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Bleeding and stent thrombosis on $P2Y_{12}$-inhibitors: collaborative analysis on the role of platelet reactivity for risk stratification after percutaneous coronary intervention

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Aims Although platelet reactivity during $P2Y_{12}$-inhibitors is associated with stent thrombosis (ST) and bleeding, standardized and clinically validated thresholds for accurate risk stratification after percutaneous coronary intervention (PCI) are lacking. We sought to determine the prognostic value of low platelet reactivity (LPR), optimal platelet reactivity (OPR), or high platelet reactivity (HPR) by applying uniform cut-off values for standardized devices.

Methods and results Authors of studies published before January 2015, reporting associations between platelet reactivity, ST, and major bleeding were contacted for a collaborative analysis using consensus-defined, uniform cut-offs for standardized platelet function assays. Based on best available evidence for each device (exploratory studies), LPR–OPR–HPR categories were defined as <95, 95–208, and >208 PRU for VerifyNow, <19, 19–46, and >46 U for the Multiplate analyser and <16, 16–50, and >50% for VASP assay. Seventeen studies including 20 839 patients were used for the analysis; 97% were treated with clopidogrel and 3% with prasugrel. Patients with HPR had significantly higher risk for ST [risk ratio (RR) and 95% CI: 2.73 (2.03–3.69), $P < 0.00001$], yet a slight reduction in bleeding [RR: 0.84 (0.71–0.99), $P = 0.04$] compared with those with OPR. In contrast, patients with LPR had a higher risk for bleeding [RR: 1.74 (1.47–2.06), $P < 0.00001$], without any further benefit in ST [RR: 1.06 (0.68–1.65), $P = 0.78$] in contrast to OPR. Mortality was significantly higher in patients with HPR compared with other categories ($P < 0.05$). Validation cohorts ($n = 14$) confirmed all results of exploratory studies ($n = 3$).

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Conclusions Platelet reactivity assessment during thienopyridine-type $P2Y_{12}$-inhibitors identifies PCI-treated patients at higher risk for mortality and ST (HPR) or at an elevated risk for bleeding (LPR).

Keywords $P2Y_{12}$-inhibitors • Platelet reactivity • Stent thrombosis, Bleeding

Introduction

Dual antiplatelet treatment (DAPT) consisting of aspirin and a $P2Y_{12}$-inhibitor is recommended in patients undergoing percutaneous coronary interventions (PCIs) to prevent thrombotic complications.[1] However, adjunctive administration of all currently available $P2Y_{12}$-inhibitors has been associated with an increased risk for bleeding.[2–4] Since both ischaemic and bleeding events are important correlates of overall patient survival, attempts minimizing both complications in PCI-treated patients are highly warranted.[5] Monitoring platelet reactivity during $P2Y_{12}$-inhibitors was hoped to help prevent bleeding and/or stent thrombosis (ST) as numerous prior studies have linked high platelet reactivity (HPR) to a greater risk for ischaemic complications, while low platelet reactivity (LPR) has been associated with greater bleeding events.[6,7] However, the published cut-offs for HPR and LPR are highly heterogeneous, usually non-validated outside of the exploratory studies, leading to controversies on the prognostic relevance of platelet function testing in patients undergoing PCI. Such methodical uncertainties might be—in part—the reasons for failures of two randomized studies[8,9] evaluating the impact of platelet function testing guidance of antiplatelet therapy.

In the setting of a collaborative analysis, we sought to analyse the data from a large number of published studies to determine the prognostic impact of platelet reactivity, classified as low (LPR), optimal (OPR), or high (HPR) by applying standard cut-off criteria in patients treated with $P2Y_{12}$-inhibitors.

Methods

Study selection and literature search

For this collaborative analysis, we identified published studies reporting the rates of major (or clinically relevant) bleeding, mortality, and ST according to different levels of platelet reactivity in PCI-treated patients. In line with the recommendations of two prior consensus papers, only studies using standardized platelet function assays, such as the VerifyNow P2Y12 assay, the Multiplate analyser with ADP test, or the VASP assay, were included.[6,7] We conducted a PubMed database search for published articles until January 2015 using the following pre-defined search terms alone or in combination: platelet reactivity, clopidogrel, ticagrelor, prasugrel, VerifyNow, Multiplate, VASP, ST, and bleeding. Abstracts from major scientific meetings and reference lists of published reviews were also checked to identify relevant studies. Authors of selected studies were contacted for collaboration, and after a positive response, original data were provided by responsible authors for all analyses (*Figure 1*).

Eligibility criteria and data extraction

We restricted our analysis to studies that met all of the following inclusion criteria: (i) patients with stable or acute coronary artery disease undergoing PCI with an assessment of platelet reactivity during or in close proximity ($\leq$30 days) to the performed intervention; (ii) patients receiving aspirin and a $P2Y_{12}$-receptor inhibitor for PCI; (iii) assessment of platelet function with the VerifyNow, Multiplate analyser, or VASP assay; and (iv) reporting clinical outcomes in relation to platelet reactivity findings including ST, major or clinically relevant bleeding, and mortality. Exclusion criteria included application of less widely available or non-standardized assays for platelet function testing (e.g. light transmission aggregometry), studies using other types of antiplatelet agents (e.g. cilostazol, vorapaxar). In addition, studies primarily conducted in non-Western patients were also excluded due to the hypothesized differences in the pharmacodynamic response to $P2Y_{12}$-inhibitors across races, also referred to as the 'East-Asian paradox'.[10] The authors agreed that the cut-offs identified by exploratory studies (defined below) might not be applicable to the non-Western population given the low number of such ancestry in these cohorts. In studies where parallel results with different platelet function assays were presented in the same cohort, the assay with the largest numbers of tested individuals was used for the analysis to prevent over-representation of the studies in the analysis.

Platelet reactivity assessment and cut-off values

To test the prognostic relevance of platelet reactivity classified as low (LPR), optimal (OPR), or high (HPR), we used the best available evidence to identify cut-off values for the included platelet function assays. The chosen cut-off values were in line with recent recommendations of two expert opinion papers,[6,7] except for the LPR cut-off of the VerifyNow assay which was based on results of the large ADAPT-DES registry that was not available at the time of the consensus papers.[11] Therefore, the selected cut-off values for LPR, OPR, and HPR categories were $<$95, 95–208, and $>$208 PRU for VerifyNow,[11,12] $<$19, 19–46, and $>$46 U for the Multiplate analyser,[13,14] and $<$16, 16–50, and $>$50% for VASP[15] assays, respectively.

Clinical endpoint definitions and subgroups

Definite or probable ST was defined according to the Academic Research Consortium (ARC) criteria. Clinically relevant major bleeding complications were recorded with the definition used in each study. Accepted bleeding scales included TIMI major, BARC type $\geq$ 2, and ADAPT-defined clinically relevant bleeding. When rates of major bleeding were not available, the combined rate of major and minor events was used in the analysis. All-cause mortality was used if available; otherwise cardiovascular mortality was substituted. Outcomes were analysed for the longest follow-up period available within each study. Pre-specified subgroup analyses were planned for different platelet function assays, ACS vs. non-ACS patients, prasugrel/ticagrelor vs. clopidogrel treatment, and exploratory vs. validation trials.

Statistical analysis

For this collaborative analysis, responsible authors were contacted individually to provide the rate of ST, bleeding, and mortality according to the standardized cut-off values for LPR, OPR, and HPR groups in their specific cohorts. Using the obtained dataset, we performed a

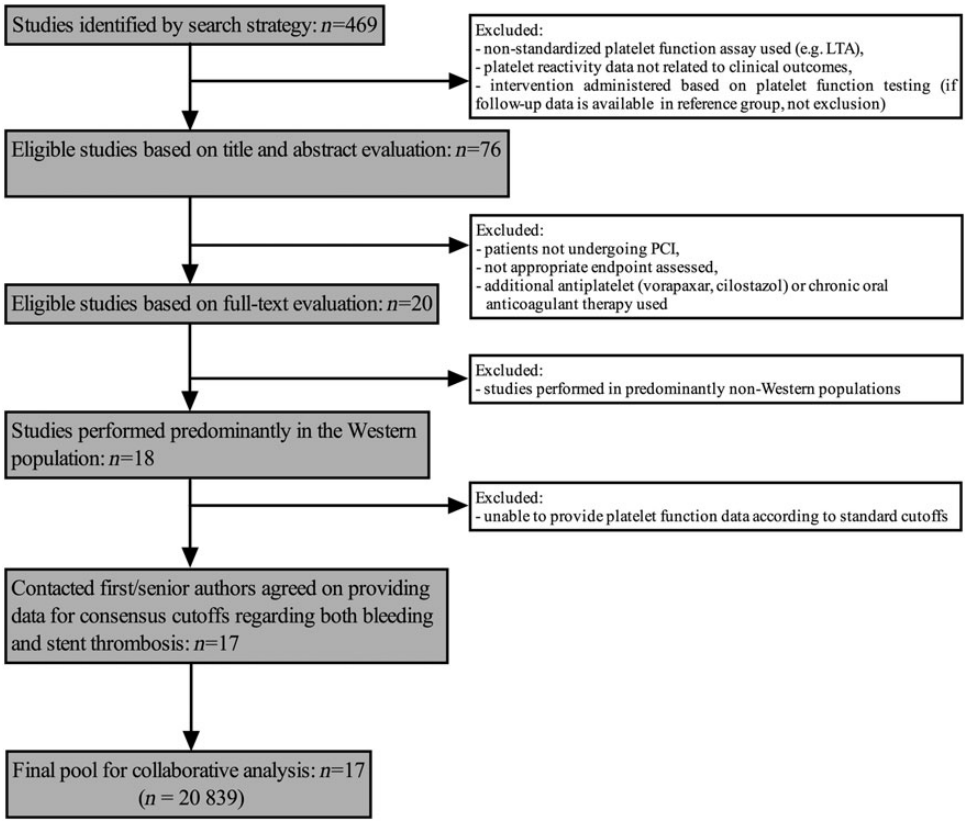

Figure 1 Flowchart of study selection. LTA, light transmission aggregometry.

weighted fixed-effect meta-analysis using the Mantel–Haenszel method to compare the relative risk (risk ratio, RR) of outcome events in the HPR and LPR groups in contrast to patients with OPR, used as reference. Sensitivity analyses were performed in all outcomes with random-effect modelling. When risks of bleeding and ST were compared according to platelet reactivity categories in the same plot, absolute risk estimates were preferred rather than RR to demonstrate the clinical relevance of the trade-off between ST and bleeding. Therefore, fixed-effect Mantel–Haenszel weighted risk differences (RDs) were calculated with the OPR group as reference. To further corroborate our statistical findings, unweighted analyses were performed to obtain RR from pooled crude event rates. Consistency of the obtained results was analysed in pre-defined subgroups by interaction testing. A two-sided *P*-value of <0.05 was considered statistically significant in all comparisons. Statistical analyses were performed with Review Manager (RevMan) computer program version 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011.) and the Comprehensive Meta-analysis software version 2 (Biostat, Englewood, NJ, USA).

Results

Study cohorts

Overall, 17 studies[9,12,13,15–28] with 20 839 patients were identified and included in the analysis (*Figure 1*). Median follow-up time was 8.5 months (minimum–maximum: 1–17). Baseline characteristics and clinical results of the included studies are summarized in *Table 1*. All studies reported definite/probable ST according to the ARC criteria. Thirteen of the 17 studies reported bleeding events according to the TIMI scale, two studies[22,28] used the BARC definition, one study reported moderate/several events on the GUSTO scale,[9] and one study used an own bleeding definition (ADAPT-DES)[12] for clinically relevant bleeding (*Table 1*). In total, data were available for 13 377 patients with the VerifyNow device, for 3908 patients with the Multiplate analyser, and for 3554 patients with the VASP assay. The vast majority of patients (97%) was treated with clopidogrel and only 3% received prasugrel. No eligible study was identified in patients on ticagrelor.

Outcome data

By applying standard cut-off values, 41% ($n = 8554$) of the patients had HPR, 20% ($n = 4073$) LPR, and 39% ($n = 8212$) OPR. Patients with HPR demonstrated a significantly higher risk for ST compared with those with OPR (2.73, 95% CI: 2.03–3.69, $P < 0.00001$, *Figure 2*, Supplementary material online, *Figure S1*), while the risk of ST did not further differ between patients with LPR and OPR (RR: 1.06, 95% CI: 0.68–1.65, $P = 0.78$, *Figure 2*, Supplementary material online, *Figure S2*) Unweighted analyses were consistent with these findings (*Figure 2*). Regarding bleeding, patients with HPR showed a slight decrease compared with those with OPR (RR: 0.84, 95% CI: 0.71–0.99, $P = 0.04$), but patients with LPR had a significant, 1.7-fold higher risk in comparison to those with OPR (RR: 1.74,

Table 1 Baseline characteristics of the 17 studies included in the collaborative analysis

First author	Acronym	Year	*n*	Expl study	Device	$P2Y_{12}$-inhibitor	Definition of bleeding	HPR (%)	LPR (%)	Age (mean)	Female gender (%)	DM (%)	ACS (%)	DES (%)	Median length of follow-up (months)
Bonello[15]	–	2012	301	Yes	VASP	Prasugrel	TIMI major	25.2	27.9	68	11	23	100	53	12
Breet[19]	POPular	2010	1052	No	VerifyNow	Clopidogrel	TIMI major	53.3	7.8	64	25	18	0	64	12
Campo[20]	–	2011	300	No	VerifyNow	Clopidogrel	TIMI major + minor	20.7	27.0	66	23	24	61	71	17
Cuisset[22]	POBA	2013	1542	No	VASP	Clopidogrel, prasugrel	BARC type ≥ 2	30.0	8.5	64	20	30	100	58	6
Freynhofer[17]	WILMAA	2011	300	No	VASP	Clopidogrel	TIMI major	75.0	3.3	62	32	27	64	65	7
Mangiacapra[23]	ARMYDA-PROVE	2012	732	No	VerifyNow	Clopidogrel	TIMI major	48.1	7.1	66	27	30	0	27	1
Marcucci[27]	–	2009	683	No	VerifyNow	Clopidogrel	TIMI major	45.1	15.8	69	24	26	100	18	12
Morel[24]	–	2011	433	No	VASP	Clopidogrel	TIMI major	6.9	57.3	65	25	37	76	45	9
Patti[26]	ARMYDA-PRO	2008	160	No	VerifyNow	Clopidogrel	TIMI major	59.4	4.4	66	19	34	54	26	1
Patti[25]	ARMYDA-BLEEDING	2011	310	No	VerifyNow	Clopidogrel	TIMI major	59.4	4.2	67	22	37	32	25	1
Palmerini[28]	GEPRESS	2014	978	No	VASP	Clopidogrel	BARC type ≥2	48.9	7.7	67	24	27	100	59	12
Price[9]	GRAVITAS	2011	1692[a]	No	VerifyNow	Clopidogrel	GUSTO mod/severe	70.0	8.0	63	30	41	15	100	5.7
Sibbing[13]	ISAR	2010	2533	Yes	Multiplate	Clopidogrel	TIMI major	16.9	38.5	68	24	29	12	100	1
Sibbing[21]	ISAR-REACT 4	2012	564	No	Multiplate	Clopidogrel	TIMI major	36.3	27.0	68	22	31	100	100	1
Siller-Matula[18]	MADONNA	2012	395[a]	No	Multiplate	Clopidogrel	TIMI major	36.2	28.4	64	24	34	37	91	1
Siller-Matula[16]	PEGASUS PCI	2012	416	No	Multiplate	Clopidogrel	TIMI major	36.3	28.6	64	24	32	34	99	12
Stone[12]	ADAPT-DES	2013	8,448	Yes	VerifyNow	Clopidogrel	ADAPT-defined	42.7	20.0	64	26	32	52	100	12

ACS, acute coronary syndrome; DES, drug-eluting stent; DM: diabetes, Expl, exploratory studies that formed the basis of the standardized cut-off definitions; HPR, high platelet reactivity; LPR, low platelet reactivity.
[a]Only patients on 75 mg clopidogrel without adjustment included from these studies.

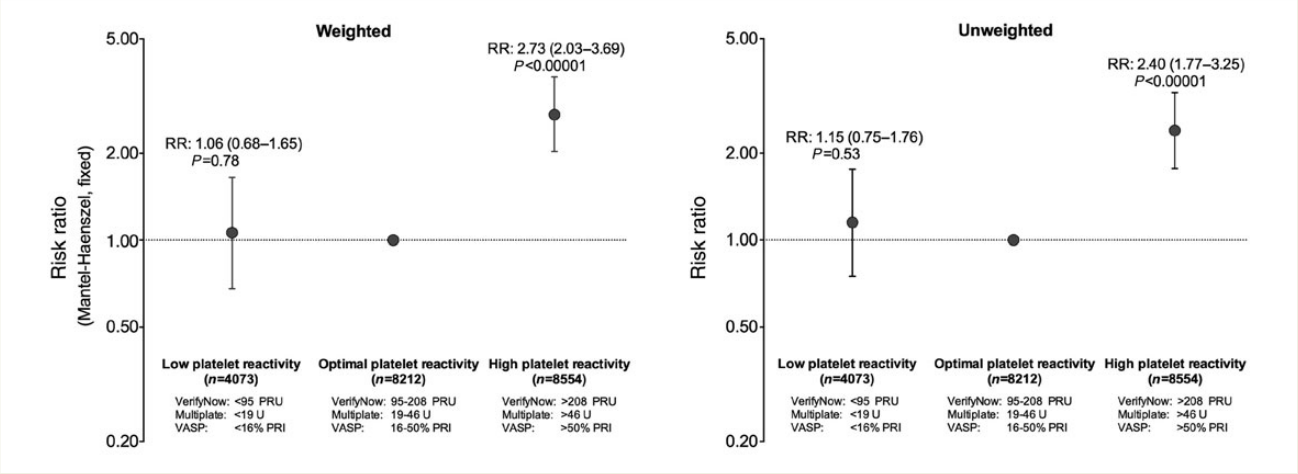

Figure 2 Relative risk of stent thrombosis according to platelet reactivity levels. RR, risk ratio.

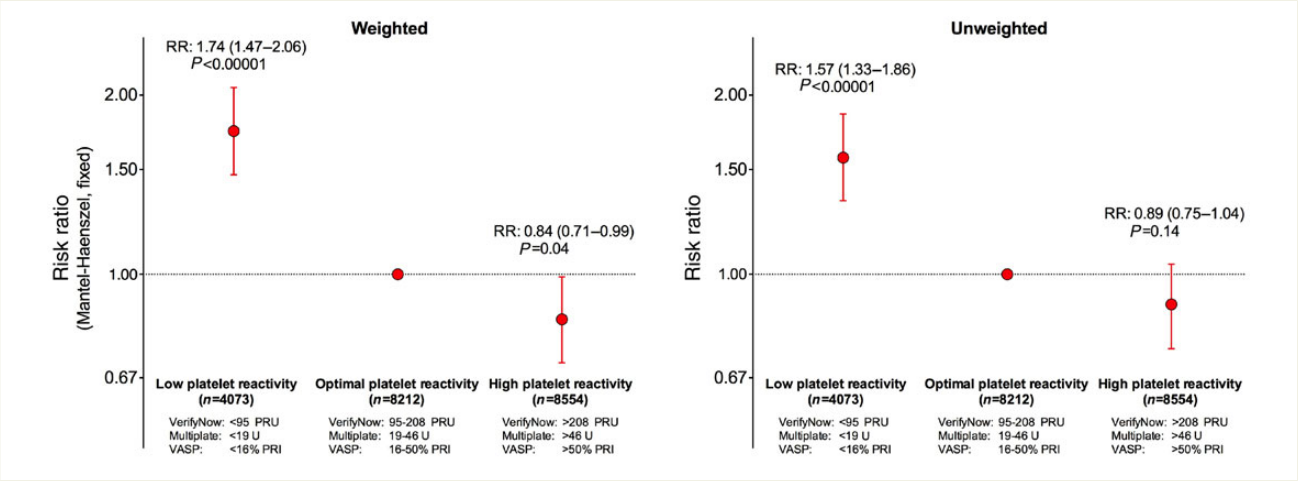

Figure 3 Relative risk of bleeding events according to platelet reactivity levels. RR, risk ratio.

95% CI: 1.47–2.06, $P < 0.00001$, *Figure 3*, Supplementary material online, *Figures S3 and S4*) Unweighted analyses confirmed the higher risk of bleeding in the LPR group; however, the risk of bleeding did not differ between patients with HPR and OPR (*Figure 3*). In case of mortality, patients with HPR had a significantly higher risk compared with patients with OPR (HR: 1.54, 95% CI: 1.22–1.94, $P = 0.0002$) or LPR (HR: 1.45, 95% CI: 1.04–2.02, $P = 0.03$, *Figure 4*, Supplementary material online, *Figures S5 and S6*).

Outcome data: rationale for the 'optimal' range of platelet reactivity

Since bleeding events were 3.4-fold more frequent than ST (total: 797 vs. 236 events) during the mean follow-up of 8.5 months, absolute RDs were used to better capture the trade-offs in bleeding and ST between various platelet reactivity groups (*Figure 5*). When platelet reactivity levels were grouped only as low or high, the results suggested that a significant reduction in ST in the non-HPR group may only be achieved at the price of a large increase in bleeding, and *vice versa*, lower risk for bleeding in the non-LPR group was associated with an excess risk of ST (*Figure 5A*). However, when an 'optimal' range of platelet reactivity (OPR) was introduced and used as a reference, a large reduction in bleeding was observed in this group without any excess in ST compared with LPR (*Figure 5B*). Similarly, the OPR group had a significantly lower risk for ST, with only a slight absolute increase in bleeding compared with patients with HPR (*Figure 5B*).

Subgroup and sensitivity analyses

Based on interaction analyses, the impact of HPR on ST was consistent in all subgroups (*Figure 6*). The predicted risk of bleeding was also directionally similar for LPR in the tested subgroups; however, significant interactions for even stronger associations were observed in some subgroups (*Figure 6*). Random-effects modelling demonstrated similar results to fixed-effect analyses for all outcomes (Supplementary material online, *Table S1*).

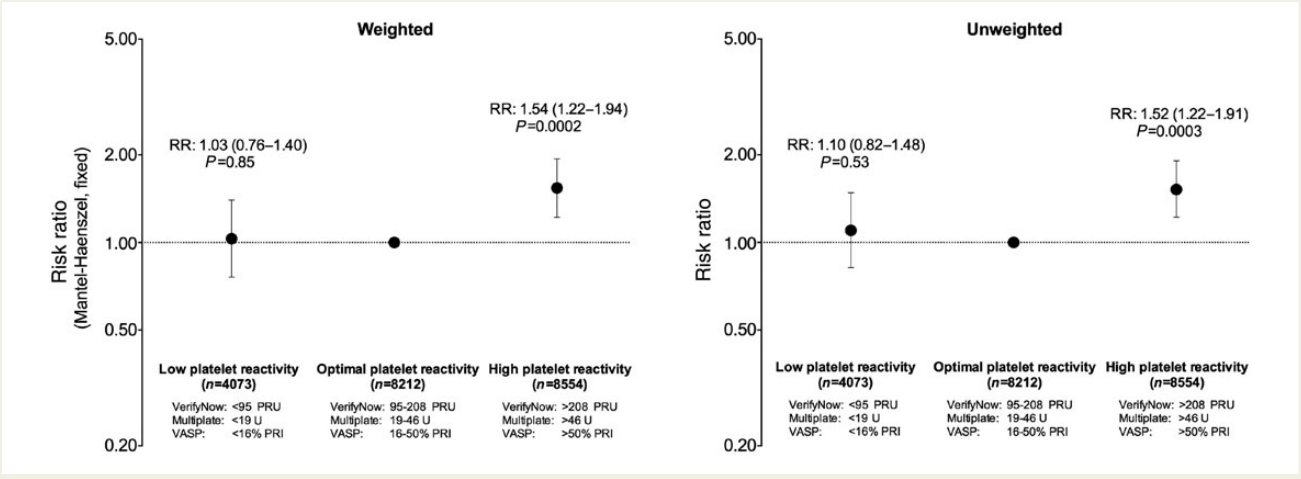

Figure 4 Relative risk of mortality according to platelet reactivity levels. RR, risk ratio.

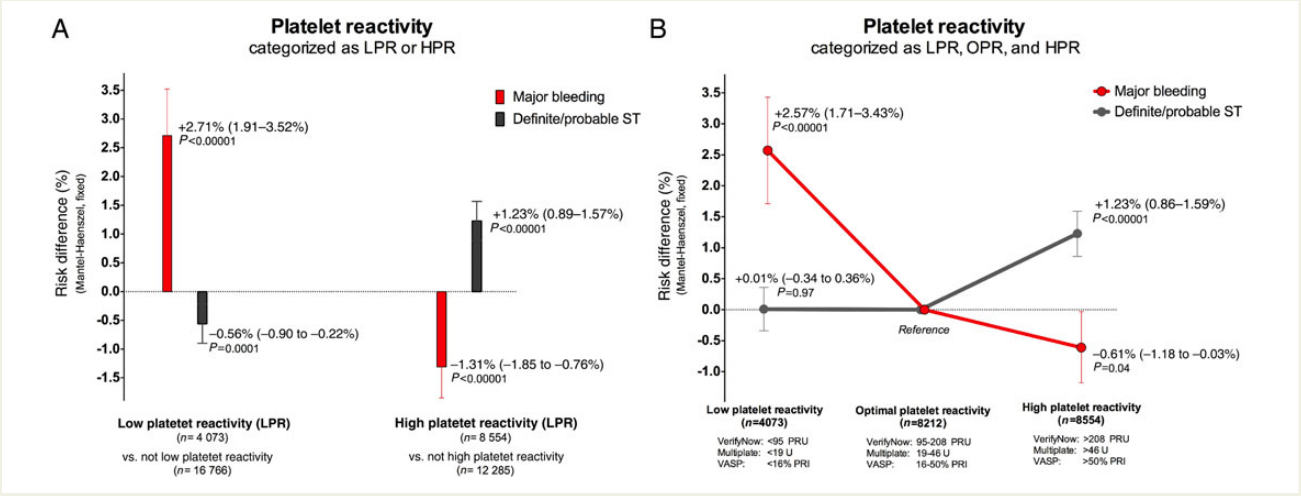

Figure 5 Absolute risk of stent thrombosis and bleeding according to platelet reactivity levels. (*A*) Risk estimates when platelet reactivity is categorized into groups of low platelet reactivity or high platelet reactivity. (*B*) A comparison of platelet reactivity categorized as low, optimal, or high. ST, stent thrombosis.

Discussion

This collaborative analysis represents the first attempt towards clinical validation of standardized cut-off points for platelet function testing in a large sample of patients undergoing PCI. Main results can be summarized as follows:

(i) Thienopyridine-treated patients with HPR have a 2.7-fold higher risk for ST and a 1.5-fold higher risk for mortality compared with those with OPR following PCI.
(ii) Patients with LPR show a 1.7-fold higher risk for major bleeding complications without any further reduction in the risk of ST compared to patients with OPR.
(iii) These results suggest the existence of an optimal range of $P2Y_{12}$-inhibition (OPR) that can be considered as a therapeutic window, within which the predicted risk of ST and major bleeding is the lowest after PCI.

Finding the balance between efficacy and safety for patients treated with $P2Y_{12}$-inhibitors is a key aspect to improve prognosis in patients after PCI. Despite demonstrated reductions in ischaemic complications (including ST) with currently available $P2Y_{12}$-inhibitors among patients undergoing PCI, the price to pay has always been a higher risk for bleeding.[2–4] Prior analyses have confirmed that the higher the level of $P2Y_{12}$-inhibition, the lower the rate of thrombotic events[29]; however, an inverse association exists for bleeding.[3,4] Moreover, active metabolite generation and platelet reactivity inhibition of available thienopyridine-type $P2Y_{12}$-inhibitors are highly variable between patients, affected by common genetic variants and clinical factors that further complicate accurate risk assessment and

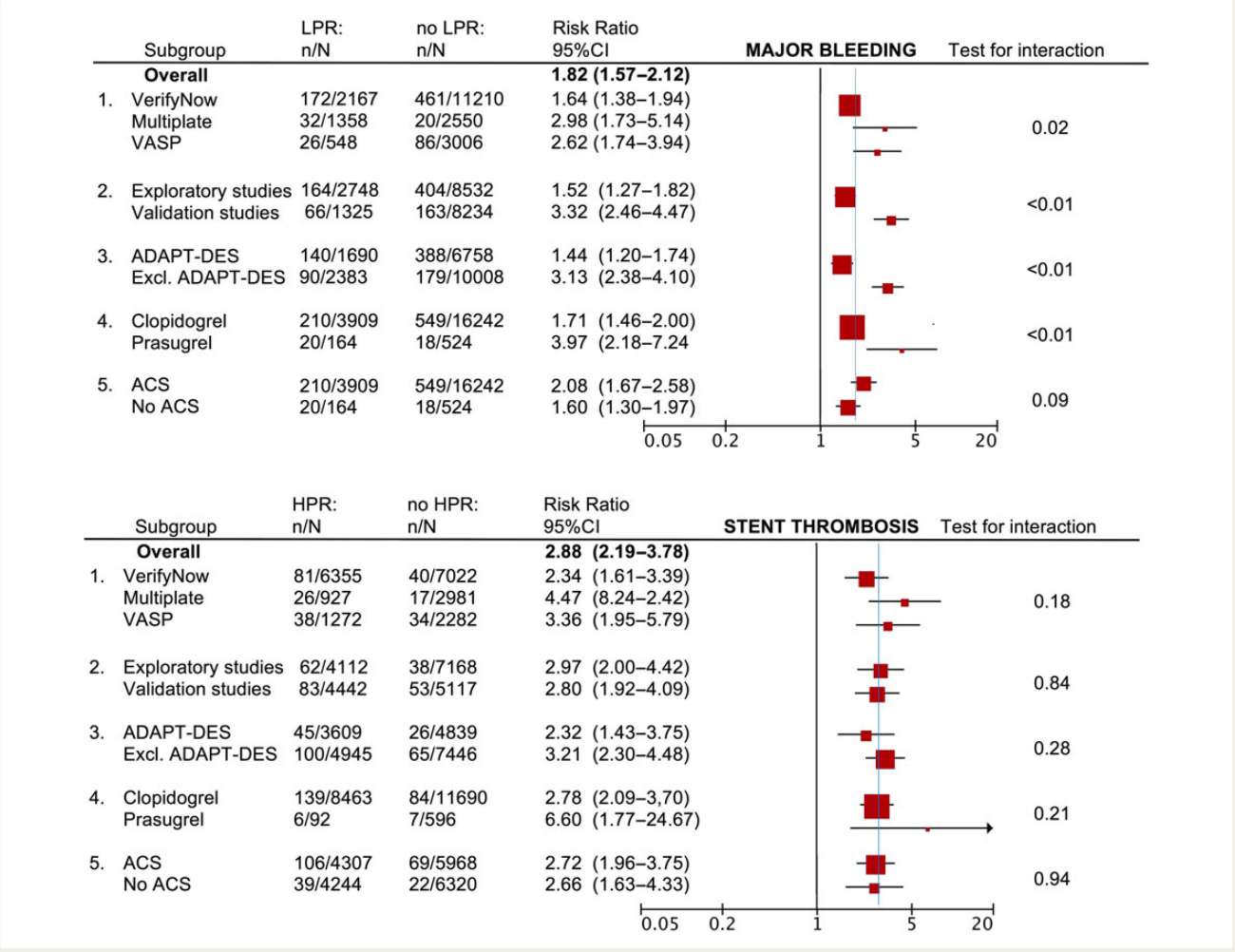

Figure 6 Interaction analysis according to pre-defined subgroups. ACS, acute coronary syndrome; HPR, high platelet reactivity; LPR, low platelet reactivity.

selection of the optimal $P2Y_{12}$-inihbitor for the individual.[7] Risk stratification by clinical scores is often flawed by the fact that most of the used risk markers for thrombotic complications are also predictors of bleeding events (such as age, hypertension, or renal failure). Therefore, clinical scores might help to predict bleeding and thrombosis, but have limited usefulness in balancing such complications in clinical practice because they do not well discriminate between the two unwanted outcomes. With this respect, measuring residual platelet reactivity during $P2Y_{12}$-inhibitor treatment was suggested as a valuable option to help stratifying patients according to bleeding and thrombosis, as several observational studies have linked HPR to higher risk for ST and LPR to increased risk for bleeding.[12,14,15]

Despite these promising observations, major drawbacks towards recommending platelet function testing for risk assessment after PCI were the large methodical heterogeneity in assessing on-treatment platelet reactivity and lack of generally applicable cut-off values to define patients with HPR and LPR. In addition, it was also unclear how the two extreme platelet reactivity categories are related to an intermediate range of platelet inhibition, hypothesized as 'optimal' (OPR).[14] In spite of these limitations, two recent expert consensus papers[6,7] proposed specific cut-off values to define HPR and LPR, acknowledging; however, the preliminary nature of these values and lack of proper validation in sufficient studies.

Therefore, the present collaborative analysis represents an important step forward in defining standard cut-off points for selected platelet function assays and validating them in a large population of patients undergoing PCI. Based on our results, the proposed and herein tested cut-off values were highly significantly ($P < 0.00001$) associated with ST (46 U for Multiplate, 208 PRU for VerifyNow, and 50% PRI for VASP) and bleeding (19 U for Multiplate, 95 PRU for VerifyNow, and 16% for VASP). According to the observed pattern of risk for bleeding and ST, patients in the OPR range had the lowest net rates of adverse events, enabling a unique and sharp discrimination of bleeding and ischaemia by a single biomarker assessment (*Figure 5B*). Interestingly, the more traditional view of platelet reactivity, categorizing it as only low (LPR) or high (HPR), suggested that the risk of bleeding and ST is complementary; i.e. a decreased risk in one side is always accompanied by a significant increase on the other (*Figure 5A*). This paradigm may be challenged by introducing

the group of OPR, where a significant reduction in bleeding was observed without an excess risk in ST compared with LPR, and also, a significant reduction in ST was observed with only a minor, even doubtful (see *Figure 3*) increase in bleeding in contrast to HPR.

Importantly, interaction testing confirmed that our findings might be relevant in several subgroups: most importantly, validation cohorts corroborated the selection of cut-off points for HPR and LPR based on exploratory trials (*Figure 6*). Based on the interaction analyses for bleeding, some subgroups showed even stronger associations between LPR and bleeding; however, we believe these differences should be viewed and interpreted carefully given the heterogeneity of patients included in various subgroups.

It is important to highlight that despite the potential value of platelet reactivity to stratify patients into categories of risk for bleeding and ST, the clinical benefits of adjusting platelet reactivity based on monitoring treatment, by targeting the optimal range considered as a therapeutic window is still unknown, and cannot be answered on the basis of our analysis. In this regard, prior randomized clinical trials (GRAVITAS, TRIGGER-PCI, and ARCTIC)[8,9,30] using the VerifyNow assay were disappointing due to lack of clinical improvements after treatment adjustments based on platelet function testing. However, none of these studies targeted an optimal range of platelet reactivity, were characterized by no[9] or only minimal utilization[8] of potent antiplatelet agents, focused mainly on stable coronary artery disease patients[30] and were underpowered.[31] Additionally, the two large, completed randomized trials used cut-off values different from those proposed and validated in our analysis.[8,9] Thus, the concept of tailored antiplatelet treatment and its possible benefits remains *unproven* but cannot be deemed *disproved* based on these trials.[8,9,30] Future studies (NCT01959451, NCT01538446) are therefore required, using the validated cut-off values, focusing on high-risk cohorts of patients and implementing the therapeutic window concept of platelet inhibition to assess the clinical relevance of tailored $P2Y_{12}$-inhibition therapy.

Although our analysis provides new evidence in a large sample of patients by validating cut-off points for risk stratification, we are aware of limitations. First, we were unable to perform adjusted Cox-proportional hazard analyses based on individual time-dependent data. This limitation does not influence our conclusions regarding the prognostic importance of platelet reactivity for risk assessment. Although adjusted analyses are useful to understand whether the associations between platelet reactivity values and outcomes are independent from confounding factors, platelet function results are never adjusted according to these at the bedside. Conversely, adjusted analyses would be useful to project whether treatment adjustments based on platelet reactivity could reduce bleeding or ST; however, such adjustments are never perfect and cannot account for all known confounders. Secondly, although there were no meaningful interaction for outcomes between prasugrel and clopidogrel-treated patients, the low number of prasugrel-treated subjects may suggest that the associations between validated cut-offs and outcomes might be relevant for clopidogrel but need further confirmation for prasugrel. Moreover, any platelet function assessment in ticagrelor-treated patients would not be capable to measure possible pleiotropic effects of the drug, which might in fact be the underlying cause of the observed reduction in mortality risk in the PLATO trial.[4] Finally, we admit that the pooled studies were heterogeneous regarding the length of follow-up that may introduce bias towards the exact determination of early-, and long-term risk of ST and bleeding in relation to platelet reactivity levels after PCI.

In conclusion, the present analysis shows in a large sample of thienopyridine-treated patients that HPR, defined by validated cut-offs using standardized platelet assays, is associated with a significantly higher risk for ST and mortality, while LPR predicts a higher risk for bleeding. The lowest rates of net adverse events in patients within an intermediate range of platelet reactivity (OPR) suggest that platelet reactivity may be a valuable marker to discriminate between ST and bleeding in patients after PCI. Further randomized trials are warranted to test the potential benefit of tailoring treatment into the optimal range of platelet reactivity.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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