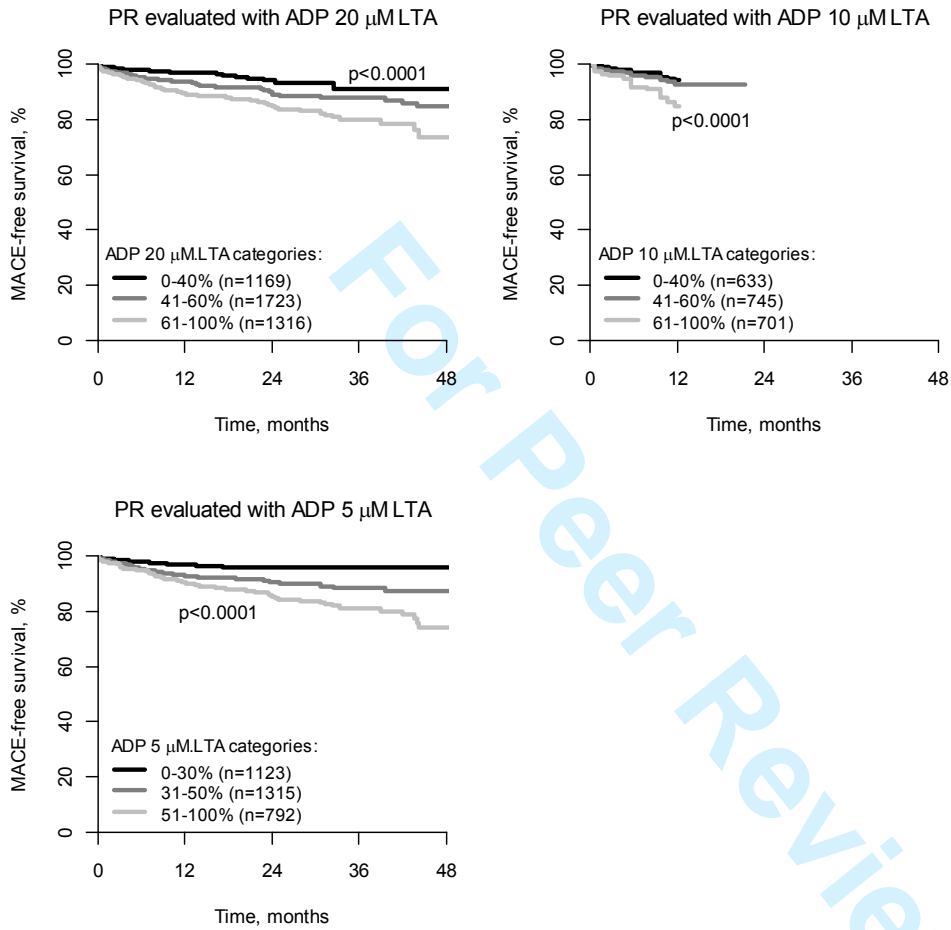
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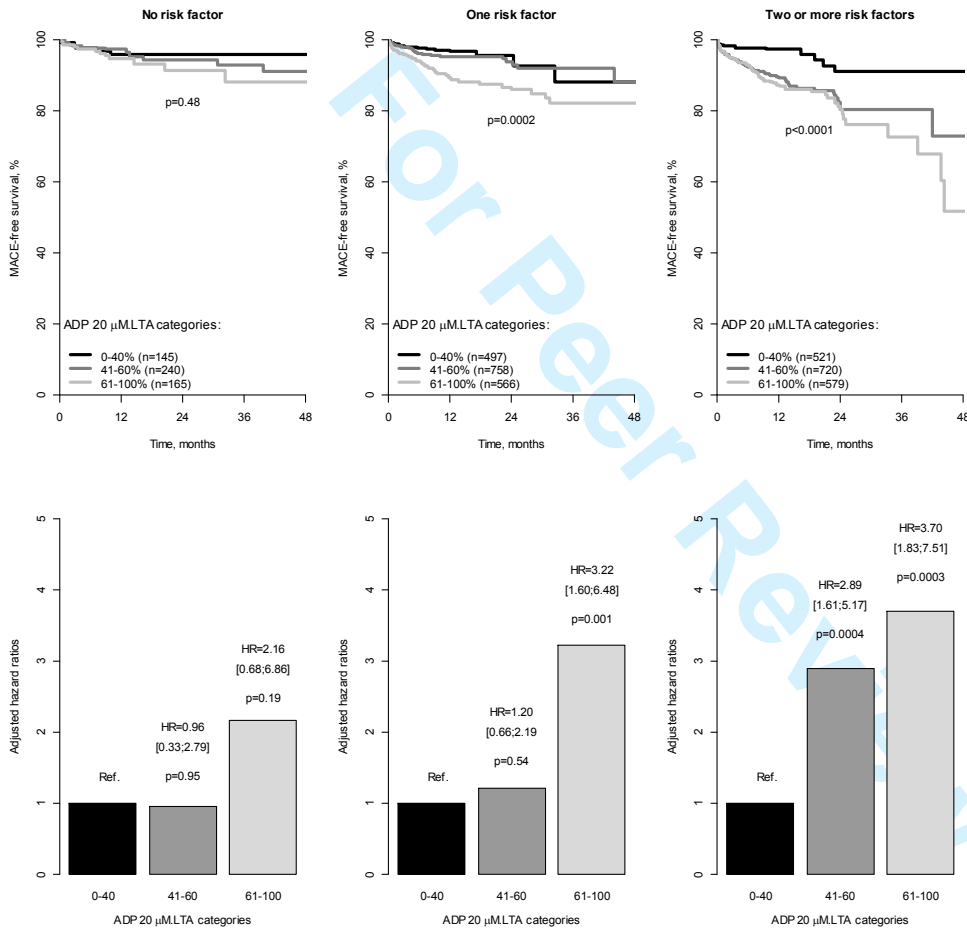
662 **Figure 2:** Kaplan-Meier survival curve for the occurrence of MACE

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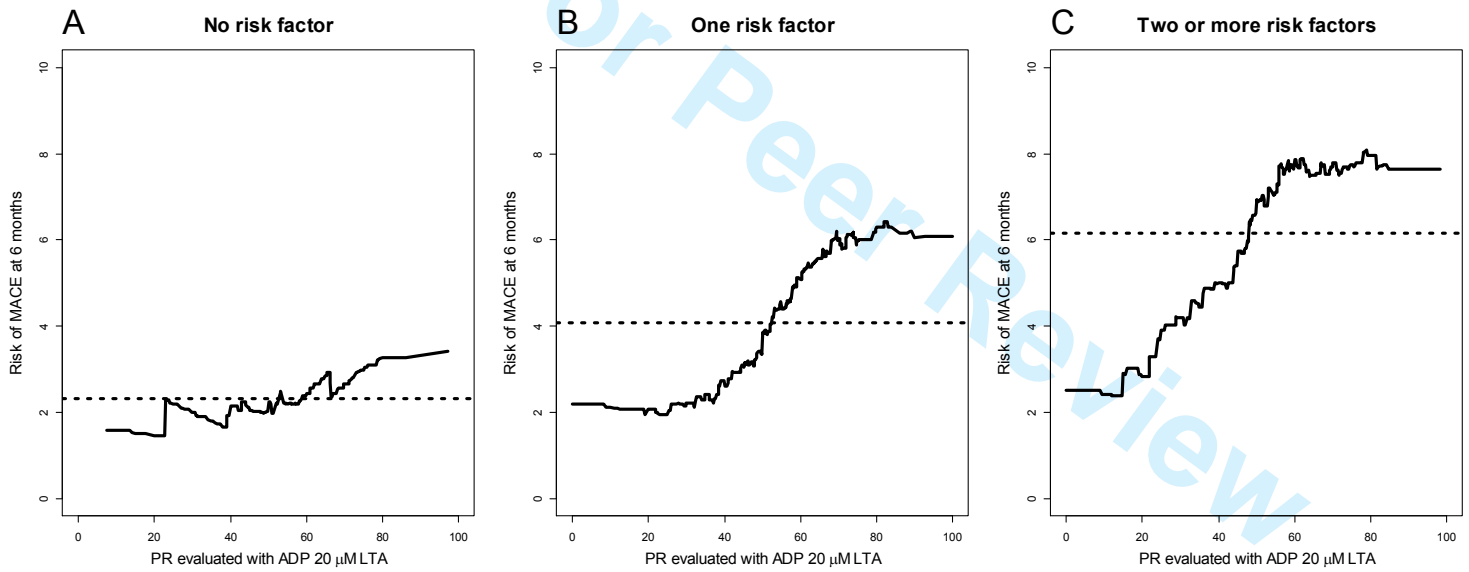
665 **Figure 3:** Association between platelet reactivity and the occurrence of MACE according to
 666 the level of risk. Low-risk patients have none of the risk factors (among age > 75 years, acute
 667 coronary syndrome at inclusion, diabetes, and hypertension), intermediate-risk patients have
 668 one risk factor and high-risk patients have two or more risk factors. PR was assessed with 20
 669 μ M ADP LTA.
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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 μ M ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).



1 Rebuttal to BMJ reviewers' comments

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3 **Reviewer: 1**

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5 **Comments:**

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

11 *Response : NA*

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15 **Reviewer: 2**

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17 **Comments:**

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

22
23 1. The background of the studies included in this meta analysis should be better clarified:
24 The differential between purely observational cohorts versus studies in patients undergoing
25 revascularization studies. In patients undergoing revascularization the risk for periprocedural
26 adverse outcome is considered especially high in patients with low reactivity but this category
27 is not clearly differentiated from the patients receiving secondary preventive medical
28 treatment only. Please make this more clear to the reader.

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

35
36
37 2. The endpoints are ACS, ischemic stroke and vascular death. Why not including any
38 death ?

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

49
50 3. Did the authors look at separate outcome parameters within this dataset; especially on
51 ACS versus stroke leaving vascular death out of the perspective ?

52 *Response : we did not look at each outcome separately or leaving vascular death aside. As mentioned*
53 *in the results section, > 90% of the events were ACS (383 ACS out of 421 MACE), thereby precluding*
54 *a reliable analysis on separate outcomes. Of note, vascular deaths were adjudicated by an*
55 *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")

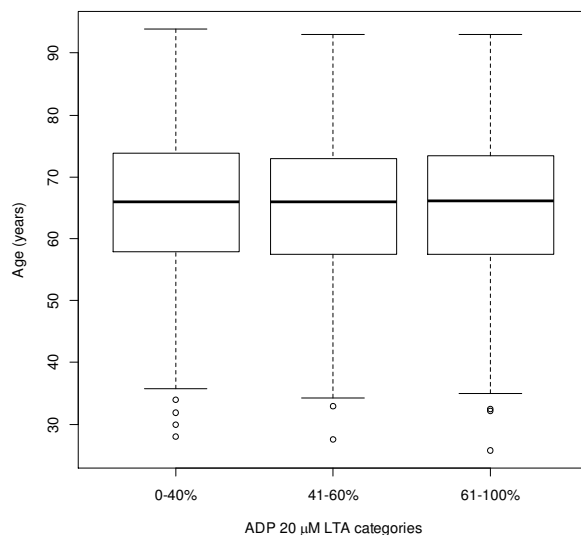
5. **A serious limitation is the use of LTA for PR only. Especially as the authors state in the introduction that verify now was never tested likewise it would have seemed easy to also look for outcome of verify Now testing. Please explain. Also, make clear in the conclusion section that observed results 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 largest effect. please extend more on the relationship 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 μ M 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 61-100% 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 μ M 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 methodological 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-

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

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

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

16
17 **3)Data presented in Table 3 and in Figure 2 could be interpreted to indicate that the**
18 **concentration of ADP used is of limited significance since the influence of risk factors appears**
19 **in all three ADP concentration groups. this should be discussed by the authors**

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

23
24 **4)Since the bulk of the work is statistical , the paper should receive careful statistical review.**

25
26 *Response : this was done within the BMJ editorial committee including a statistician, Rafael Perera,*
27 *who did not have any methodological or statistical issues related to this meta-analysis. In addition we*
28 *provide a detailed description of the methods used and several sensitivity analyses that support the*
29 *robustness of the findings (supplemental online material). Finely, we included a quality assessment*
30 *tool, PROBAST, that was recently and specifically designed for meta-analysis or prognostic studies.*
31 *The PROBAST tool was kindly made available to us by Dr Penny Whitting as acknowledged in the*
32 *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 of 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

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**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**

Jean-Luc Reny; Pierre Fontana; Willibald Hochholzer; Franz Josef Neumann; Jurrën ten Berg; Paul W. Janssen; Tobias Geisler; Meinrad Gawaz; Rossella Marcucci; Anna-Maria Gori; Thomas Cuisset; Marie-Christine Alessi; Philippe Berdagué; Paul A. Gurbel; Gerald Yong; Dominick J. Angiolillo; Daniel Aradi; Roy Beigel; Gianluca Campo; and Christophe Combescure.

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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 20 μ M 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 μ M; (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.

1
2 Corresponding authors of selected studies were contacted to collaborate to the meta-
3
4 analysis and to provide their individual patient's data.

5
6 **Results:** Thirteen prospective studies totalled 6,478 clopidogrel-treated patients who
7
8 experienced 421 MACE (6.5%) during a median follow-up of 12 months. The risk of MACE
9
10 associated with PR increased differentially according to the number of risk factors present
11
12 (age>75 years, ACS at inclusion, diabetes, and hypertension; interaction $p=0.04$): no
13
14 association to PR in low-risk patients (no risk factor) ($p=0.48$); 3.2 (1.6 to 6.5, $p=0.001$) times
15
16 greater risk of MACE in high PR intermediate-risk patients (one risk factor); 2.9 (1.6 to 5.2,
17
18 $p=0.0004$) and 3.7 (1.8 to 7, $p=0.0003$) times greater risk of MACE in medium PR and high
19
20 PR high-risk patients (≥ 2 risk factors). PR allowed the reclassification of 44% (1837/4193
21
22 patients) of the total population to a different risk level for the outcome of MACE, mostly in
23
24 patients originally identified as intermediate or high risk.

25
26 **Conclusion:** The magnitude of the association between PR and MACE risk is strongly
27
28 dependant on the level of cardiovascular risk faced by patients on clopidogrel suggesting that
29
30 PR-tailored strategies may be most effective in higher-risk patients.

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34 **Keywords:** clopidogrel, drug response, platelets, cardiovascular diseases, ischemic events.
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4 Atherosclerotic diseases account for more than 40% of deaths in Western countries, and
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6 antiplatelet therapy is a major preventive strategy in this setting.¹ Clopidogrel, a P2Y₁₂
7
8 receptor blocker, inhibits the activation of platelets by adenosine diphosphate (ADP), and is
9
10 widely prescribed for secondary prevention in patients with atherosclerotic diseases. When
11
12 combined with aspirin, clopidogrel is particularly effective in patients with acute coronary
13
14 syndromes (ACS),² and has proved superior to aspirin alone in several other large
15
16 randomised controlled trials. The pharmacodynamic response to clopidogrel shows a wide
17
18 inter-individual variability.^{3,4} Numerous cohort studies, often performed on patients with acute
19
20 coronary syndrome (ACS) and/or undergoing percutaneous coronary interventions (PCI),
21
22 have shown an association between high on-treatment platelet reactivity (PR) and the risk of
23
24 recurrent major adverse cardiovascular events (MACE).⁵⁻⁷ However, recent studies in cohorts
25
26 of stable cardiovascular outpatients^{8,9} or in medically managed ACS patients¹⁰ failed to
27
28 confirm these results. Several randomised trials aimed at reducing the recurrence of
29
30 ischemic events have compared standard clopidogrel treatment to a P2Y₁₂-inhibitor strategy
31
32 tailored according to the presence of high PR. Although initial small trials were promising,^{11,12}
33
34 more recent larger trials showed no benefit from adjusting clopidogrel doses or switching to
35
36 prasugrel based on PR testing in low-risk coronary patients undergoing PCI.^{13,14} These
37
38 contrasting results, both from observational studies and randomised intervention trials, may
39
40 be explained by different patient characteristics including the level of risk, but to date few
41
42 data substantiate these hypotheses. We previously showed, in a study-level meta-analysis,
43
44 that the risk of recurrent MACE associated with high PR was greater in studies using
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46 GpIIb/IIIa inhibitors (a marker of high-risk patients) than in studies which did not.⁷ Another
47
48 meta-regression from a study-level meta-analysis of randomised trials suggested that the
49
50 higher the incidence of coronary stent thrombosis in a given study, the larger the net clinical
51
52 benefit from a PR-tailored strategy.¹⁵ Finally, the ADAPT-DES registry of patients undergoing
53
54 PCI showed that high PR was predictive of stent thrombosis mostly in ACS patients, but
55
56 there was no interaction reported between PR and the presence of an ACS at inclusion.¹⁶
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1 This information suggests the hypothesis that high PR might be more relevant in high-risk
2 populations, but convincing data at the individual level are lacking. To date, the only meta-
3 analysis on individual patient data performed on 6 studies totalling 3,059 patients assessed
4 with the VerifyNow P2Y₁₂ assay did not explore this hypothesis.¹⁷ Similarly, one of the
5 largest and more recent meta-analysis on 8 studies and 4817 patients did not explore this
6 interaction due to the lack of individual data.¹⁸ To further investigate this interaction on a
7 larger population we performed a collaborative meta-analysis of individual patient data and
8 focused on the interaction between relevant vascular risk factors and PR, assessed with
9 ADP induced light transmission aggregometry (LTA), in order to better define the risk of
10 MACE. ADP-induced LTA is the assay upon which all P2Y₁₂ receptor inhibitors have been
11 developed, thus supporting its use in the present meta-analysis. In addition, among several
12 available assays to evaluate PR, LTA is the historical gold standard with which most platelet
13 function assays were compared.
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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 μ M 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

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

27 MACE-free survival curves were derived from individual patient data using the Kaplan-Meier
28 estimator; curves were compared using log-rank tests stratified by study. Associations
29 between conventional risk factors, PR strata, and risk of MACE were analysed using
30 multivariate, mixed-effect Cox models. The amount of heterogeneity was assessed by the
31 size of the random effects (Tau²) which is an estimate of the between study variability.²³ The
32 presence of heterogeneity was tested by comparing models with and without random effects
33 (likelihood ratio test). The interactions between the level of risk and PR strata were tested.
34 MACE-free survival according to PR, as a continuous variable, was assessed using the R
35 package prodlim using the symmetrical nearest neighbourhoods method.²⁴ Sensitivity
36 analyses were conducted to check the robustness of the findings with respect to: the risks of
37 bias and concerns about the applicability of studies; the definition of MACE, including target
38 vessel revascularisation or PCI at inclusion, and; the influence of a given specific study. The
39 net reclassification index (NRI) for survival data²⁵ was computed to quantify the contribution
40 of PR testing for the prediction of the 6-month risk of MACE in patients with increasing
41 numbers of traditional risk factors. The event and non-event continuous NRIs were reported.
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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 reporting²⁶ 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

1 factors was used as a surrogate for the individual risk of MACE. Patients with none of these
2 factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with
3 factors were classified 'low-risk', patients with one factor 'intermediate-risk', and patients with
4 two or more factors 'high-risk' (global p-value <0.0001 for the trend).
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8 9 *MACE and PR*

10 Nine studies (n = 4,438 patients) performed LTA using 20 µM ADP, four studies (n = 2,144
11 patients) used 10 µM ADP, and eight studies (n = 3,317 patients) used 5 µM ADP. Figure 2
12 shows the MACE-free survival curves by category of ADP concentration. Risk of MACE
13 increased significantly with PR with 20 µM ADP, 10 µM ADP, and 5 µM ADP.
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20 With adjustment, high PR was still significantly associated with an increased risk of MACE
21 (Table 3). However, for PR evaluated using 10 µM ADP, risk only increased for the highest
22 PR category, corresponding to LTA values greater than 60%.
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28 *Interaction between risk level and PR for the outcome of MACE*

29 Platelet reactivity assessed with 20 µM ADP

30 Patients with none of the four risk factors showed no significantly increased risk associated
31 with PR, while for patients with one risk factor only, the higher strata of PR was associated
32 with an increased risk of MACE. Patients with two or more risk factors showed an increased
33 risk of MACE for both the medium and higher strata of PR. (Figure 3). In a Cox model, the
34 interaction between PR strata and the risk level was statistically significant (p=0.04). The
35 corresponding hazard ratios (HRs) are shown in Figure 3. Heterogeneity was not detected
36 for the overall interaction (p=0.81), as well as when it was restricted to each risk level
37 category (intermediate versus low risk level, p=0.45, and high versus low risk level, p=0.90).
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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

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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 μ M ADP

A total of only five low-risk patients in four studies performing 10 μ M 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 μ M ADP

The direction of interaction between PR using 5 μ M ADP and the risk level was similar to that observed for PR using 20 μ M 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 μ M ADP than in those using 20 μ M 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

1 for the overall interaction ($p=0.19$). Figure 4C shows that the risk of MACE was unaffected by
2
3 PR in low-risk patients while it increased for PR values above 30% in intermediate-risk
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5 patients and for PR values above 10%–20% in high-risk patients.
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8 9 *Sensitivity analyses*

10 Sensitivity analyses were performed for PR using 20 μ M ADP to assess: the robustness of
11
12 the association between PR and risk of MACE and its interaction with the level of
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14 cardiovascular risk; the robustness of the results in the population of PCI patients and when
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16 target vessel revascularisation is added to the composite outcome. All analyses showed that
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18 the sizes of the effects remained similar, and whilst in some instances the statistical
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20 significance of the interactions could be lost, there was no impact on their magnitudes
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22 (supplemental Tables 1 and 2). Notably, when PR was categorised in quartiles (20 μ M ADP
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24 maximal aggregation quartiles = 0%–38.1%, 38.2%–51.3%, 51.4%–63.0%, 63.1%–100%)
25
26 the interaction between PR and the number of risk factors remained significant ($p=0.01$).
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28 When restricted to the population of 3,564 patients treated with PCI and tested using 20 μ M
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30 ADP the interaction was of similar magnitude but no longer significant (supplemental Table3).
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36 *Publication and availability biases*

37 A check for potential publication bias was made for PR using 20 μ M ADP, on which the main
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39 analyses were performed. A funnel plot was obtained by representing the HR of PR using 20
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41 μ M ADP and the standard error, assessed in each separate study (supplemental Figure 4).
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44 Two studies with a negative association between PR using 20 μ M ADP and the risk of MACE
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46 (with small sample sizes) were detected as missing using the ‘trim and fill’ method for making
47
48 the funnel plot symmetrical. When these missing studies were added, the pooled HR was not
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50 significantly modified. These findings suggested that the publication bias in our meta-analysis
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52 was minor.
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54 Seven qualifying studies could not provide individual patient data. It is of note that in five of
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56 these, the relation between clopidogrel non-response and ischemic events was not a study
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1 objective (pharmacokinetic-pharmacodynamic studies or randomised trials of different
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4 clopidogrel loading doses). The two remaining studies (n = 101 and 111 patients) were
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6 specifically interested in the prognostic value of PR for MACE.
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Confidential For Review Only

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 μ M 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 μ M 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 registry¹⁶ 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 μ M 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 μ M ADP, and a

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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 P2Y₁₂ 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 P2Y₁₂ 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 P2Y₁₂ 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 P2Y₁₂ assay were negative. Indeed, the GRAVITAS¹³ and ARCTIC¹⁴ 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%.

1 Similarly, the ARTIC study population was also at a low absolute risk of subsequent
2 cardiovascular events because the prevalence of ACS patients was low, and the composite
3 endpoint also included other events that may not be related to platelet function. The
4 interaction of PR and the number of risk factors, as identified in the present meta-analysis,
5 substantiates the hypothesis that the risk associated with high PR was not clinically relevant
6 in low-risk patients, and that any measure aiming to lower PR is unlikely to lead to a
7 beneficial reduction of MACE for these low-risk patients. Based on these observations we
8 speculate that higher risk patients are more likely to benefit from a therapy tailored to their
9 initial PR. This may explain why early interventions designed to efficiently blunt high PR in
10 ACS patients with multiple conventional risk factors translated into a reduction of MACE.^{11 12}
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24 In the current new antiplatelet era, prasugrel and ticagrelor have a major part to play in the
25 management of ACS, leaving clopidogrel as an alternative for patients with high bleeding risk.
26 However, a recent cost-effectiveness analysis for six European perspectives showed that the
27 universal use of newer P2Y₁₂ inhibitors for ACS patients is probably not as cost-effective as
28 strategies based on PR.⁴⁴ It should also be kept in mind that ticagrelor and prasugrel
29 increase the risk of bleeding and that a therapeutic medium-PR window is associated with
30 optimal net clinical benefit.²² The net benefits of newer P2Y₁₂ inhibitors could also probably
31 be improved not only by testing for PR, but also by incorporating patient risk levels in the
32 decision-making process. Although ongoing trials on tailored P2Y₁₂ strategies, including
33 TROPICAL-ACS ([ClinicalTrials.gov identifier: NCT01959451](https://clinicaltrials.gov/ct2/show/study/NCT01959451)) and ANTARCTIC⁴⁵ partly
34 include this concept of risk levels, further efforts in this direction are needed.
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46 This meta-analysis has several strengths, such as the good overall quality of the studies
47 included, as assessed using a quality tool specifically adapted to prognostic studies. The
48 availability of individual patient data allowed a reliable evaluation of the risk associated with
49 PR and of the interaction with vascular risk factors. Readily available risk factors relevant to a
50 secondary prevention population were thus identified. The consistency of results across the
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1 different ADP concentrations used in the different studies to assess PR, as well as the
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3 sensitivity analyses, indicated that the results were robust.
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5 Despite the advantages related to the availability of individual patient data, this meta-analysis
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7 also had some limitations, including a low proportion of women (25%). This did not allow a
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9 stratification of the analyses by gender, as is usually the case in risk assessment tools such
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11 the European SCORE or the Framingham risk score. Indeed, in these latter scores gender is
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13 not considered as one of traditional risk factors, but is rather presented in separate charts for
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15 women and men. There were incomplete data on concomitant medications or other relevant
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17 risk factors such as the left ventricular ejection fraction, cholesterol levels or renal
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19 insufficiency. Finally, information on bleeding was limited to five studies and a low number of
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21 events, thus precluding a reliable analysis of bleeding events and their relation to PR.
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24 In conclusion, high PR in patients on clopidogrel is associated with an increased risk of
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26 MACE in patients with vascular risk factors, but not in low-risk patients. These findings
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28 suggest that trials on tailored PR treatment strategies should be primarily stratified on the
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30 individual vascular risk factors in order to assess a truly personalized approach.
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47 development and is available from the PROBAST team (www.systematic-reviews.com/probast).
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53 **Contributors:**

54 Reny JL, Fontana P, and Combescure C are guarantors for the study, had full access to the
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56 data and take responsibility for the integrity of the data and the accuracy of its analysis
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19 All authors had full access to all of the data (including statistical reports and tables) in the
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21 study and can take responsibility for the integrity of the data.
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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 (%)	Hypertension (%)	Hypercholesterolemia (%)	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 diphosphate concentration used for the evaluation of platelet reactivity

* Median (maximum)

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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.

<i>Factors collected in studies</i>	<i>Adjusted HR [95% CI]</i>	<i>p</i>	<i>Level of risk of MACE *</i>	<i>HR [95% CI]</i>	<i>p</i>
<i>Current smoking status</i>	0.92 [0.71;1.18]	0.50	<i>Low risk (n=579)</i>	1	
<i>Age (> 75)</i>	1.56 [1.25;1.95]	<0.0001	<i>Intermediate risk (n=2444)</i>	1.61 [1.05;2.45]	0.03
<i>Diabetes</i>	1.58 [1.27;1.96]	<0.0001	<i>High risk (n=3435)</i>	2.58 [1.69;3.94]	<0.0001
<i>Hypercholesterolemia</i>	0.86 [0.69;1.06]	0.15			
<i>Hypertension</i>	1.23 [0.98;1.54]	0.07			
<i>ACS at inclusion</i>	2.00 [1.27;3.16]	0.003			
<i>Gender (Male)</i>	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 μ M		ADP 10 μ M		ADP 5 μ M	
	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 μ M are 0%-40%, 41%-60%, 61%-100%,

and for ADP 5 μ M 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 20 μ M ADP in addition to risk factors. The predicted risk was stratified in three levels (low: $\leq 3\%$, intermediate: $>3\%$ and $\leq 5\%$, 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 20 μ M ADP			Total
		Low risk ($\leq 3\%$)	Intermediate risk ($>3\%$ and $\leq 5\%$)	High risk ($>5\%$)	
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 μ M 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 μ M ADP LTA, in patients with no risk factors (A), one risk factor (B) and two or more risk factors (C).

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Figure 1

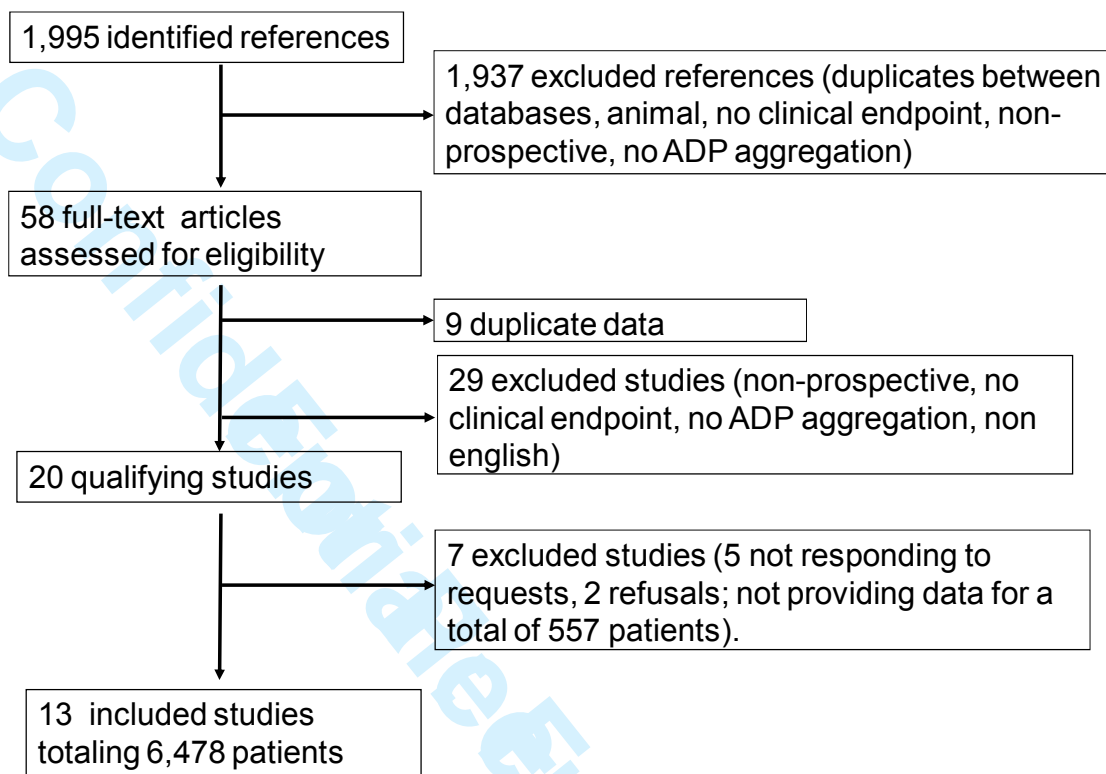
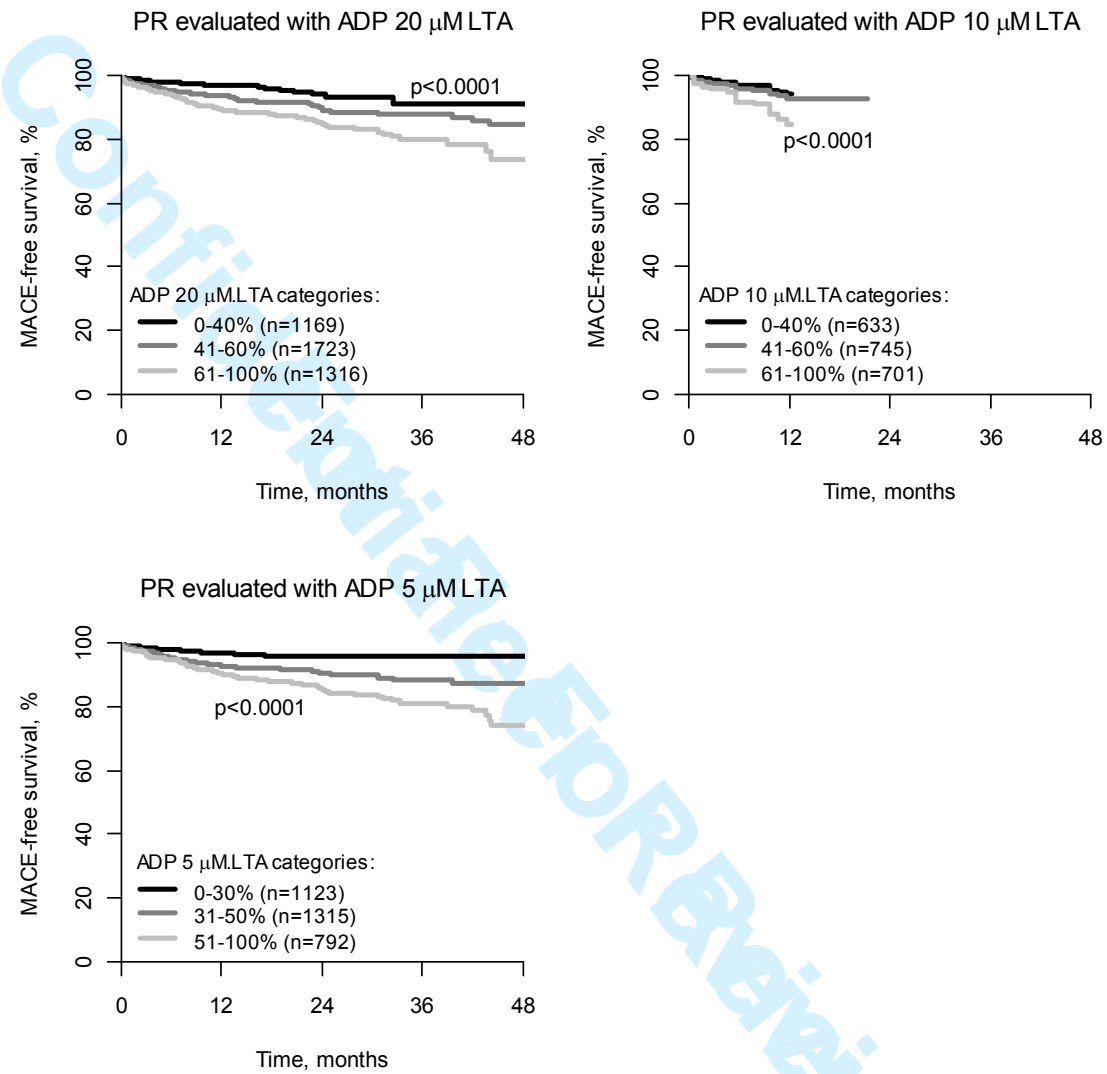


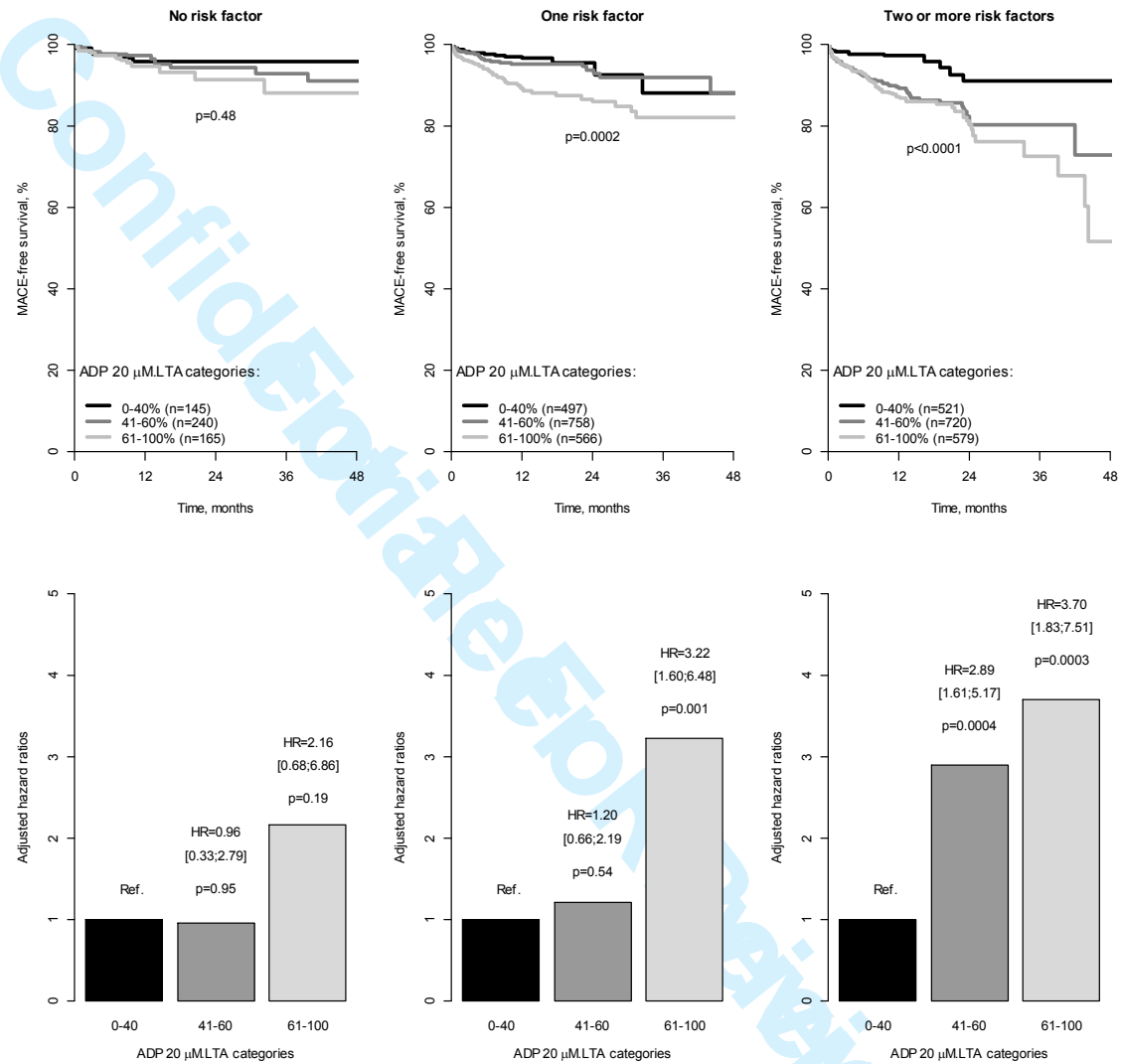
Figure 2



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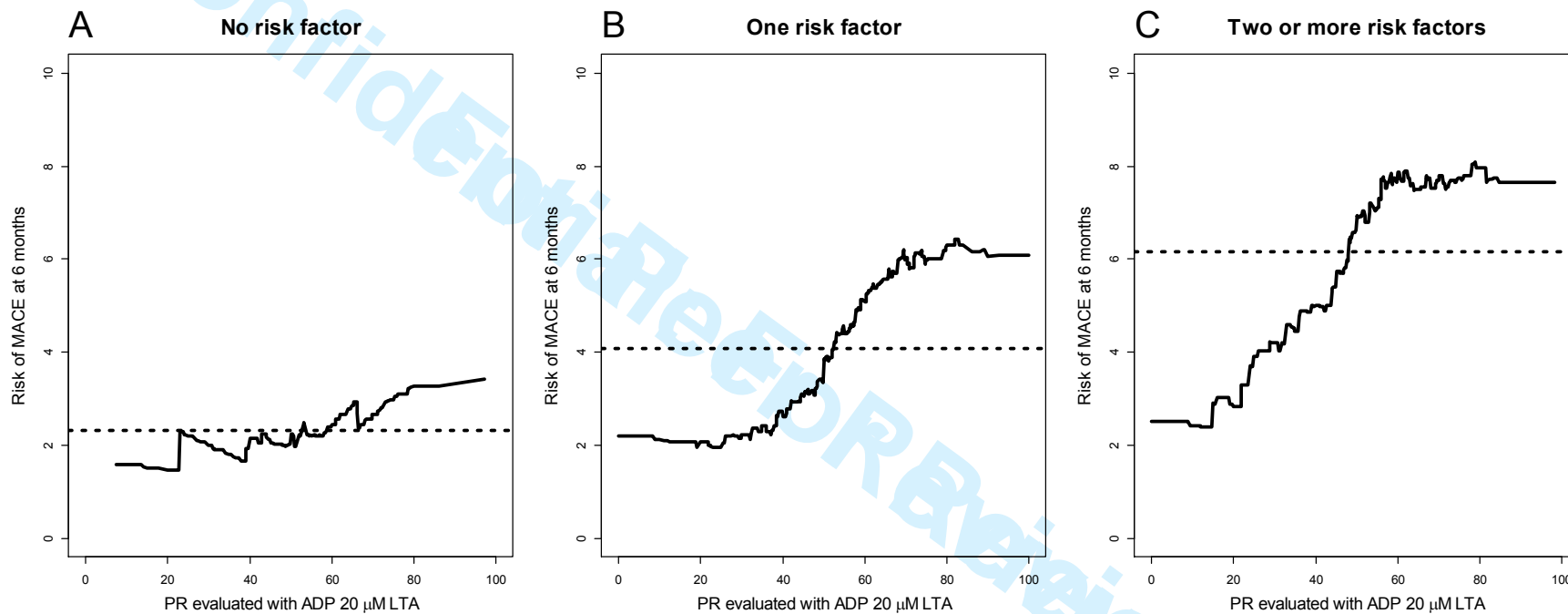
Figure 3



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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

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

Supplemental Figure 1. Criteria to assess risks of bias and concerns about applicability

Supplemental Figure 2. Result of the assessment by domains for risk of bias and concerns about applicability

Cox models and assumption of proportionality of hazards

Complementary results on heterogeneity

Detailed results on studies using 5 μ M ADP to assess platelet reactivity

Supplemental Figure 3. Interaction between 5 μ M ADP LTA values and the level of risk

Sensitivity analyses

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

Supplemental Table 2. Sensitivity analysis for the modification of the association between PR, assessed using 20 μ M ADP and risk of MACE

Supplemental Table 3. Sensitivity analysis in patients with PCI at inclusion and when target vessel revascularisation (TVR) is included in the composite outcome

Supplemental Figure 4. Funnel plot for detection of a potential publication bias

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 method²⁴. 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

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

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

25 26 **Complementary characteristics of studies, risks of bias, and concerns regarding** 27 **applicability**

28 In several instances, data on covariates were not available. For example, data on the use of
29 proton pump inhibitors and statins were not available from eight studies. Aspirin was part of
30 the treatment for all patients in ten studies, and was given to 79%, 90%, and 95% of the
31 patients in the three remaining studies. All but one study included coronary artery disease
32 (CAD) patients exclusively; this last study⁸ included 85% CAD patients, 10% peripheral
33 arterial disease patients, and 5% ischemic stroke patients. PCI was performed on 87% of
34 patients. The studies differed markedly with respect to the frequency of diabetes (19%–
35 100%), smoking (11%–55%), the use of GpIIb/IIIa inhibitors (0%–100%), the ADP
36 concentration used for aggregation tests (5–20 μM) to assess PR, and the presence of acute
37 ischemia at inclusion (0%–100%). The median follow-up was 12 months, and in all the
38 studies the modalities of the follow-up were identical for clopidogrel responders and non-
39 responders. LTA was most frequently performed using ADP 20 μM .

40 Overall risks of bias and concerns about applicability of the studies were low (Figures 1 and
41 2). In one study using LTA with ADP 10 μM , MACE was defined as stent thrombosis during a
42 30-day follow-up period;³³ however, with no specific information on myocardial infarction or
43 stroke (as was the case in most of the studies), this lead to a potential high risk of outcome
44 bias. In three studies,^{27 36 38} the risk of outcome bias was unclear because either they did not
45 include stroke in the composite outcome of MACE or they did not mention whether
46 adjudication of the outcome was performed blinded to PR test results. In another study, using
47 LTA using ADP 20 μM , the risk of bias with respect to flow and timing was unclear as 13% of
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3 patients had been lost to follow-up.³¹ The characteristics, PR test results, and treatments of
4 these patients were similar to the rest of the cohort. Regarding their applicability, only two
5 studies caused concern, because of an exclusively diabetic population in one,²⁹ and because
6 of the absence of upper and lower limits to the platelet counts of the patients included in the
7 second.³⁸
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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

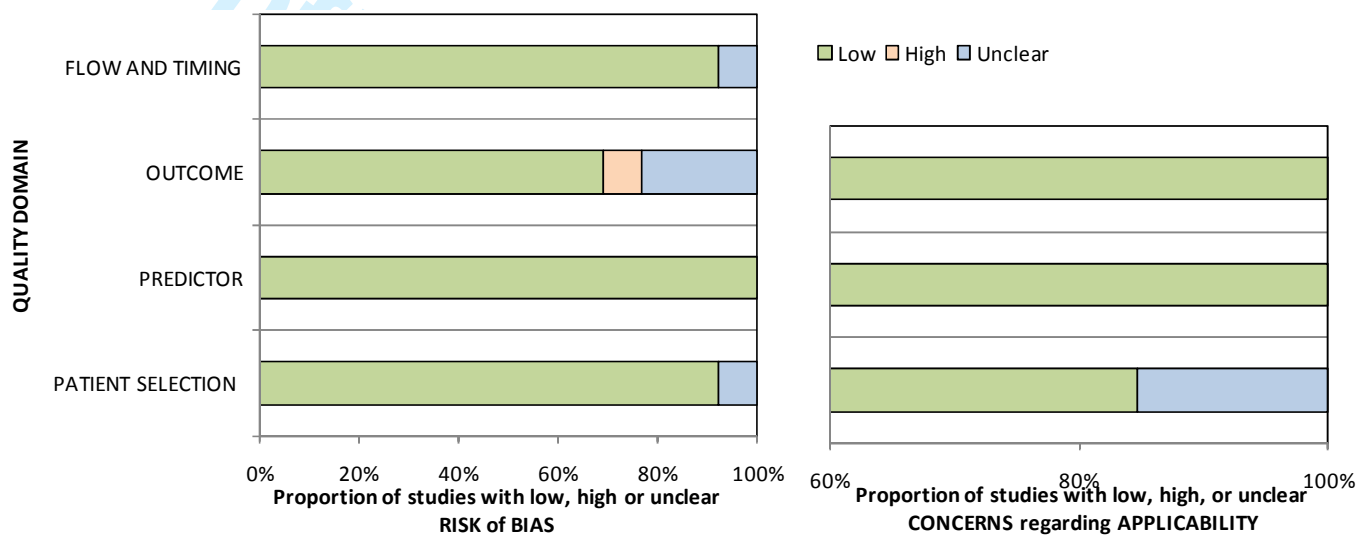
Concerns about applicability

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 effects²³ corresponding to the between-study variance (Tau^2). These variances are reported in the following table for the mixed-effects Cox model when PR is evaluated using ADP 20 μM .

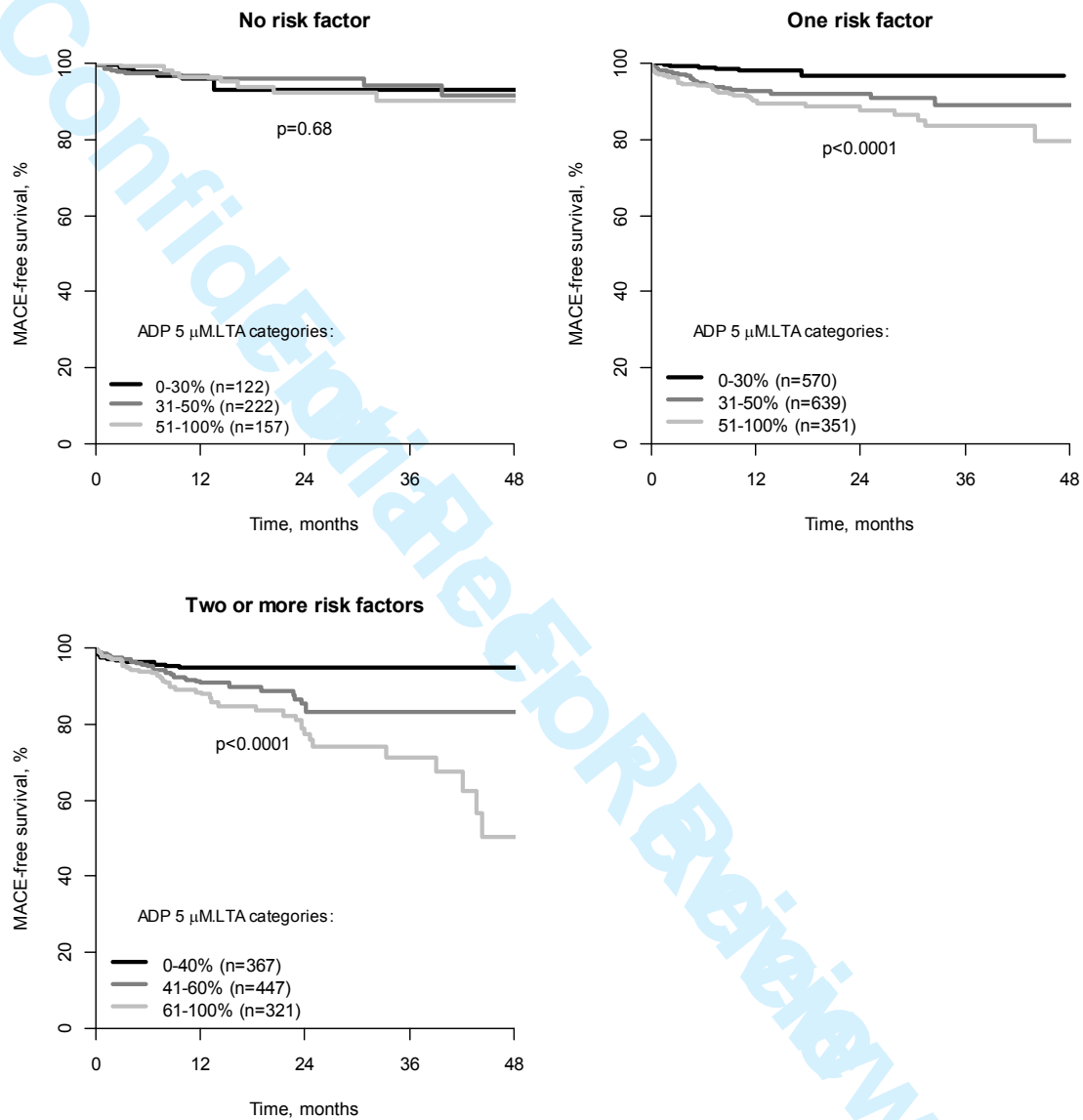
Random effects	Tau^2
<i>Intercept</i>	0.112
<i>PR categories</i>	
41%–60%	0.000
61%–100%	0.324
<i>Interaction terms</i>	
ADP category 2 * Intermediate risk level	0.134
ADP category 3 * Intermediate risk level	0.086
ADP category 2 * High risk level	0.043
ADP category 3 * High risk level	0.002

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

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3 [2.29;10.10] and $p < 0.0001$ for ADP 5 μM 51%–100%), while in high risk patients (two or
4 more risk factors), the direction of the effect was the same but only significant for the high PR
5 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]
6 and $p = 0.002$ for ADP 5 μM 51%–100%). Figure 4C, in the main text, describes the influence
7 of PR, analysed as a continuous variable, on the two-year risk of MACE for the different
8 patient risk levels. In low-risk patients, the MACE-free survival fluctuates between 90% and
9 95% with no pattern of decreased survival with higher PR. Intermediate risk patients have a
10 reduced MACE-free survival corresponding to an increased risk of MACE for PR values
11 above 30%, while the risk of MACE in high-risk patients increases earlier (for PR values
12 above 20%).
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Supplemental Figure 3. Interaction between 5 μ M 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 μ M. A leave-one-out approach was applied to check the robustness of the association between PR using ADP 20 μ M and risk of MACE. Whichever study was removed, the association remained significant. The HR for the intermediate category of maximal aggregation using ADP 20 μ M (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 (Table 1).

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 μ M, 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 μ M 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 already present in patients with one risk factor compared to those with none ($\text{HR}=1.61$ [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 20 μM ADP, 10 μM ADP and 5 μM ADP (after exclusion of patients with missing data for PR).

	20 μM ADP	10 μM ADP	5 μM 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 according to PR within each risk level.

<i>Risk level</i>	20μM ADP				
	0 - 40	41 - 60		61 - 100	
	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 μ M ADP and risk of MACE (leave-one-out procedure)

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

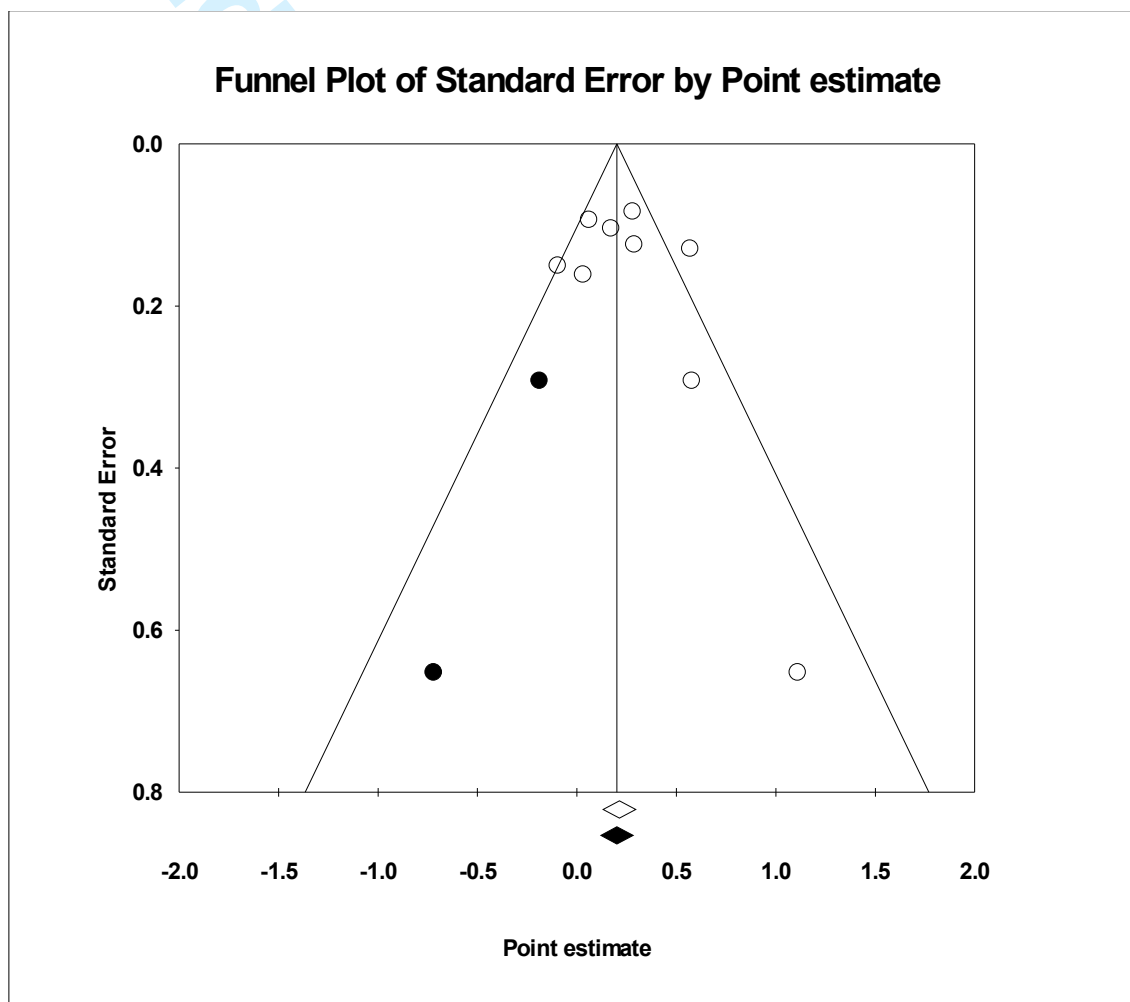
	Reny study removed		Geisler study removed		Campo study removed		Geisler & Campo studies removed		Angiolillo study removed	
	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p	HR [95% CI]	p
Interaction with Nb Risk factors										
No risk factors										
ADP 20 0%-40%	Ref		Ref		Ref		Ref		Ref	
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 μ M 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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