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ORIGINAL COMMUNICATION

Platelet function/reactivity testing and prediction of risk of recurrent vascular events and outcomes after TIA or ischaemic stroke: systematic review and meta-analysis

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Abstract

Background The prevalence of ex vivo 'high on-treatment platelet reactivity (HTPR)' and its relationship with recurrent vascular events/outcomes in patients with ischaemic cerebrovascular disease (CVD) is unclear.

Methods A systematic review and meta-analysis was performed in accordance with the PRISMA statement. MEDLINE, EMBASE and Cochrane Library were searched for completed manuscripts until May 2019 on TIA/ischaemic stroke patients, ≥ 18 years, treated with commonly-prescribed antiplatelet therapy, who had platelet function/reactivity testing and prospective follow-up data on recurrent stroke/TIA, myocardial infarction, vascular death or other cerebrovascular outcomes. Data were pooled using random-effects meta-analysis. Primary outcome was the composite risk of recurrent stroke/TIA, myocardial infarction or vascular death. Secondary outcomes were recurrent stroke/TIA, severe stroke (NIHSS > 16) or disability/impairment (modified Rankin scale ≥ 3) during follow-up.

Results Antiplatelet–HTPR prevalence was 3–65% with aspirin, 8–56% with clopidogrel and 1.8–35% with aspirin–clopidogrel therapy. Twenty studies (4989 patients) were included in our meta-analysis. There was a higher risk of the composite primary outcome (OR 2.93, 95% CI 1.90–4.51) and recurrent ischaemic stroke/TIA (OR 2.43, 95% CI 1.51–3.91) in patients with vs. those without 'antiplatelet–HTPR' on any antiplatelet regimen. These risks were also more than twofold higher in patients with vs. those without 'aspirin–HTPR' and 'dual antiplatelet–HTPR', respectively. Clopidogrel–HTPR status did not significantly predict outcomes, but the number of eligible studies was small. The risk of severe stroke was higher in those with vs. without antiplatelet–HTPR (OR 2.65, 95% CI 1.00–7.01).

Discussion Antiplatelet–HTPR may predict risks of recurrent vascular events/outcomes in CVD patients. Given the heterogeneity between studies, further prospective, multi-centre studies are warranted.

Keywords Platelet function/on-treatment platelet reactivity · Transient ischaemic attack · Ischaemic stroke · Systematic review · Meta-analysis

Introduction

An important proportion of patients with ischaemic cerebrovascular disease (CVD) are not protected from recurrent vascular events with commonly prescribed 'non-monitored' antiplatelet therapy. Because the risk of recurrent events is highest early after a non-cardioembolic TIA/ischaemic stroke [1, 2], early institution of an effective preventive antiplatelet regimen is very important [3].

Monitoring the effects of antiplatelet therapy with reliable ex vivo platelet function/reactivity tests has the potential to

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facilitate precision-based medical treatment of CVD patients [4]. Prior data suggesting that ex vivo antiplatelet–HTPR increases the risk of subsequent vascular events are mostly derived from patients with ischaemic heart disease (IHD) [5–10], but data in this cohort are conflicting [11–15]. One meta-analysis found a higher incidence of stent thrombosis, myocardial infarction (MI) or death in patients with antiplatelet–HTPR compared with those with lower on-treatment platelet reactivity following percutaneous coronary intervention (PCI) on a device called the VerifyNow® which assesses platelet reactivity in whole blood ex vivo at low shear stress [16]. Altering antiplatelet therapy based on platelet function/reactivity testing with the VerifyNow did not improve 'vascular outcomes' in two large trials in IHD patients [17, 18]. However, a subsequent meta-analysis of 10 randomized clinical trials ($N=4213$ patients) revealed that intensifying antiplatelet therapy based on HTPR testing was associated with reduced cardiovascular mortality and stent thrombosis after PCI ($P=0.02$), with no difference in the risk of major haemorrhagic complications between 'intensified' and 'standard treatment' groups ($P=0.44$) [19]. A more recent substudy of the TROPICAL-ACS randomized trial also showed that the 1 year risk of stroke, MI or vascular death was higher in patients with compared with those without prasugrel-HTPR who underwent percutaneous coronary intervention for an acute coronary syndrome (4.8% vs. 2.2%; Hazard Ratio 2.16, 95% CI 1.01–4.65; $P=0.049$) [20].

Due to the heterogenous aetiology of TIA and ischaemic stroke [21] and the higher risk of intracerebral haemorrhage after stroke than after an acute coronary syndrome, one cannot simply extrapolate data on ex vivo HTPR from IHD patients to those with CVD. This area of translational research has received much less attention in the important population of patients with CVD, as outlined in prior systematic reviews [22, 23]. Therefore, there is now a compelling need to determine whether there is sufficient evidence to indicate that platelet function/reactivity monitoring could enable optimised secondary prevention to reduce the morbidity, costs of care or mortality specifically in patients with TIA or ischaemic stroke.

Aims

The aims of this updated systematic review and innovative meta-analysis were to establish the latest prevalence ranges of antiplatelet–HTPR, and to determine the potential role of ex vivo platelet function/reactivity testing in predicting the risk of 'recurrent vascular events' and 'neurological outcomes' following TIA or ischaemic stroke.

Hypothesis

We hypothesised that antiplatelet–HTPR status would be associated with the risk of these outcome events in a 'CVD-specific' patient population.

Methods

Search Strategy

The systematic review and meta-analysis were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [24]. This study protocol is registered with the International Prospective Register of Systematic Reviews (PROSPERO) (Registration No. CRD42018104210). MEDLINE (OVID and PubMed), EMBASE (via OVID) and The Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials (CENTRAL), Cochrane Methodology Register) were searched for completed, peer-reviewed manuscripts in English from the establishment of these databases until 1st May 2019. The search strategy encompassed subject headings or thesaurus terms, and a comprehensive, relevant text-word strategy utilizing truncations and 'wildcards'. Searches were combined using Boolean operators 'and', 'or' and 'not'. The following search terms were used in different combinations: transient ischaemic attack, TIA, stroke, platelet function, platelet reactivity, platelet aggregation, platelet aggregometry, antiplatelet resistance, high on-treatment platelet reactivity, aspirin, clopidogrel, dipyridamole, and three commonly available whole blood platelet function/reactivity testing platforms at the time of planning the review (Platelet Function Analyser-100 (PFA-100®), VerifyNow®, Multiplate®). In addition, reference lists of included papers and systematic reviews were critically evaluated to search for articles not identified with the above search strategy.

Study Selection and Data Extraction

We used the following *inclusion criteria*:

1. Studies including patients with TIA or ischaemic stroke, ≥ 18 years, treated with aspirin or clopidogrel monotherapy, aspirin–dipyridamole or aspirin–clopidogrel combination therapy, who had platelet function/reactivity testing with aggregometry or any of the above commonly available whole blood platelet function/reactivity platforms;

2. Any prospectively collected outcome data recorded during follow-up *after* platelet function/reactivity testing;
3. We included pharmacogenetic studies only if they had simultaneously collected antiplatelet–HTPR data because e.g. clopidogrel may potentially have less clinical efficacy in 'poor' or 'intermediate metabolizers' and greater efficacy in 'extensive metabolizers' without CYP2C19 loss-of-function alleles [25, 26].

Exclusion Criteria

We excluded studies assessing platelet function in vitro or 'platelet activation status' ex vivo, pharmacogenetic studies not linked to antiplatelet–HTPR testing, or reports in which it was unclear whether haemorrhagic stroke patients were included. Studies which did not prospectively collect data on the risk of recurrent vascular events or outcomes during follow-up **after** ex vivo platelet reactivity/function testing were excluded. We also excluded studies purely focused on subgroups of patients with moderate–severe symptomatic or asymptomatic carotid atherosclerotic stenosis because this is the subject of a separate systematic review in preparation. However, some included studies on CVD patients overall did of course incorporate data on some patients with recently symptomatic carotid stenosis.

The majority of studies assessing antiplatelet–HTPR in CVD used a 'cross-sectional/case–control' definition of HTPR where patients' results at a single time point were compared with those from a group of healthy controls or the manufacturer's normal range [22]. Prospective longitudinal studies which assessed alteration of platelet function/reactivity ex vivo in matched samples from individual CVD patients who were tested prior to and following commencement or modification of their antiplatelet regimen are limited [27–34]. Members of our group have proposed the novel concept of 'longitudinal antiplatelet–HTPR status', which we defined as failure to alter a patient's platelet function/reactivity data compared with their own 'baseline value' before undergoing a change in antiplatelet therapy by more than twice the coefficient of variation of the assay [27]. Therefore, available data pertaining to cross-sectional and longitudinal definitions of HTPR in CVD were analysed as both have the potential to be clinically informative [27, 28].

Two independent reviewers (STL and SYL) screened the title and/or abstract of retrieved citations. If the abstract suggested the article met our inclusion criteria, the full-text article was reviewed by two reviewers who extracted the following data on pre-specified forms: authors; journal; year of publication; geographical location of the study; study design; inclusion and exclusion criteria; baseline clinical and demographic data; sample size; clinical indication for antiplatelet therapy; prescribed antiplatelet regimens and doses; type of platelet function test/platform utilised and definition of antiplatelet–HTPR employed; duration of follow-up; comparison of outcomes between those with vs. those without antiplatelet–HTPR. Any discrepancies were resolved by consensus between reviewers. Each manuscript was also critically-appraised by the supervising author who finally adjudicated on any disagreements between reviewers.

Some studies contained relevant data which were only suitable for inclusion in our systematic review. Others reported sufficiently-detailed data which were suitable for inclusion in both the systematic review and in the meta-analysis to calculate the potential value of antiplatelet–HTPR testing in prospectively predicting the risk of our pre-specified primary and secondary outcomes. The *primary outcome* for our meta-analysis was defined as the composite risk of recurrent stroke, TIA, myocardial infarction or vascular death. *Secondary outcomes* were: (a) the risk of having a recurrent ischaemic stroke or TIA; (b) severe stroke (NIHSS score > 16) or disability/impairment (defined as a modified Rankin scale [MRS] score ≥ 3) during follow-up because antiplatelet–HTPR could theoretically contribute to progressive ischaemia or infarction following stroke onset.

Statistical Methodology

We used standard descriptive statistical methodology for the systematic review. VT coordinated the meta-analyses using the STATA/IC 15.1 statistical package. We calculated unadjusted odds ratios (ORs) for each individual study based on reported binary data. In studies requiring OR calculations from binary data which included arms with zero events, 0.5 was added to all cells. These outcomes were pooled on the log-scale using the DerSimonian–Laird random-effects model with an inverse-variance calculation method, and the pooled effect exponentiated with resulting ORs. A secondary analysis utilizing a fixed-effects model was performed to complement the random-effects approach. Heterogeneity was assessed using the I^2 statistic. To explore possible sources of heterogeneity, we undertook subgroup sensitivity analyses based on factors which might influence outcomes between patients with and without antiplatelet–HTPR: antiplatelet regimen (aspirin, clopidogrel, aspirin–clopidogrel, aspirin–dipyridamole combination therapy); aspirin dose (≤ 100 mg/day, > 100 mg/day); geographical location/'likely ethnicity' (arbitrarily defined as 'Non-Asian' versus 'Asian' study populations) based on Country of origin of respective studies. Furthermore, studies were categorized and analysed according to methods used to assess HTPR status, including 'platelet aggregation under low shear stress', 'platelet adhesion and aggregation under high shear stress' and 'other methods'. We also performed random-effects meta-regression analyses of data based on common variables, such as the effect of year of study publication, patient age and sex on the risk of subsequent stroke/TIA, MI or vascular death.

Quality Assessment and Risk of Bias

The Risk Of Bias In Non-randomized Studies-of Interventions (ROBINS-I) tool [35] was employed by two independent reviewers (STL and SYL) to assess quality and risk of bias of included studies. The ROBINS-I domain encompassing 'deviation from interventions' was not relevant to this review.

Data Availability

The data extraction tables, which were populated from the studies included in the systematic review and meta-analysis, are available on request to be shared by the first author with qualified scientific collaborators for relevant research projects.

Ethical Considerations

Because these data were collated as part of a systematic review and meta-analysis, our Local Research Ethics Committee (LREC) hospital guidelines indicate that formal LREC approval for such a study is not required. However, all data were securely stored in electronic format and no individual patient could be identified from the data contained within. The study and analyses were conducted in accordance with best ethical practice and supervised by experienced Consultants who are International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP)-certified.

Results

The search identified 34 studies which were suitable for inclusion in our comprehensive systematic review, 20 of which also met inclusion criteria for both qualitative and quantitative synthesis in our meta-analysis (Fig. 1). A summary of data from our systematic review of 34 studies will be presented first, followed by data from the meta-analysis of the aforementioned 20 studies.

Systematic Review of the Prevalence of Antiplatelet–HTPR

Most studies used 'cross-sectional/case–control' definitions of antiplatelet–HTPR [22] and prevalence varied according to the device used, time interval after TIA or stroke onset, definition employed [36, 37] and doses of prescribed therapy [22, 38]. The following figures represent the range for the minimum–maximum reported prevalence of antiplatelet–HTPR on each antiplatelet regimen based on the definition of antiplatelet–HTPR used in the individual studies included in our systematic review. The prevalence of antiplatelet–HTPR across all studies ranged from 3 to 65% with aspirin, 8–56% with clopidogrel, 1.8–35% with aspirin and clopidogrel in patients on aspirin–clopidogrel combination therapy, and 56–59% with dipyridamole in patients who were also on aspirin (Supplemental Tables I and II). In our systematic review, thirteen independent studies found a clear association ($P < 0.05$; Supplemental Table III), and eleven studies found no clear independent association between antiplatelet–HTPR status and the risk of recurrent vascular events (Supplemental Table IV). Twelve studies revealed an association between antiplatelet–HTPR status and more severe strokes, early neurological deterioration or adverse outcomes ($P < 0.05$; Supplemental Table V). One study found a relationship between clopidogrel–HTPR status and CYP2C19*2 and CYP2C19*3 pharmacogenetic profiles, and revealed that clopidogrel–HTPR, but not CYP2C19 genotype, was associated with an increased risk of recurrent ischaemic stroke/TIA, cardiac events or vascular death [39]. Four studies showed an association between CYP2C19 genotype and the risk of clopidogrel–HTPR [39–42]. One study suggested that patients with a 'CYP2C19 extensive metaboliser genotype' were more likely to have good functional outcomes (MRS ≤ 2) at 3 and 6 months follow-up [40].

Meta-analysis

The meta-analysis was performed on pooled data from 20 studies in 4989 patients (1507 patients with antiplatelet–HTPR and 3482 without antiplatelet–HTPR), 56.7% of whom were men, with a mean age of 65.8 years (standard deviation: 4.9 years). The mean duration of follow-up was 14.9 months for all studies combined in the meta-analysis (supplemental table VI).

Quality Assessment and Risk of Bias

Fourteen of 20 included studies were considered to have a 'very low risk' of bias, with 3 studies potentially influenced by 'missing data' and 4 studies had some domains categorized as 'sound for a non-randomized study but not comparable to a rigorous randomized trial'. If one accepts that it is difficult to assess the precise impact of the missing data in 3 studies, the overall risk of bias was deemed to be low or very low in 17/20 (85%) included studies (supplemental figure I).

Meta-analysis of Data on HTPR Status and Risk of Composite Vascular Outcomes

There was an increased risk of the composite primary outcome of recurrent stroke/TIA, myocardial infarction or vascular death in patients with vs. those

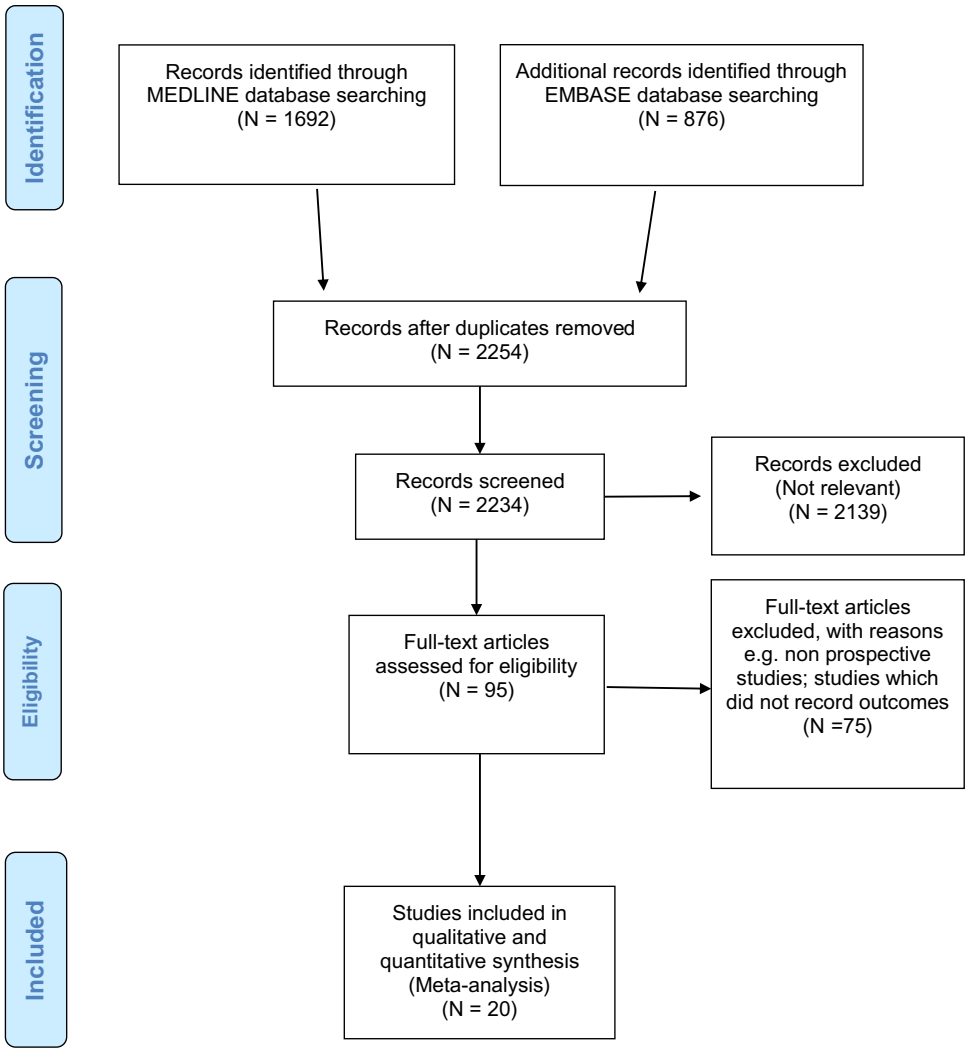


Fig. 1 Flow chart summarising search strategy used in line with PRISMA statement

without antiplatelet–HTPR on any antiplatelet regimen (OR = 2.93, 95% CI 1.90–4.51 (Fig. 2a), with high heterogeneity between studies ($I^2 = 80.7\%$, $p < 0.001$). Subgroup analysis by antiplatelet regimen demonstrated a significant risk of the composite outcome in patients with vs. those without aspirin–HTPR (OR = 3.13, 95% CI 1.77–5.56) and dual antiplatelet–HTPR (OR = 3.14, 95% CI 1.86–5.31) (Fig. 2b). Patients with clopidogrel–HTPR did not have a significantly higher risk of the composite outcome, but the number of included studies was very small (OR = 1.98, 95% CI 0.89–4.4). There was high heterogeneity between aspirin studies ($I^2 = 84\%$, $p < 0.001$), moderate heterogeneity between clopidogrel studies ($I^2 = 67.3\%$, $p = 0.047$), and low heterogeneity between dual antiplatelet studies ($I^2 = 0\%$, $p = 0.78$). Separate subgroup analysis of outcomes in studies on lower dose aspirin (≤ 100 mg daily) and higher dose aspirin (> 100 mg daily) revealed that there was a statistically significantly increased risk of the composite outcome in patients with vs. those without aspirin–HTPR on lower dose aspirin (OR = 3.38, 95% CI 1.77-6.45), but not on higher dose aspirin although the 95% CIs were very wide (OR = 2.24, 95% CI 0.46–10.99) (Fig. 2c). Antiplatelet–HTPR on any regimen was associated with a higher risk of the composite outcome in 'Asian' and 'Non-Asian' populations, with no difference between these subgroups (Fig. 2d). However, there was high heterogeneity in each subgroup. Studies employing the principle of platelet aggregometry (e.g. conventional aggregometry, VerifyNow and Multiplate) showed a higher risk of composite outcomes in those with vs. those without antiplatelet–HTPR (OR = 3.27, 95% CI 2.02–5.31; supplemental figure II). Studies which assessed platelet adhesion/aggregation under high shear stress (PFA-100) did not show a significant association between HTPR status and risk of the subsequent composite outcome, but the number of subjects included in this analysis was limited ($N = 521$ in total; supplemental figure II).

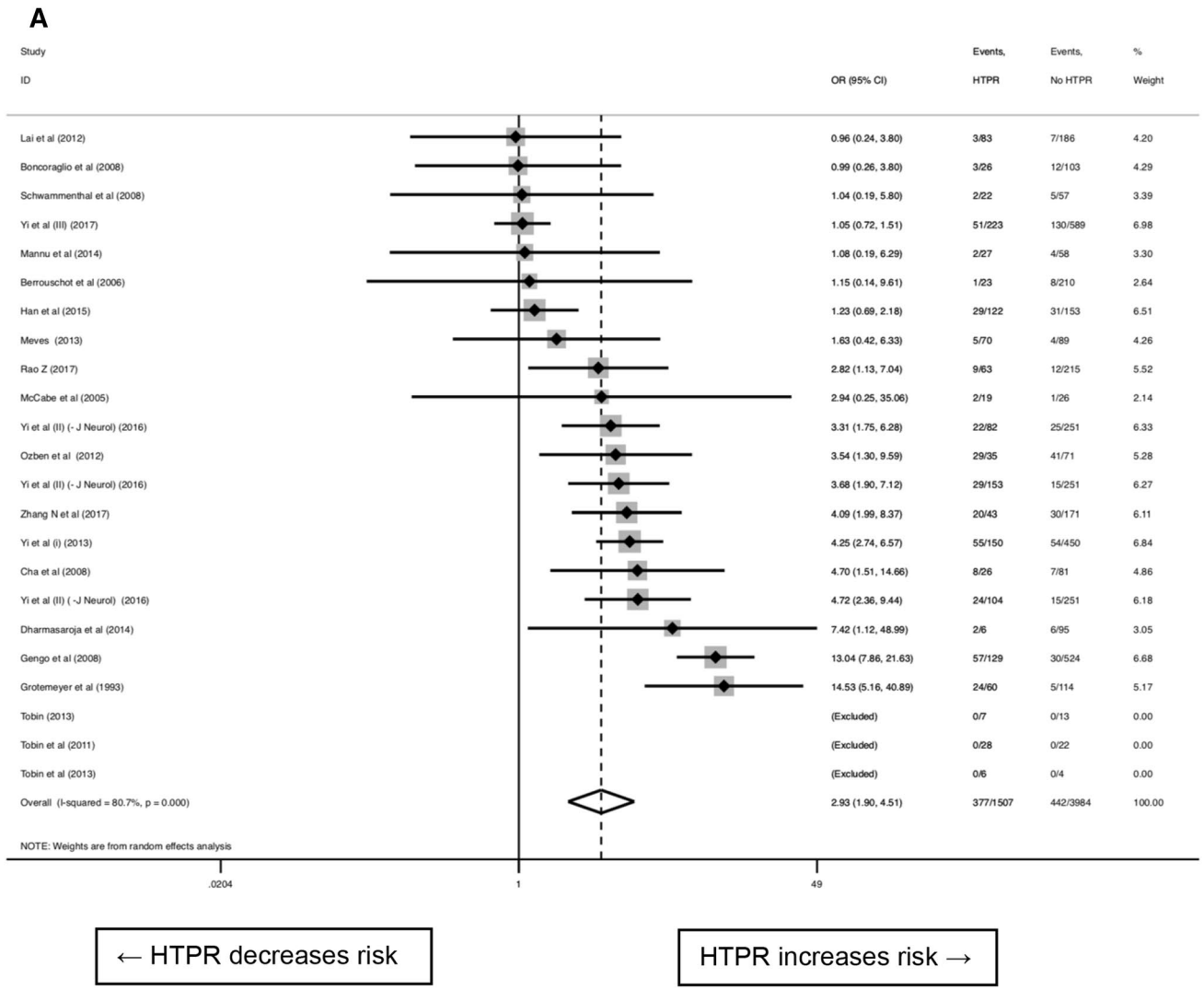


Fig. 2 **a** Combined analysis of the composite risk of subsequent ischaemic stroke/TIA, myocardial infarction or vascular-related death in those with vs. those without antiplatelet–HTPR on any antiplatelet regimen. **b** Subgroup analysis based on antiplatelet regimen on the composite risk of subsequent ischaemic stroke/TIA, myocardial infarction or vascular death in those with vs. those without HTPR on aspirin, clopidogrel or dual antiplatelet therapy. (Tobin et al. 2011 study included patients changing from aspirin to aspirin–dipyridamole combination therapy, but no outcome events were observed during follow-up. All other 'dual antiplatelet therapy' studies were on aspirin–clopidogrel). **c** Subgroup analysis based on aspirin dose on the composite risk of subsequent ischaemic stroke/TIA, MI or vascular death in those with vs. those without aspirin–HTPR on 'Lower dose' ($\leq$100 mg daily) and 'Higher dose' (>100 mg daily) aspirin. **d** Subgroup analysis based on geographical study location on the composite risk of subsequent ischaemic stroke/TIA, myocardial infarction or vascular death in those with vs. those without antiplatelet–HTPR

Meta-analysis of Data on HTPR Status and Risk of Recurrent Ischaemic Stroke/TIA

Meta-analysis of studies which included data on the risk of recurrent ischaemic stroke/TIA showed a significantly higher risk of ischaemic stroke/TIA recurrence in patients with vs. those without antiplatelet–HTPR on any antiplatelet regimen (OR = 2.43, 95% CI 1.51–3.91) (Fig. 3a). However, there was considerable heterogeneity between studies ($I^2 = 81.6\%$, $p < 0.001$). Analysis of specific antiplatelet regimens showed that aspirin–HTPR (OR = 2.26, 95% CI 1.19–4.30) and dual antiplatelet–HTPR (OR = 4.78, 95% CI 2.65–8.62) were associated with a higher risk of recurrent ischaemic stroke/TIA. However, there was no statistically significant association between clopidogrel–HTPR and risk of subsequent ischaemic stroke/TIA (OR = 1.98, 95% CI 0.89–4.42). There was high heterogeneity between studies assessing aspirin–HTPR ($I^2 = 84.9\%$, $p < 0.001$), moderate heterogeneity between clopidogrel–HTPR studies ($I^2 = 66.1\%$, $p = 0.05$) and low heterogeneity between studies of dual antiplatelet therapy ($I^2 = 0\%$, $p = 0.38$) (Fig. 3b). Aspirin–HTPR was associated with

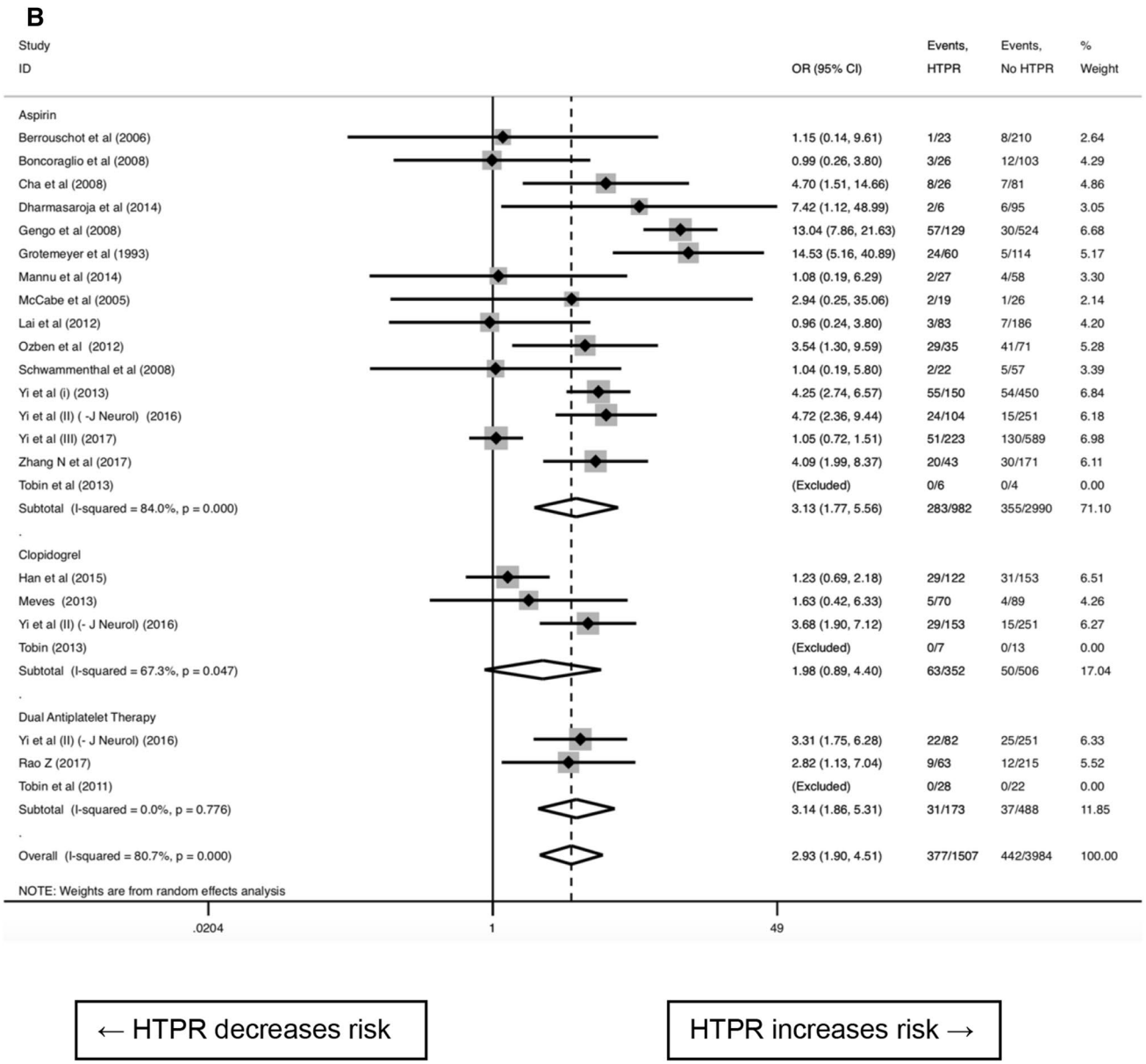
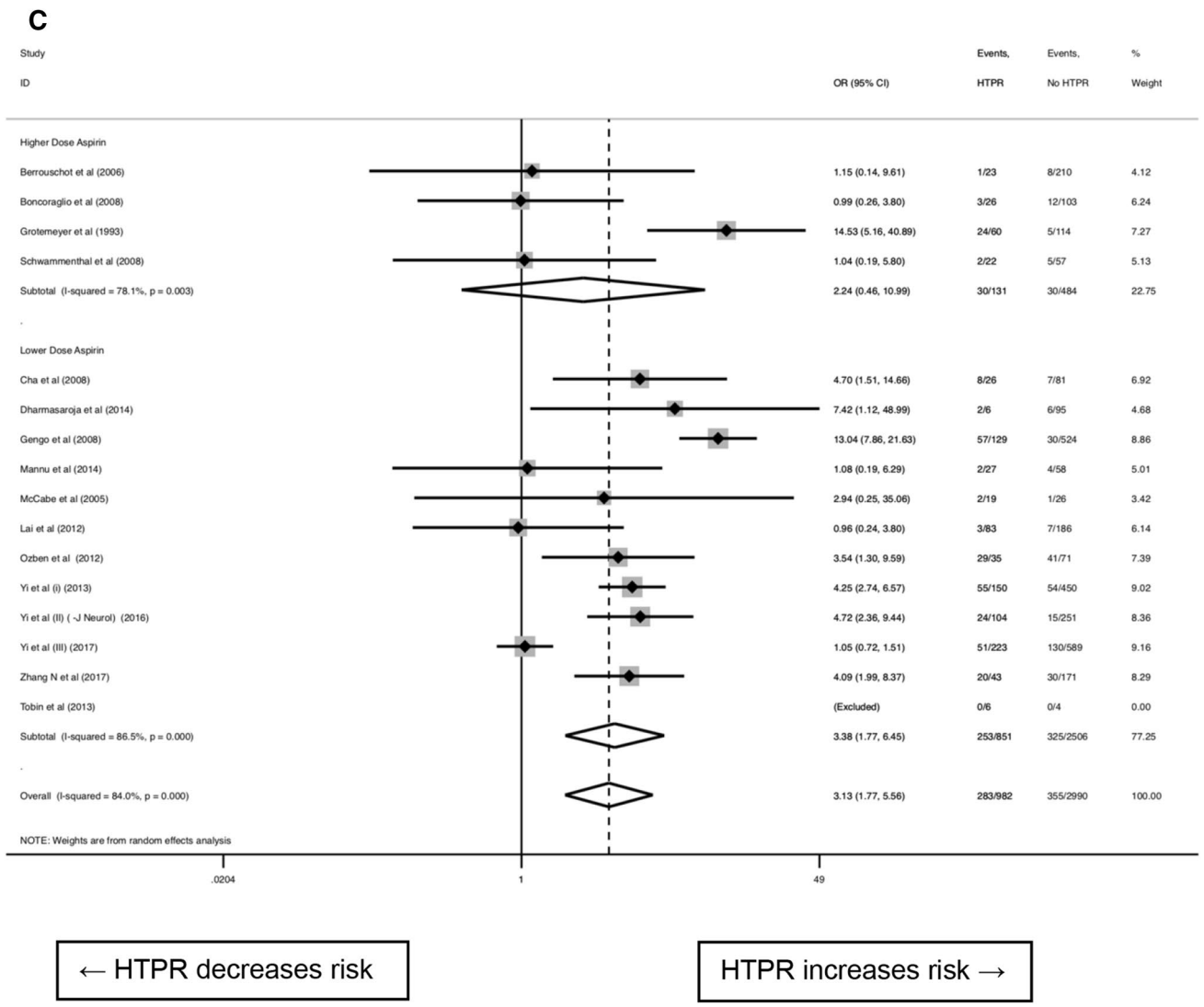


Fig. 2 (continued)

a significantly increased risk of subsequent ischaemic stroke/TIA in patients on lower dose aspirin (OR = 2.70, 95% CI 1.30–5.59), but not in those on higher dose aspirin (Fig. 3c). There was high heterogeneity between studies of lower dose aspirin ($I^2 = 88.1\%$, $p < 0.001$), but low heterogeneity in studies with higher dose aspirin ($I^2 = 0\%$, $p = 0.99$). There was a higher risk of subsequent ischaemic stroke/TIA in patients with antiplatelet–HTPR in the 'Asian' subgroup' (OR = 2.48, 95% CI 1.56–3.93), but the differences were not statistically significant in the 'Non-Asian' subgroup (OR = 2.02, 95% CI 0.62–6.61), with high heterogeneity in each subgroup (Asian: $I^2 = 77.2\%$; Non-Asian I^2: 80.1%; $p < 0.001$) (Fig. 3d).

Meta-analysis of Data on the Relationship between HTPR Status and Stroke Severity or Disability during Follow-up

The risk of having a severe stroke during follow-up (NIHSS > 16) was higher in those with vs. those without HTPR (OR = 2.65, 95% CI 1.00–7.01) (Fig. 4), but there were insufficient data to comment on MRS outcomes in those with vs. those without HTPR. There was moderate heterogeneity between studies ($I^2 = 63.2$, $p = 0.099$).



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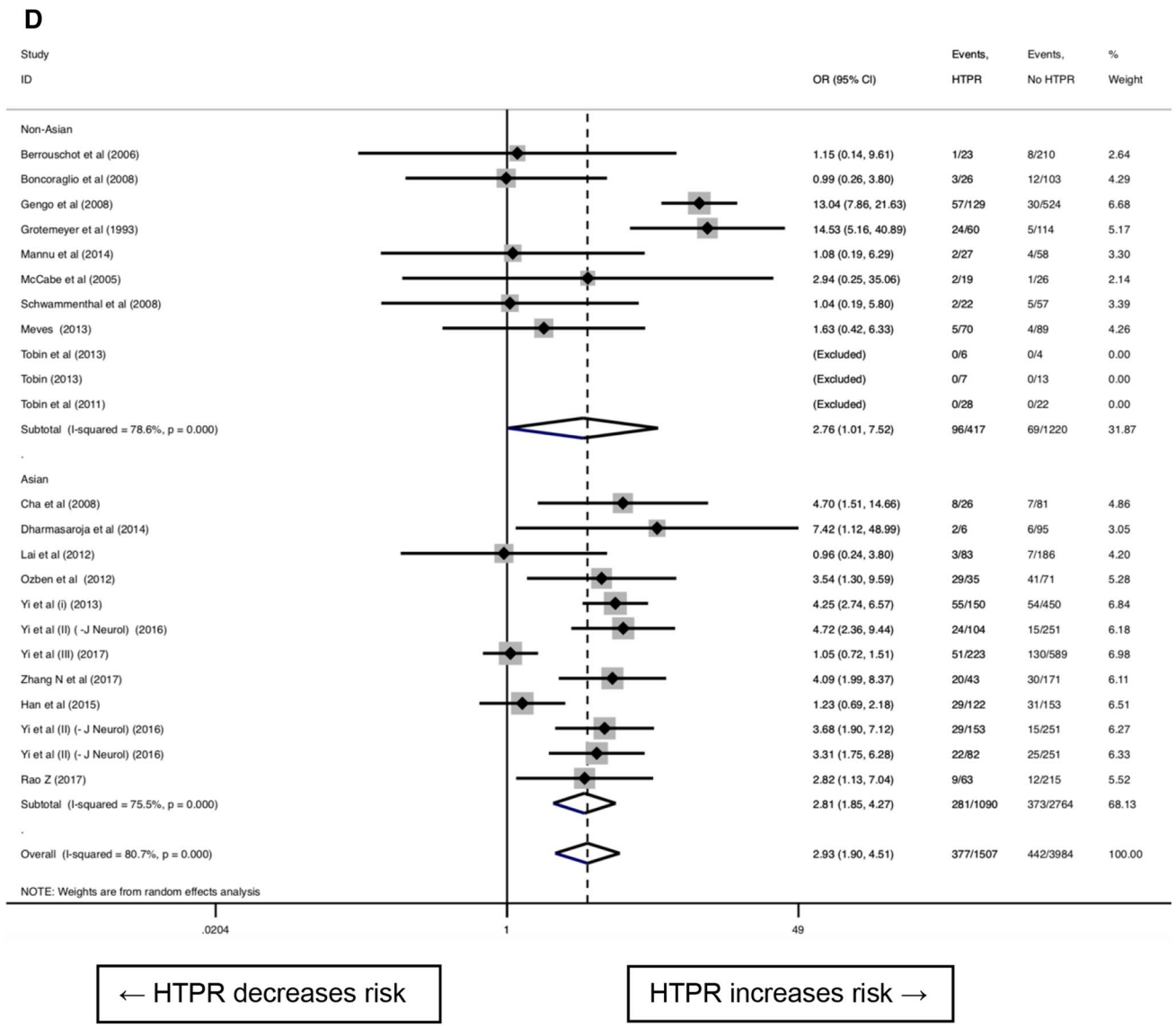
Meta-regression Analysis

Meta-regression analysis revealed that the only common variable which influenced the relationship between antiplatelet–HTPR status and the risk of the composite outcome was the 'year of publication', with older studies more likely to reveal a significant relationship between antiplatelet–HTPR status and outcomes ($p = 0.01$). However, post hoc analysis, after excluding data from the study by Grotemeyer et al. [7] to focus on the era of 'more modern secondary preventive therapy' from 2005 onwards, negated the impact of the year of publication on our results ($p = 0.7$). Nevertheless, it is important to note that the higher risk of composite outcomes with antiplatelet–HTPR overall, or aspirin–HTPR in particular, persisted after post hoc meta-analyses which excluded Grotemeyer's data (supplemental figures III and IV).

Discussion

Our comprehensive systematic review revealed a wide range of prevalence of antiplatelet–HTPR of 3–65% with aspirin, 8–56% with clopidogrel, 1.8–35% with either aspirin or clopidogrel in patients on aspirin–clopidogrel combination therapy, and 56–59% with dipyridamole when it was added to aspirin in the early, subacute or late phases after TIA/ischaemic stroke [22]. The highest absolute prevalence of aspirin–HTPR was observed in a case–control study assessing platelet adhesion/aggregation with the PFA-100 under moderately-high shear stress [43]; the highest prevalence of clopidogrel–HTPR was noted with a modified aggregometry paradigm (VerifyNow) [44] (Supplemental Table I). Case–control definitions may underestimate effects of antiplatelet therapy in individuals compared with



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novel longitudinal definitions [22, 27], but most data in this review were derived from studies employing traditional case–control definitions. Variability in HTPR prevalence rates between studies and devices emphasises the importance of ideally assessing platelet reactivity with more than one device in future studies to clarify which testing platforms are most likely to predict outcomes.

Our meta-analysis indicates that CVD patients with antiplatelet–HTPR on any regimen, based on HTPR definitions employed in individual studies, had at least 2–3 times the risk of experiencing the composite outcome or recurrent ischaemic cerebrovascular events alone during prospective follow-up compared with patients without antiplatelet–HTPR. These findings also applied to patients on aspirin monotherapy or aspirin–clopidogrel combination therapy, but there was high heterogeneity between studies in patients on aspirin alone and fewer studies in patients on aspirin–clopidogrel dual antiplatelet therapy. Our outcome data on aspirin are broadly in keeping with a prior systematic review and meta-analysis by Fiolaki et al. which reported an increase in the relative risk of recurrent ischaemic stroke/TIA in patients with vs. those without aspirin–HTPR [23]. However, not all recurrent vascular events were recorded *during prospective follow-up after HTPR testing* by Fiolaki et al., which is a major strength or our current, more focused prospective meta-analysis, and the value of assessing HTPR status to predict the risk of the composite outcome of recurrent stroke, TIA, myocardial infarction or vascular death was not pre-planned or analysed in that prior study [23]. In contrast to the findings by

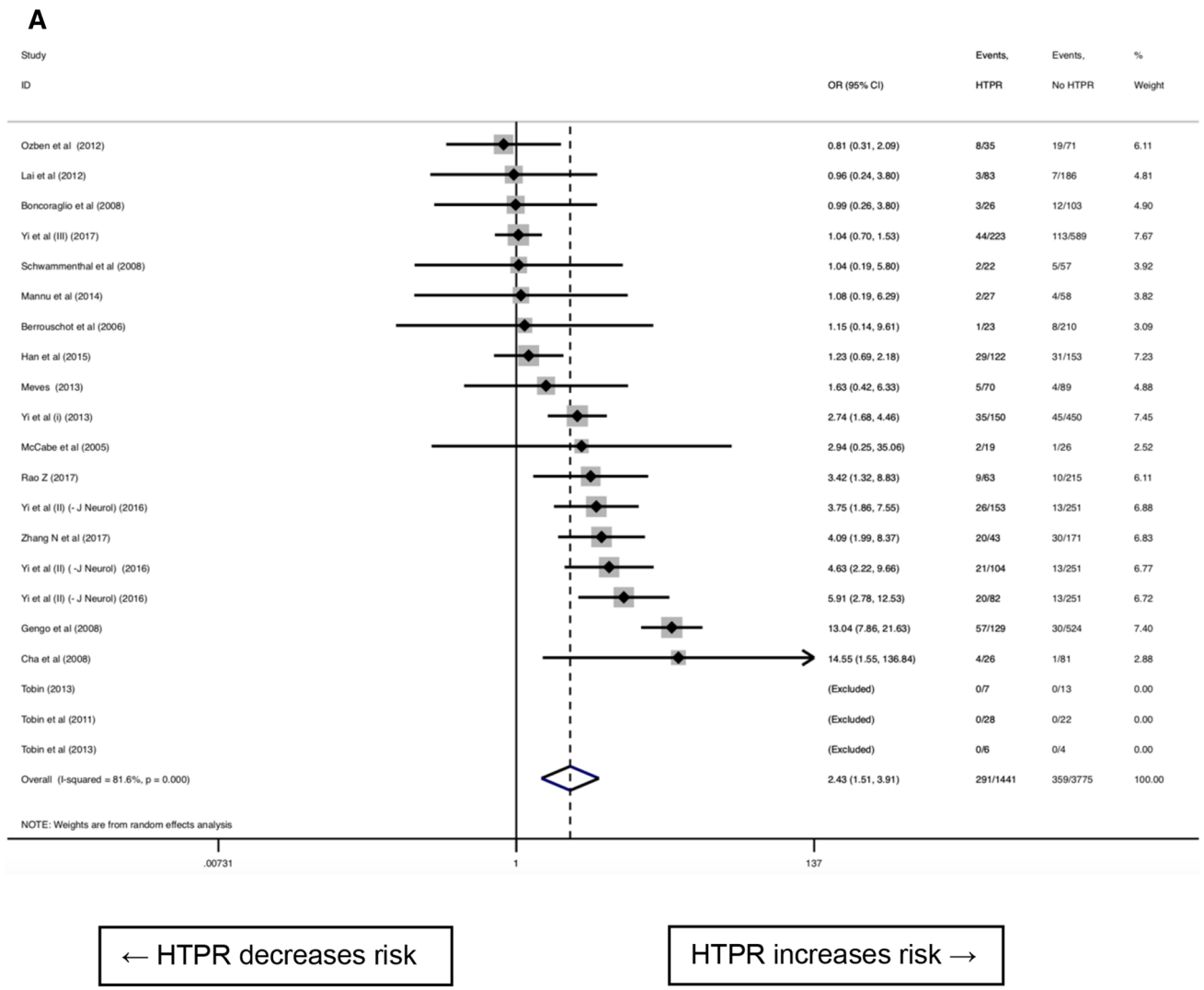
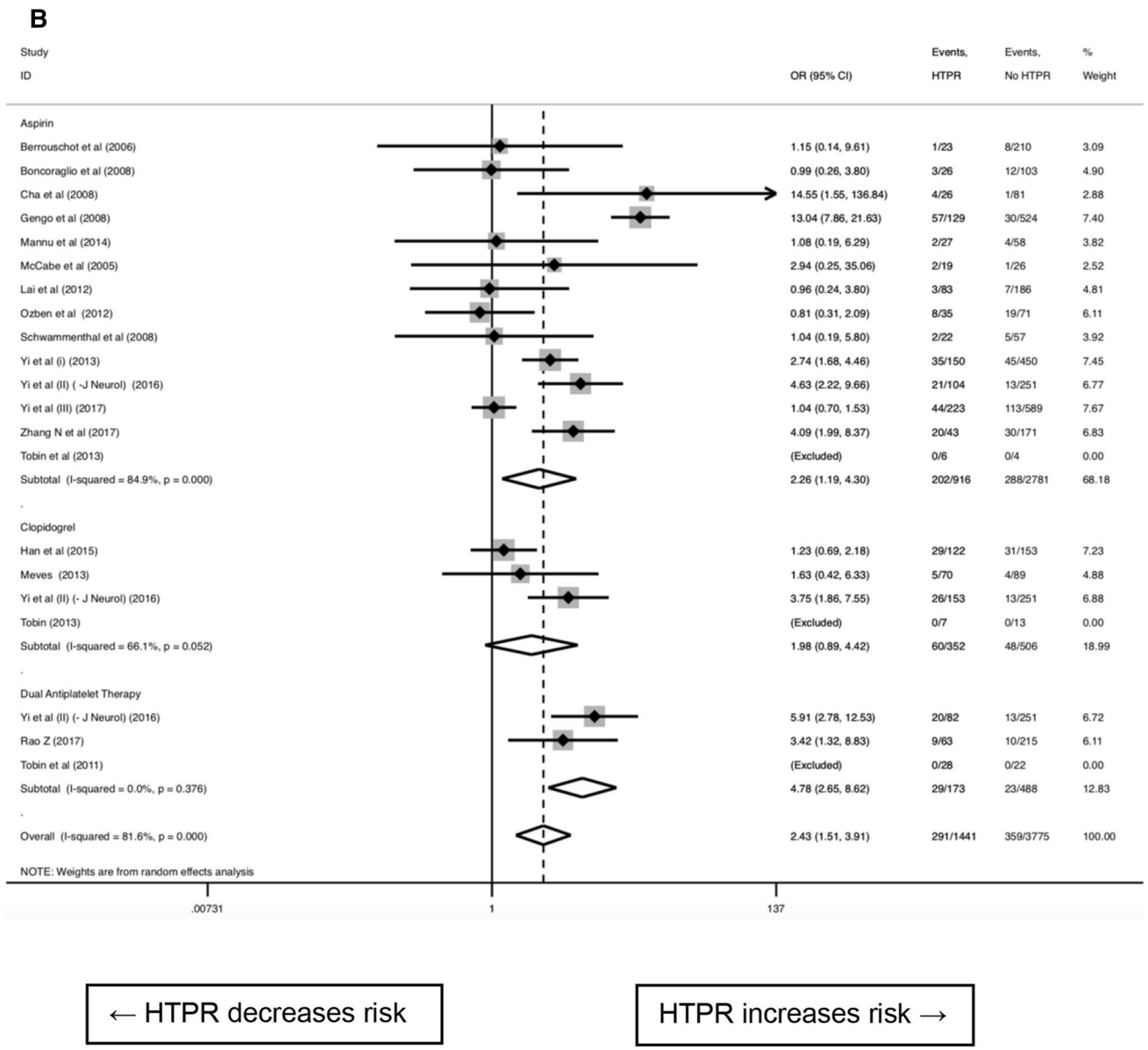


Fig. 3 **a** Combined analysis of the risk of recurrent ischaemic stroke/TIA in those with vs. those without antiplatelet–HTPR on any antiplatelet regimen (aspirin, clopidogrel or dual antiplatelet therapy) during follow-up. **b** Subgroup analysis of the risk of recurrent ischaemic stroke/TIA in those with vs. those without HTPR on various antiplatelet regimens (Aspirin, Clopidogrel, Dual Antiplatelet therapy). (Tobin et al. [28] study included patients changing from aspirin to aspirin–dipyridamole combination therapy, but no outcome events were observed during follow-up. All other 'dual antiplatelet therapy' studies were on aspirin–clopidogrel). **c** Subgroup analysis on the risk of recurrent ischaemic stroke/TIA in those with vs. those without aspirin–HTPR on 'Lower dose' (≤ 100 mg daily) and 'Higher dose' (> 100 mg daily) aspirin

Fiolaki et al. [23], the presence of clopidogrel–HTPR was not significantly associated with the risk of ischaemic cerebrovascular outcomes (or composite vascular outcomes in our study) using different, carefully-selected prospective studies in our meta-analysis. However, our clopidogrel–HTPR findings should be interpreted with caution because they might reflect a type II error due to the lower number of patients on clopidogrel with prospective data available for analysis, and there was moderate heterogeneity between studies. Larger prospective studies assessing the value of clopidogrel–HTPR at predicting outcomes after TIA/ischaemic stroke are warranted.

Pre-planned subgroup analyses revealed that the relationship between aspirin–HTPR and risk of recurrent vascular outcomes overall or cerebrovascular outcomes in particular pertained to studies on 'lower dose' (≤ 100 mg/day) but not 'higher dose' aspirin (> 100 mg/day). This highlights the need to conduct adequately-powered, multi-centre studies assessing the predictive value of aspirin–HTPR status in CVD patients in routine clinical practice on a range of aspirin doses. Most patients included in this review received lower dose aspirin, in keeping with recommendations from meta-analyses of clinical trials [45]. However, because a minority of studies assessed patients on higher dose aspirin,



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one should not conclude that assessment of aspirin–HTPR status is unwarranted in patients on higher dose aspirin because the 95% CIs of the analyses of data from lower and higher dose aspirin studies overlapped, and the 95% confidence intervals in the higher dose aspirin group in particular were wide, as emphasised in the results section above.

Our novel subgroup data also indicate that the potential value of antiplatelet–HTPR status in predicting the risk of recurrent composite vascular outcomes applies to both 'Asian' and 'non-Asian' study populations, which to our knowledge, had not been comprehensively addressed before. However, the absence of information on the precise ethnic background of study patients is a limitation of this review and precludes further specific comment. HTPR data were predictive of the composite outcome in studies using aggregometry or modified aggregometry techniques, but not in studies assessing platelet adhesion or aggregation at high shear stress. It must be acknowledged that the limited number of outcome data from prospective studies with the PFA-100 could also have led to a type II error, and thus warrants further study.

The recently published Platelet Reactivity in Acute Stroke or Transient Ischaemic Attack (PRINCE) trial revealed a lower prevalence of antiplatelet–HTPR with ticagrelor than with clopidogrel on the VerifyNow P2Y12 assay in Chinese patients at 90 days following a 'high risk TIA' or minor

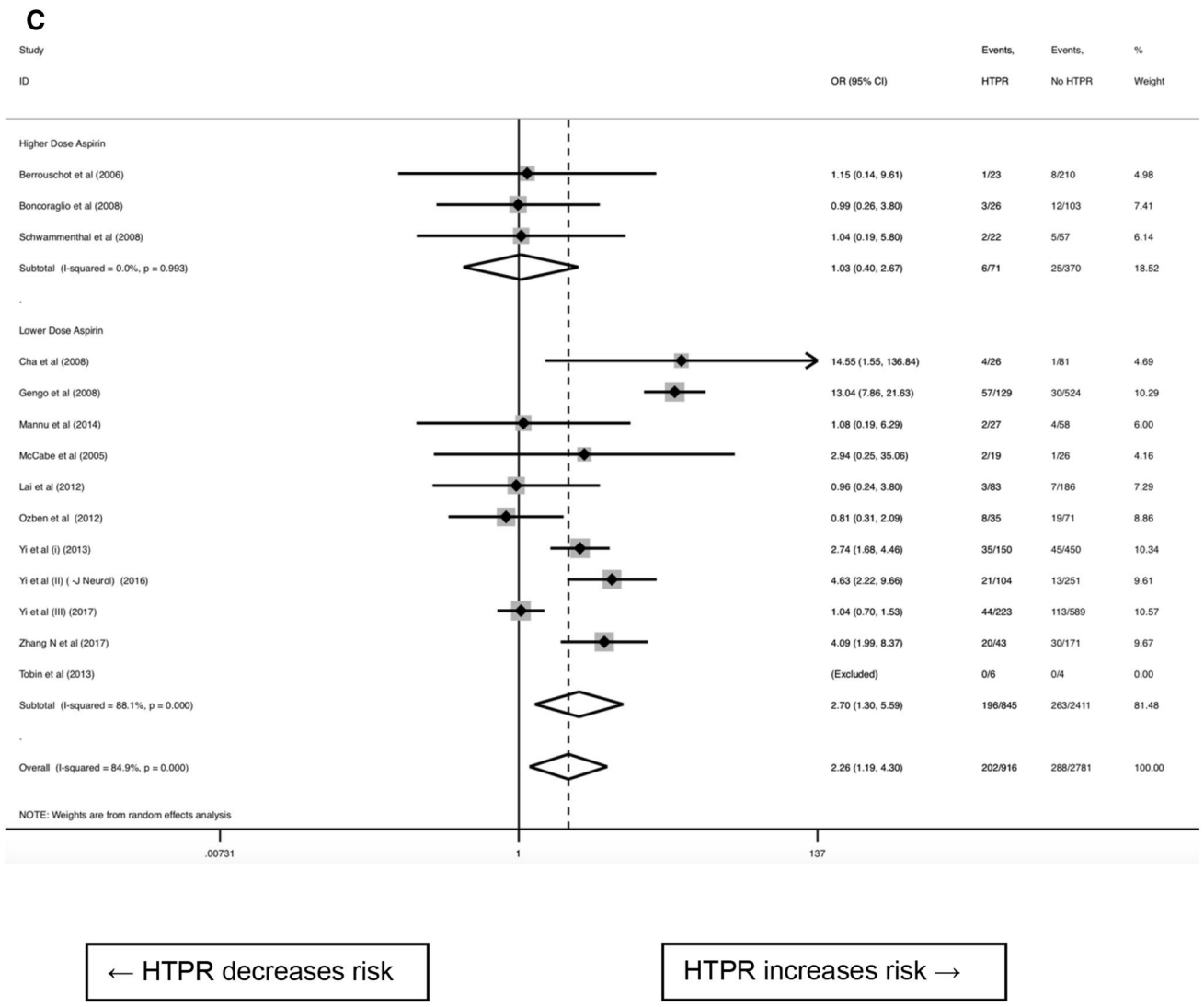

Fig. 3 (continued)

ischaemic stroke (12.5 vs. 29.7%, $p < 0.001$) [46]. However, the authors did not report the risks of recurrent vascular events in the subgroups of patients with vs. those without ticagrelor-HTPR and clopidogrel–HTPR, respectively, and the study was not designed or powered to detect such differences. Therefore, these trial data, which were included in our systematic review but did not meet criteria for inclusion in our meta-analysis, do not provide evidence that one should change antiplatelet treatment based on clopidogrel–HTPR or ticagrelor-HTPR status.

An emerging body of evidence indicates that the presence of antiplatelet–HTPR in particular might have an adverse effect on baseline stroke severity, early deterioration, poorer functional outcome or higher mortality during follow-up (Supplemental Table V) [47–53]. These studies do not prove that antiplatelet–HTPR is directly responsible for the pathogenesis of more severe strokes at presentation or poorer outcomes during follow-up because the dose and duration of therapy varied between studies, but might partly reflect 'secondary platelet hyper-reactivity' following larger strokes which is not inhibited by prescribed doses of aspirin or clopidogrel. We identified an increase in the risk of having a persistently severe stroke during follow-up in those with vs. those without antiplatelet–HTPR. However, there were insufficient data to perform separate subgroup analyses on aspirin or clopidogrel, respectively due to the limited number of studies in which these dichotomous NIHSS outcome data were included. Future meta-analyses should explore the impact of antiplatelet–HTPR on 'dynamic NIHSS changes' in the early phase after ischaemic stroke. These preliminary findings are still informative and indicate that assessment of the combined risk of recurrent vascular events **or** 'adverse functional outcomes' could enhance the statistical power of future studies.

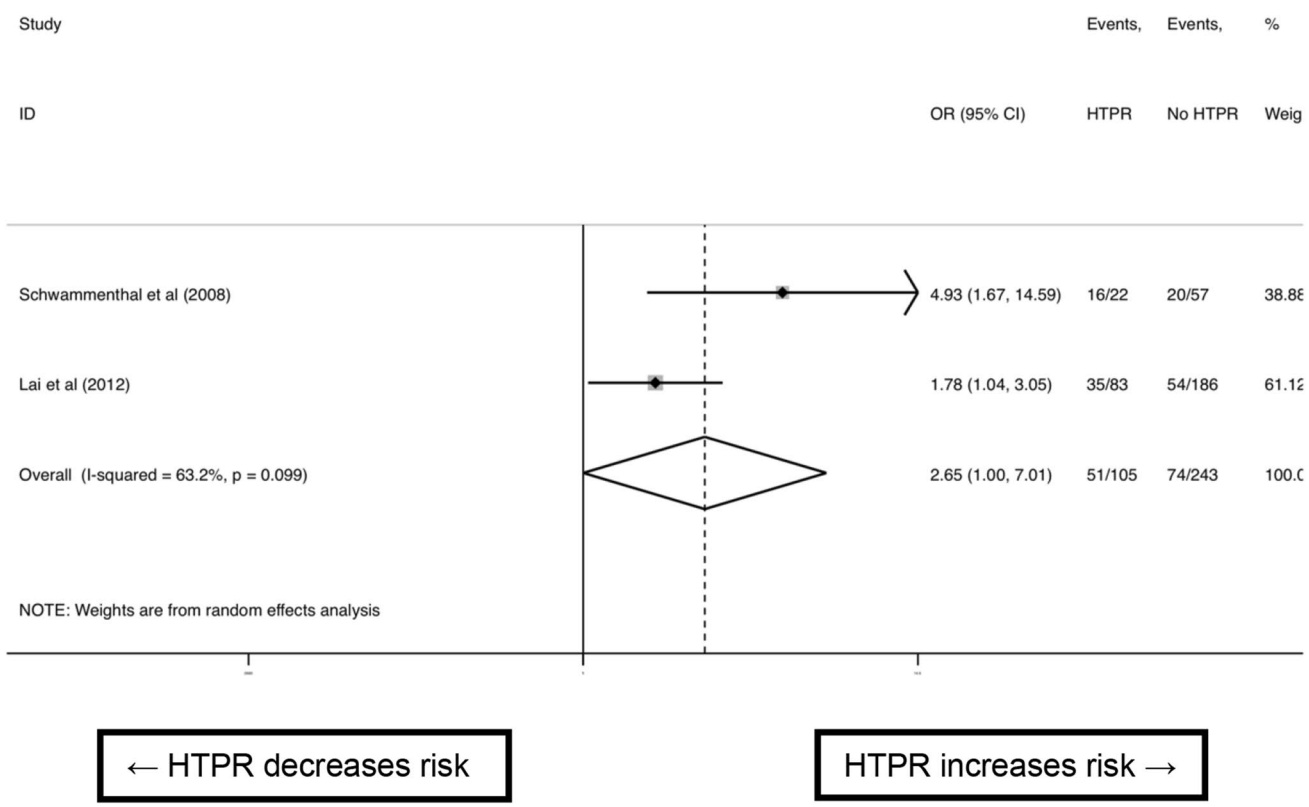

Fig. 4 Analysis of the odds of having a severe disabling stroke (defined as having an NIHSS > 16) in those with vs. those without antiplatelet–HTPR on aspirin or clopidogrel

Limitations

Our study had some limitations. The meta-analysis was limited by fundamental constraints inherent in some studies, e.g. small sample size and discernible clinical heterogeneity between individual studies which we have outlined above, and we did not conduct an individual patient data meta-analysis. There were differences between studies in the definitions of antiplatelet–HTPR, the timing of measurement of HTPR status following index TIA/stroke and whether or not initial 'loading doses' of antiplatelet agents were prescribed; available information regarding these variables have been described in detail in our supplemental tables. Further analyses are warranted to determine whether time from symptom onset influences results because some prior studies have shown a higher absolute prevalence of aspirin–HTPR in the early vs. late phase after TIA/ischaemic stroke [36]. Because we only included studies which assessed recurrent events during *prospective follow-up after antiplatelet–HTPR testing*, and as recurrent vascular events may occur very early after initial TIA or stroke onset [1], this robustly designed meta-analysis might potentially have underestimated the predictive value of antiplatelet–HTPR testing at predicting early recurrent vascular events in CVD patients. We do not have data on the precise timing of recurrent events/outcomes after symptom onset in patients on individual antiplatelet regimens, but our inclusive approach nevertheless provides useful information that antiplatelet–HTPR testing after TIA or ischaemic stroke overall may predict outcomes. The inherent problem with hyper-acutely assessing antiplatelet–HTPR status is that e.g. aspirin may not have fully inhibited thromboxane biosynthesis if testing is performed too early in the first week after commencing treatment, so assessment of the prevalence of antiplatelet–HTPR status during this time period could produce misleadingly high values. However, future studies and meta-analyses could address the issue of the predictive value of antiplatelet–HTPR testing e.g. after 7 days of treatment and in the more subacute and later phases after symptom onset. The use of enteric coated aspirin might have led to an increased prevalence of aspirin–HTPR overall in studies on CVD patients included in this systematic review and meta-analysis, as has been observed in healthy controls [54], and in obese patients with type II diabetes [55]. We did not have sufficient data on the formulation of aspirin prescribed in several studies to reliably comment further on this issue, but future studies should document information on the formulation of aspirin used in individual CVD patients. We could not entirely control for the possibility of

positive publication bias if groups did not publish findings if their data were not predictive of recurrent events/outcomes. However, this comprehensive review included a wide range of positive and negative predictive studies, including those not powered or designed to predict outcomes, thus minimising selection bias in our own systematic review process. Although available published data in some studies may also have limited our ability to perform a thorough assessment of the risk of bias, assessment of studies with the ROBINS-I tool revealed that the majority (85%) had a low or very low risk of bias.

Conclusions

In conclusion, monitoring antiplatelet–HTPR status with platelet function/reactivity assays, in combination with pharmacogenetic testing [22], has the potential to predict the risk of recurrent vascular events and functional outcomes in CVD patients on commonly prescribed antiplatelet regimens. Given the moderate–high heterogeneity between studies, further prospective, adequately-powered, multi-centre studies in diverse geographical populations are urgently needed to address this issue. Such information would facilitate progression to a definitive interventional trial to determine whether altering therapy in individual patients with antiplatelet–HTPR reduces the risk of recurrent vascular events, adverse functional outcomes or improves survival following TIA/ischaemic stroke. However, the current evidence-base does not yet support routine alteration of antiplatelet therapy in clinical practice based on ex vivo antiplatelet–HTPR testing in CVD patients outside of a research study or clinical trial.

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Compliance with ethical standards

Conflicts of interest On behalf of all authors, we state that there are no conflicts of interest to declare.

Ethical approval These data were collated as part of a systematic review and meta-analysis, our Local Research Ethics Committee (LREC) hospital guidelines indicate that formal LREC approval for such a study is not required. However, all data were securely stored in electronic format and no individual patient could be identified from the data contained within. The study and analyses were conducted in accordance with best ethical practice and supervised by experienced Consultants who are International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP)-certified.

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