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The effects of different aspirin dosing frequencies and the timing of aspirin intake in primary and secondary prevention of cardiovascular disease: a systematic review

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INTRODUCTION

Enhancing the effectiveness of aspirin by tailoring administration regimens is an important question amongst health professionals. We conducted a systematic review to evaluate the evidence on the effects of different aspirin regimens in terms of timing (chronotherapy) or frequency of dosing in the prevention of cardiovascular disease. Only 2 out of the 28 included studies reported long-term cardiovascular outcomes highlighting an evidence gap that future research should address. The remaining 26 studies used surrogate outcomes.

INTRODUCTION

Cardiovascular disease (CVD) continues to be a leading clinical and public health problem worldwide, accounting for around 17.5 million deaths each year (1). Once-daily administration of low-dose aspirin (around 75-100mg) is the most commonly used antiplatelet treatment for secondary prevention of CVD as it reduces the risk of major cardiovascular events (MACE) by 25% (2, 3). The use of aspirin in primary prevention of CVD has a more controversial risk-benefit profile and is not routinely recommended (4-6).

Despite the known benefits of aspirin, some patients experience recurrences of ischemic events (4). Poor compliance with treatment may be one explanation (7, 8), however, differences in co-morbidities, co-medications and kinetics of aspirin targets could also account for a variable response (9, 10). Accelerated platelet function recovery may also account for variability in platelet responsiveness, especially in patients with increased platelet turnover, e.g. in diabetes, essential thrombocythaemia (ET) and coronary artery disease (CAD) (11-16).

Chronotherapy studies have postulated that aspirin intake at bedtime instead of on awakening could potentially lead to greater benefits in some patients by reducing morning platelet reactivity, improving blood pressure (BP) profile, and subsequently reducing incidence of cardiovascular events during the high-risk morning hours (17-19). In addition, some evidence has suggested that increased dosing frequency may benefit patients with suboptimal response to aspirin or where aspirin treatment appears to have been ineffective (20, 21). In contrast, a less frequent administration of aspirin, e.g. every other day, could minimise long-term adverse events, such as bleeding, though it is currently not known in which patient groups this might be beneficial.

Scoping searches identified no recent, methodologically robust systematic reviews on the timing and frequency of dosing of aspirin administration in primary and secondary prevention of CVD. A broad review with some systematic methodological elements was identified, which covered the chronotherapy aspect of a range of drugs, including aspirin (searches up to 2011) (22). However, the robustness of the overall findings was uncertain

due to methodological limitations in the review, including restrictions placed on publication language and date, and a lack of quality assessment of reviewed studies. A further systematic review and meta-analysis from 2011 on timing of aspirin administration was identified, however, this appears to be published in abstract form only and full details on methodology could not be ascertained (23). Thus, the aim was to undertake an up-to-date, methodologically robust systematic review of the evidence on alternative timing and dosing regimens of aspirin used in primary and secondary prevention of CVD. This was split into two research questions:

- The effect of timing of aspirin intake (e.g. morning versus evening) on primary and secondary prevention of CVD.
- The effect of altering the frequency of aspirin intake (e.g. once- versus twice-daily (or more) or alternate-day dosing) on primary and secondary prevention of CVD.

TAXONOMY OF STUDIES ASSESSING ASPIRIN REGIMENS' EFFECTIVENESS

The search strategy identified 4,272 records; 28 studies were eligible for inclusion and informed the analysis (see Figure 1). 12 studies (19, 24-34) investigated the effects of aspirin when administrated once-daily in the morning/after awakening versus in the evening/at bedtime; 12 studies (15, 35-45) compared aspirin administration once-daily with two or more times daily; and 4 studies (46-49) compared once-daily versus alternate-day (or less frequent) aspirin dosing. Table 1 shows the main study characteristics.

Studies reporting primary outcomes

Morning versus evening administration

No studies were identified that compared morning versus evening administration of aspirin and reported cardiovascular events or mortality in any population.

Once- versus twice-daily (or more) administration

Only one study was identified. The UK transient ischemic attack (UK-TIA) trial published in 1991 (44) randomised patients with a transient ischaemic attack (TIA) or minor ischaemic stroke to twice-daily aspirin (2x 600mg = 1200mg), once-daily aspirin (300mg) or placebo. There were no significant differences between aspirin regimens for vascular and non-vascular deaths, stroke, myocardial infarction or MACE, though there were significantly fewer upper gastrointestinal symptoms with the lower once-daily dose regimen. This study was deemed to be at low risk of bias overall (see supplementary file Table 1); however, due to the substantial difference in overall daily dose, it was not possible to derive any conclusions for a twice- versus once-daily dosing regimen.

Once-daily versus alternate-day (or less frequent) administration

One RCT (48) from 1999 found a statistically significant reduction of MACE in patients with primary atrial fibrillation when 125mg aspirin was administered on alternate-days compared to once-daily; the difference between regimens was not statistically significant for ischaemic stroke. Due to the unclear risk of bias of the current study (see supplementary file Table 1) and the absence of additional studies supporting a possible benefit from an alternate-day aspirin administration in patients with atrial fibrillation, no firm conclusions could be drawn.

Adverse events

Five studies (19, 25, 28, 33, 35) (4 from morning versus evening, 1 from once- versus twice-daily group) reported that patients did not experience any adverse events following different aspirin regimens but without any further details. Specific adverse events, including heartburn, headache, gastric and haemorrhagic side effects, were reported in 6 studies (24, 26, 35, 36, 44, 48) (2 from morning versus evening, 3 from once- versus twice-daily, 1 from alternate-day group) with event frequencies similar across different aspirin regimens. The remaining 18 studies provided no details on adverse events during aspirin treatment.

Studies reporting secondary/surrogate outcomes

24 of the 28 studies included in the analysis reported surrogate outcomes such as BP and PFTs. 2 further studies reported other outcomes such as frequency of cutaneous flushing (24) and incidence of colorectal cancer (47) in people with diabetes.

Effect on blood pressure

Morning versus evening administration

12 studies (see Table 1) were identified: 9 were RCTs (4 parallel (19, 25, 28, 29) and 5 cross-over (26, 27, 30, 31, 33)) and 3 non-randomised controlled trials (1 parallel (24) and 2 cross-over (32, 34)). Studies were heterogeneous in terms of population (untreated or treated hypertension, CVD), duration of treatment (from 5-7 days to 1 year) and outcome (BP, PFT or cutaneous flushing).

9/12 studies reported 24h or 48h mean ambulatory blood pressure measurements (ABPM) (19, 25-30, 33, 34). Of those, 4 parallel RCTs (19, 25, 28, 29) conducted by the same research group favoured aspirin administration in the evening, with most results being statistically significant (see Figure 2). All 4 studies included untreated grade 1 (mild) hypertensives or pre-hypertensives that were on average much younger compared to the populations in the cross-over trials discussed below. It has been suggested that lack of nocturnal BP decline ("non-dipping") may be an independent indicator of increased cardiovascular risk (50); only 1/4 studies (19) performed a sub-group analysis in dipper versus non-dipper patients, which showed a similar benefit for evening intake. A further small, short-term cross-over trial (33), also in pre-hypertensives, found no difference between morning and evening intake.

The 4 remaining cross-over studies (26, 27, 30, 34) found that the effect of aspirin was not influenced by the timing (see Figure 2). The populations included in these studies were treated hypertensives (with co-morbidities such as diabetes/renal failure (27) and obesity (34)) or individuals with already established CVD (26, 30). Patients in these studies were older compared to those in the parallel RCTs, and on co-medication for hypertension.

Analysis of the mean nocturnal measurements in 8/9 studies (19, 25-28, 30, 33, 34) (data not shown) mirrored the 24hr mean ABPM results. Data from studies reporting outcomes other than ABPM are shown in supplementary file Table 2 (24, 26, 30-32).

It was speculated that study design could have an effect on findings, as the 4 studies (19, 25, 28, 29) showing a benefit on BP from evening aspirin administration were parallel trials, whilst the 5 studies (26, 27, 30, 33, 34) showing no difference had a cross-over design. However, 3/5 (26, 30, 33) cross-over trials either had a washout period or accounted for potential treatment period effects in their analysis (see supplementary file Table 1 for full details of quality assessment). There were no other obvious methodological differences, e.g. in terms of % of drop-outs, between the parallel and cross-over studies, with most being open-label trials, but with blinded endpoint assessment (PROBE design). All but 1 study (30) did not conduct an intention-to-treat analysis. Overall it appears more likely that differences in study population account for the differences between studies as opposed to methodological issues, though a lack of rigour in some methodological aspects may have influenced the robustness of findings.

Effect on platelet function

Morning versus evening administration

3 cross-over studies (26, 30, 32) reported platelet function inhibition as related to timing of aspirin administration (see supplementary file Table 2). In one trial (30), no difference was apparent, while in another study (26), evening administration of aspirin statistically significantly reduced morning platelet reactivity in all patients except those with diabetes. An inhibitory effect of evening administration on platelet reactivity was also observed in the Li et al. trial (32).

Once- versus twice- daily (or more) administration

The 11 studies (15, 35-43, 45) (see Table 1) comparing a different frequency of daily dosing were heterogeneous in terms of study design, duration of treatment and population

(diabetes, CAD, CVD, ET); all were short-term studies with up to 2 months per treatment period reporting as their main outcome PFT results, mainly light transmission aggregometry (LTA), serum thromboxane levels and VerifyNow Aspirin. Where possible, these results have been presented in forest plots (see Figures 3, 4), with the remaining results tabulated (see supplementary file Tables 3-7). A distinction has been made between studies comparing the same or a different overall daily dose.

For comparisons of the same overall dose, most results across 5 studies (36, 37, 39, 40, 42) did not show statistically significant differences in platelet function; 2 studies (15, 39) found a significant difference (favouring twice-daily dosing) with one but not the other of two PFTs used respectively. There is thus little evidence to suggest a potential benefit from twice-daily dosing in these populations (Type2 diabetes mellitus (T2DM) with or without CAD/CVD, or ischemic heart/cerebrovascular disease (IHD/ICD)). The daily dose in all the studies was higher (≥100mg) than what could be considered standard-of-care (up to 162mg depending on the country of study).

Two studies (38, 41) included only ET patients and suggested a potential benefit from twice-daily dosing based on PFTs (see Figure 3 and supplementary file Tables 4, 5), with most findings statistically significant. Increased platelet turnover may explain a potential benefit from twice-daily dosing in this population (51, 52).

Where the combined (split) dose was higher than the single dose, there were mostly statistically significant differences in platelet function in favour of split dosing across populations: for 4/5 studies (15, 36, 41, 42) (serum thromboxane; see Figure 4 and supplementary file Table 4), 3/4 studies (36, 37, 41) (VerifyNow; see Figure 4 and supplementary file Table 5), 2/2 studies (35, 36) (PFA-100; see supplementary file Table 6) and 3/3 studies (36, 42, 43) (WBA; see supplementary file Table 7). This was not the case for LTA as 4/5 studies (36, 37, 41, 43) did not find a significant difference between aspirin regimens (see Figure 3 and supplementary file Table 3). One study (43) found a significant difference in favour of twice-daily dosing even though the overall dose (2x 75mg) was smaller than the once-daily dose (320mg). Another study (45) suggested that aspirin once-

daily (125mg) compared to three times daily (3x 125mg) improved circadian rhythm fluctuations of haemocoagulation. Overall it was not possible, however, to distinguish between the potential impacts from a different daily dose and/or the split dosing element. There were a number of methodological concerns across studies, which may influence the robustness of findings, e.g. a lack of washout period and a lack of detail on blinding and intention-to-treat analysis (see supplementary file Table 1). The overall findings of a potential benefit of twice-daily dosing in an ET population compared to the other populations should therefore be seen as indicative only; however, it is also unlikely that the difference observed was due to particular methodological differences between study designs.

Once-daily versus alternate-day (or less frequent) administration

The 3 studies (46, 47, 49) identified in this group that reported secondary outcomes were extremely heterogeneous in terms of study design (prospective versus retrospective, cohort, non-randomised trials), population (cerebral thrombosis, high on-aspirin treatment platelet reactivity patients, diabetes), aspirin dose/frequency, duration of treatment and outcome measure (PFT, colorectal cancer; see Table 1). That limited evidence precluded any conclusions regarding the effectiveness of a daily versus alternate-day regimen.

CONCLUSIONS AND FUTURE PROSPECTS

Summary of evidence

Despite analysing 28 controlled studies, this systematic review has failed to find any substantial evidence on the effect of different aspirin regimens on long-term clinical outcomes in individuals prescribed aspirin for primary or secondary prevention of CVD. Only 2/28 studies reported long-term cardiovascular outcomes: the large UK-TIA trial (44) (once- versus twice-daily), and found no overall difference in cardiovascular events or deaths during a 4 years follow-up in a TIA population; there was a substantial difference in overall daily

aspirin dose (300mg versus 2x 600mg). In the Posada et al. trial (48) low-dose aspirin given on alternate-days was proven to be more efficient than daily dosing in preventing MACE in people with atrial fibrillation. The remaining 26 studies encompassed a range of different populations with a variety of co-morbidities; these studies presented secondary/surrogate outcomes mainly relating to BP and/or PFTs. There was some evidence, based on 4 parallel RCTs (total n=835), that evening compared to morning intake of aspirin significantly reduced ambulatory BP in untreated mild hypertensives and pre-hypertensives. In a population of treated hypertensives or in those with established CVD, aspirin administration either in the morning or in the evening did not seem to have a differential effect on ambulatory BP levels (based on 4 cross-over studies, total n=432).

A limited amount of evidence (from 2 cross-over trials n=47-53) suggested a potential benefit from twice-daily dosing for ET patients based on PFT results. There was little evidence to suggest a potential benefit from twice-daily (or more) dosing in other populations (T2DM (with or without CAD/CVD) or with IHD/ICD). Several studies reporting once- versus twice-daily (or more) dosing did not compare the same overall daily dose, therefore confounding evaluation of the split dosing aspect.

There was very limited evidence on once-daily versus alternate-day aspirin intake and studies were clinically and methodologically heterogeneous; meaningful conclusions could not be drawn.

Strengths and limitations of the systematic review and available data

To our knowledge, this is the first comprehensive systematic review looking at different timing and dosing frequencies of aspirin administration in a diverse patient population. A robust systematic review methodology and sensitive search strategy mean that it is unlikely that relevant studies have been missed, though formal assessment of publication bias was not feasible. Heterogeneity between studies, particularly in terms of population, precluded pooling in meta-analysis, but results were presented graphically where possible and supplemented with tabulated results.

Some methodological concerns were noted across included studies but the main limitation of the available evidence was the lack of long-term studies in patients prescribed aspirin for primary or secondary prevention that report clinical outcomes. Whilst surrogate outcomes such as BP and PFTs might be considered to be associated with future risk of cardiovascular events, these cannot replace traditional clinical endpoints and have their own limitations. However, findings from such studies can be used to inform the feasibility and design of longer-term studies. Further, compliance may be an issue in studies assessing dosing frequency regimens. Although compliance was assessed in most of the included studies, findings were generally not clearly reported or not reported at all.

Implications of findings

Despite the large number of patients on aspirin currently being managed in primary care, this systematic review has highlighted the lack of evidence on the effect of different aspirin regimens, in terms of timing and frequency of administration, on long-term cardiovascular outcomes. Those differences in effect observed based on surrogate end points should be interpreted with caution due to the limited evidence in different populations and some methodological concerns within studies. Thus, the current level of evidence does not warrant a change in clinical practice.

The studies conducted by the Hermida et al. group were suggestive of a favourable effect of evening aspirin intake on BP in untreated hypertensives; however, these are not necessarily representative of patients most at risk of cardiovascular events and such findings are unlikely to have an impact on current recommendations. Indeed, according to European and American guidelines, aspirin is not recommended in low-to-moderate risk hypertensives (without co-morbidities) aged below 50 years (3, 53, 54). The studies finding no difference in effect of morning or evening dosing on BP were in patients already treated with BP lowering agents. It may be that any potential differences in effect from morning versus evening aspirin intake are too small to be observed where BP is already controlled by another agent, i.e. there may be a ceiling effect to how much difference timing of aspirin can make.

A limited amount of evidence suggests that patients with ET may benefit from twice-daily dosing. There currently appear to be no recommendations on the frequency of aspirin administration in this population, and the evidence identified in this systematic review is relatively sparse, but future research focusing on longer-term outcomes may be worthwhile in patients suffering from ET.

Unanswered questions and future research

Whether or not aspirin enhances the effects of hypertensive medication in a population of essential hypertensives is still uncertain. A systematic review summarising the data on the potential antihypertensive effects of aspirin found that short-term use of low-dose aspirin doesn't seem to modify the effect of antihypertensive drugs (55); however, an increase in the risk of hypertension (about 20%) among long-term aspirin users was observed. Therefore, the effect of aspirin on BP is unclear, and this mechanism is unlikely to be a major contributor to aspirin's efficacy in prevention of major adverse cardiovascular events, in addition to its well-characterised effect on platelet function.

A significant number of studies investigating the split dosing regimen neither kept the overall daily dose the same between once- and twice-daily groups nor used the standard care low-dose of aspirin; thus, this is something that investigators need to consider when designing new trials. While most guidelines recommend doses of 75-100mg daily (3, 53, 56), some of the studies have used doses in excess of 325mg daily. At low doses, the effect of aspirin is predominantly on the platelet cyclooxygenase I (COX-1) enzyme, with little to no effect on inflammatory pathways mainly mediated through inducible COX-2 (57). However, at doses in excess of 325mg daily, especially when multiple doses per day are administered, the antithrombotic effect cannot be dissociated with the anti-inflammatory effect of aspirin. Although most included studies used anti-thrombotic aspirin doses (75-325mg daily), some studies in the context of cerebrovascular disease used doses in excess of 325mg (44, 46). It is therefore possible that the effect of aspirin in this context may be due to other mechanisms of action than its intended use as an antithrombotic agent. In addition, although

most of the studies mention that patients did not show any adverse events during aspirin therapy, the use of high doses could potentially increase the risk for bleeding and change the balance between any positive and harmful effects that aspirin might have.

A thorough search in ongoing trial registries has identified only one study that could potentially address some of the questions above. An ongoing trial by Herimida R.C. and Ayala D.E. (NCT 00725127) is investigating the effects of chronotherapy with low-dose aspirin in a population with impaired fasting glucose or T2DM on primary prevention of CVD. This study is unique in focusing on cardiovascular, cerebrovascular and renal fatal and non-fatal events after 5 years of aspirin chronotherapy. No ongoing trials were found in patients in other important risk categories (such as AF, stroke and heart failure) or in populations using aspirin for secondary prevention and this could be an unmet research need.

In conclusion, enhancing the effectiveness of aspirin for the prevention of CVD by tailoring administration regimens is an important question, and one that has been addressed in 28 studies with heterogeneous populations. The vast majority used surrogate outcomes and based on these there is limited evidence indicative of a benefit from evening administration in a primary prevention population; this could not, however, be demonstrated in a population taking aspirin for secondary prevention. There is also a clear evidence gap in terms of the effect of different aspirin regimens on long-term cardiovascular outcomes in both primary and, perhaps more importantly, in secondary prevention. Future randomised controlled trials, which control for daily aspirin dose in addition to timing and frequency, could assess the long-term clinical utility of alternative aspirin dosing strategies in this population.

SYSTEMATIC REVIEW STRATEGY

Systematic review methodology and reporting were based on the Cochrane Collaboration handbook (58) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (59). The review protocol was registered with PROSPERO (CRD42014010596) and published in BMC Systematic Reviews (60).

Search strategy

MEDLINE, MEDLINE In Process, EMBASE, CINAHL, The Cochrane Library, Science Citation and Conference Proceedings Citation Index (Web of Science), and ZETOC (British Library) were searched with no language restrictions to June 2015 (see supplementary file Appendix 1 for sample search strategy). Reference lists of relevant studies were checked. Selected websites and clinical trials registries were searched for unpublished and ongoing studies.

Selection criteria

Two reviewers independently screened articles for eligibility using predetermined criteria. Any controlled (non-)randomised studies were eligible if they included patients prescribed aspirin for primary or secondary prevention of CVD. Study selection was not restricted by underlying conditions (e.g. established CVD, diabetes, hypertension, dyslipidaemia, essential thrombocythaemia or atrial fibrillation). Studies involving patients in an acute (post-operative) setting were not analysed (~~59~~ 61-63).

There were no restrictions on doses being compared provided there was a difference in dosing timing (e.g. in the morning compared to the evening) or frequency (e.g. twice or more per day versus once per day, alternate-day versus every day). There were no restrictions on study selection by outcome report. For the review, outcomes such as cardiovascular events, mortality, and adverse events (e.g. bleeding) were considered of primary importance, and surrogate end points such as BP and platelet function measured with a platelet function test (PFT) were secondary.

Data extraction and quality assessment

Data extraction was conducted by one reviewer using a standardised, piloted data extraction form and checked by a second reviewer. Study authors were contacted if further information or clarifications were required. Quality assessment was based on the Cochrane risk of bias

tool (64). For cross-over trials additional risk of bias, such as carry-over effects, were assessed (see supplementary file Table 1).

Data synthesis and analysis

Data for analysis was taken as reported from the published articles or as supplied by the authors. Heterogeneity between studies in design, population characteristics and duration of treatment precluded meta-analysis. However, where sufficient data were available, results for each outcome were presented in forest plots for illustrative purposes without a pooled summary estimate. Results not represented in forest plots were tabulated and described. It was not possible to formally assess the potential for publication bias.

ABBREVIATIONS

ABPM: ambulatory blood pressure measurements

BP: Blood pressure

CAD: coronary artery disease

CVD: Cardiovascular disease

ET: essential thrombocythaemia

IHD/ICD: ischemic heart/cerebrovascular disease

MACE: major cardiovascular events

PFT: Platelet function test

RCT: Randomised controlled trial

T2DM: Type 2 diabetes mellitus

TIA: transient ischemic attack

UK-TIA: United Kingdom - transient ischemic attack

STUDY HIGHLIGHTS

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? Routine use of daily low-dose aspirin is known to be beneficial for secondary prevention of cardiovascular disease, though there is uncertainty regarding primary prevention.

WHAT QUESTION DID THIS STUDY ADDRESS? Are there more effective aspirin regimens – in terms of timing and frequency – than once-daily morning dosing?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE? The first comprehensive systematic review of the evidence on different aspirin regimens used in primary and secondary prevention of cardiovascular disease. Limited evidence based on surrogate endpoints is suggestive of a benefit of evening/twice-daily dosing regimens in specific (primary prevention) populations; a small amount of evidence does not suggest a differential effect in secondary prevention.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE? There is an evidence gap in terms of the effect of different aspirin regimens on long-term cardiovascular outcomes. This calls for better-standardised studies to assess the long-term clinical utility of alternative aspirin dosing strategies in primary and secondary prevention of cardiovascular disease. The current level of evidence does not warrant a change in clinical practice.

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CONFLICT OF INTEREST

The authors declared that they have no competing interests to disclose.

AUTHOR CONTRIBUTIONS

DB, JD, SS and DM developed the methodological strategy of the project. SB developed the search strategy. ML and JH provided clinical and methodological advice. DB was the main reviewer; ML, JH, SS and DM contributed to study selection and data extraction; JD led all aspects of the review. DB, ML and JD drafted the manuscript. All authors contributed to the research, and approved the final manuscript. DF is the principal investigator and guarantor.

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FIGURE LEGENDS

Figure 1. PRISMA flow diagram for study selection

Figure 2. 24hr mean systolic and diastolic ambulatory BP differences between morning and evening aspirin intake in chronotherapy studies.

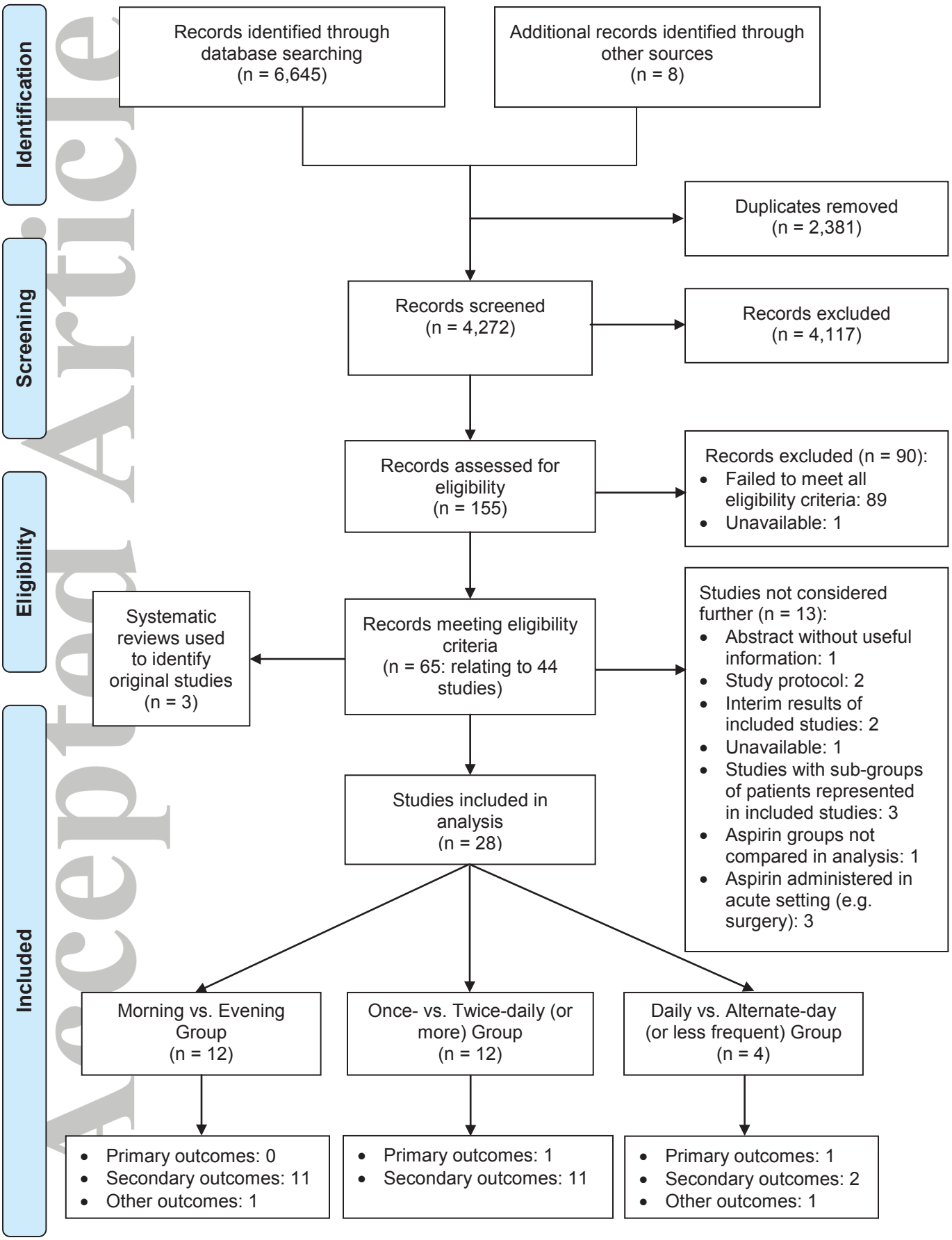
CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic blood pressure; HTN, hypertension; RCT, randomised controlled trial; SBP, systolic blood pressure; SD, standard deviation; WMD, weighted mean difference; [a]studies reporting 48hr mean systolic and diastolic ambulatory BP.

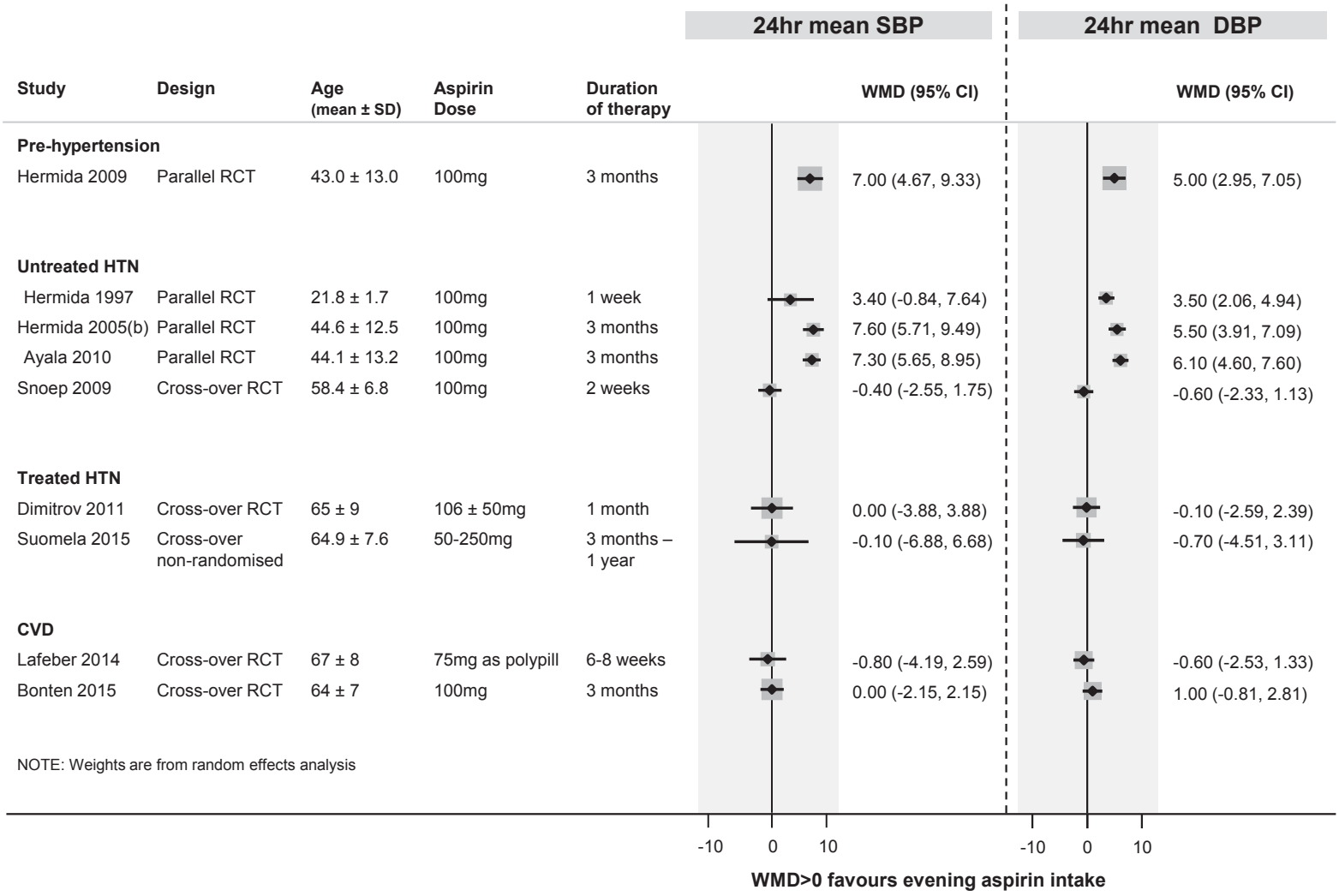
Figure 3. Light transmission aggregometry (LTA) data from studies looking at different aspirin dosing frequencies.

Forest plot illustrating mean difference in percentage of platelet aggregation in response to 0.5-1.3mM arachidonic acid (AA) as measured by LTA. CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; ET, essential thrombocythaemia; RCT, randomised controlled trial; T2DM, type 2 diabetes mellitus; WMD, weighted mean difference.

Figure 4. Serum TxB_2 and VerifyNow data from studies looking at different aspirin dosing frequencies.

Forest plot on the left illustrating mean difference in serum thromboxane levels (ng/ml) and forest plot on the right illustrating mean difference in aspirin reaction units (ARU) as measured with the VerifyNow analyser. CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; ICD, ischemic cerebrovascular disease; IHD, ischemic heart disease; RCT, randomised controlled trial; T2DM, type 2 diabetes mellitus; WMD, weighted mean difference. [a]no VerifyNow data available for those studies; [b]no serum TxB_2 data available for that study.

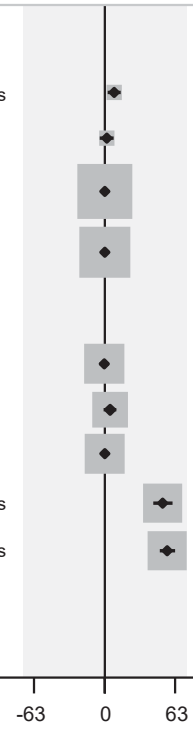




LTA (% aggregation)

Study	Main underlying disease	Aspirin Dose	Duration of therapy	WMD (95% CI)
Same overall dose				
Dillinger 2012(a) (cross-over RCT)	T2DM with CAD	150mg vs 2x 75mg	mean 10 ± 2 days	8.50 (2.83, 14.17)
Bethel 2015 (cross-over RCT)	T2DM without CVD	200mg vs 2x 100mg	2 weeks	2.00 (-4.12, 8.12)
Capodanno 2011 (cross-over non-random.)	T2DM with CAD	162mg vs 2x 81mg	1 week	0.00 (-0.38, 0.38)
Capodanno 2011 (cross-over non-random.)	T2DM with CAD	325mg vs 2x 162mg	1 week	0.00 (-0.69, 0.69)
Different overall dose				
Spectre 2011 (cross-over RCT)	T2DM + vascular complications	75mg vs 2x 75mg	2 weeks	-0.20 (-4.06, 3.66)
Bethel 2015 (crossover RCT)	T2DM without CVD	100mg vs 2x 100mg	2 weeks	2.00 (-3.73, 7.73)
Capodanno 2011 (cross-over non-random.)	T2DM with CAD	81 mg vs 2x 81mg	1 week	0.00 (-0.45, 0.45)
Dillinger 2012(b) (cross-over non-random.)	ET	250mg vs 2x 100mg	mean 15 ± 5 days	52.00 (43.39, 60.61)
Dillinger 2012(b) (cross-over non-random.)	ET	100mg vs 2x 100mg	mean 15 ± 5 days	56.00 (49.07, 62.93)

NOTE: Weights are from random effects analysis



WMD>0 favours twice-daily aspirin intake

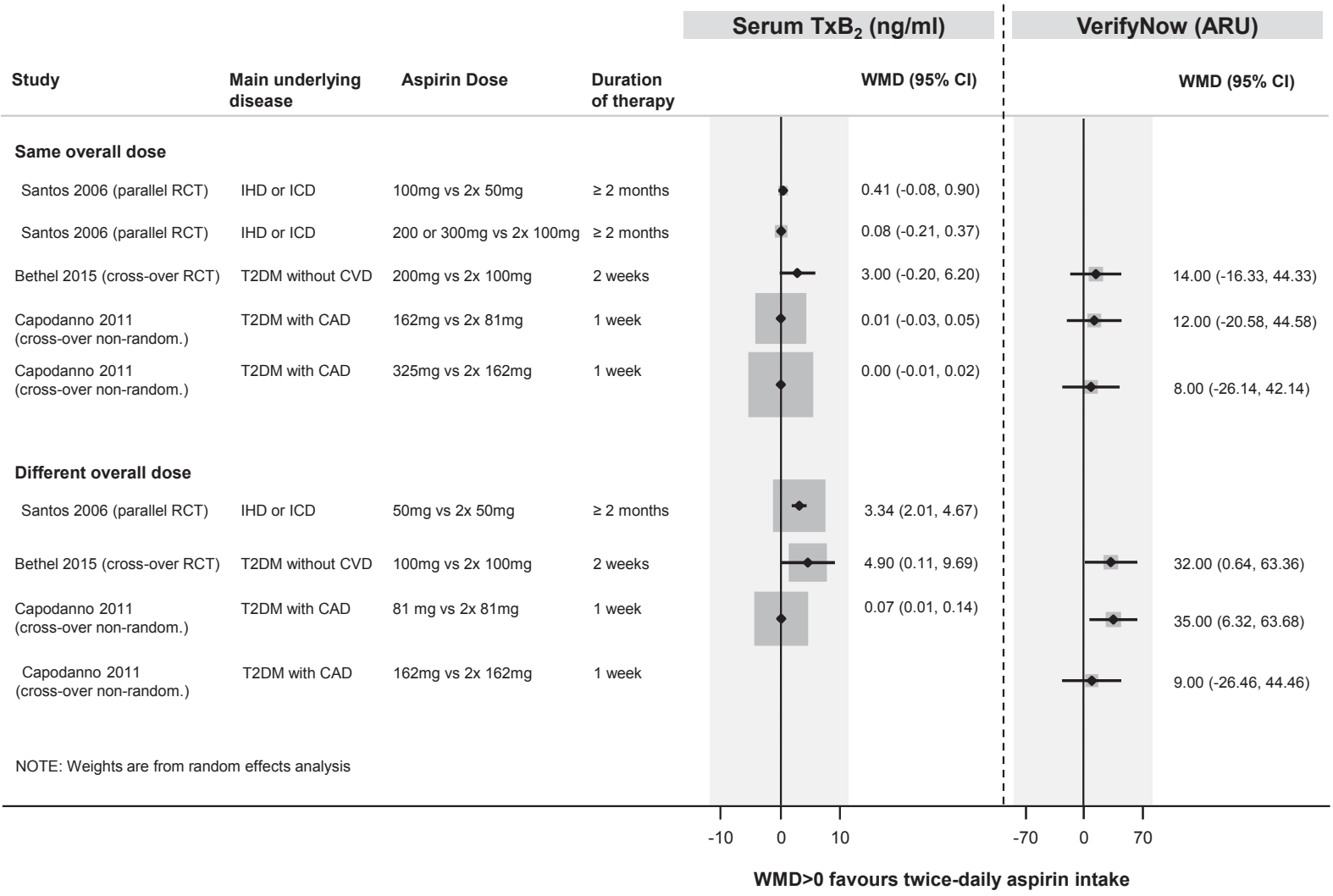


Table 1. Main characteristics of included studies

Table 1. Main characteristics of included studies

a. Morning versus evening aspirin administration

Study/ year/ country	CVD prevention	Main underlying condition	Age, years (mean ± SD)	Study arms (n=)	Aspirin dose/ frequency	Duration of therapy	Outcome measure
Parallel RCTs							
Hermida et al., 1997, Spain [29]	Primary	Untreated grade 1 (mild) essential hypertension	21.8 ± 1.7	1. 2hr after awakening (n=4) 2. 7-9hr after awakening (n=6) 3. 2hr before bedtime (n=8)	100mg OD	1 week	Ambulatory BP
Hermida et al., 2005(b), Spain [19]	Primary	Untreated grade 1 (mild) essential hypertension	44.6 ± 12.5	1. On awakening (n=126) 2. At bedtime (n=131) Subgroup for (non-)dippers	100mg OD	3 months	Clinic and ambulatory BP
Hermida et al., 2009, Spain [28]	Mainly Primary	Pre-hypertension	43.0 ± 13.0	1. On awakening + HDR (n=61) 2. At bedtime + HDR (n=59) 3. HDR only (n=124)	100mg OD	3 months	Clinic and ambulatory BP
Ayala & Hermida, 2010, Spain [25]	Mainly Primary	Untreated grade 1 (mild) essential hypertension	44.1 ± 13.2	1. On awakening (n=159) 2. At bedtime (n=157) Also subgroup for sex	100mg OD	3 months	Clinic and ambulatory BP
Cross-over RCTs							
Snoep et al., 2009, Netherlands [33]	Primary	Untreated grade 1 (mild) essential hypertension	58.4 ± 6.8	1. On awakening (n=16) 2. At bedtime (n=16)	100mg OD	2 weeks	Ambulatory BP
Dimitrov et al., 2012, France [27]	Primary	Treated essential hypertension	65.0 ± 9.0	1. On awakening (n=75) 2. At bedtime (n=75)	106 ± 50mg OD (mean ± SD)	1 month	Ambulatory BP
Lee et al., 2011, Korea [31]	Mainly Primary	Treated essential hypertension	54.8 ± 7.8	1. On awakening (n=109) 2. At bedtime (n=108) Subgroup for (non-)dippers	100mg OD	12 weeks	Clinic BP
Lafeber et al., 2015, Netherlands [30]	Mainly Secondary	Established CVD or at high risk of having a CV event	67.0 ± 8.0	1. Morning polypill (n=78) 2. Evening polypill (n=78) 3. Polypill individual agents(n=78)	Polypill containing 75mg aspirin OD	6-8 weeks	Clinic and ambulatory BP; PFT (VerifyNow)
Bonten et al., 2015, Netherlands [26]	Primary and secondary	Mixed population already using low-dose aspirin for prevention of CVD	64.0 ± 7.0	1. 1hr after awakening (n=263) 2. 1hr before bedtime (n=263)	100mg OD	3 months	Clinic and Ambulatory BP; PFT (VerifyNow)
Parallel non-randomised controlled trials							
Alves et al., 2008, Austria/ Germany/ Ireland/ Portugal/ Switzerland [24]	Primary and secondary	Elevated cardiovascular risk mainly due to CVD or T2DM	61.4 ± 10.6 (morning study arm), 60.4 ± 10.7 (evening study arm)	1. In the morning (n=227) 2. In the evening (n=312)	75-100mg OD	15 weeks	Frequency of cutaneous flushing
Cross-over non-randomised controlled trials							
Li et al., 2010, China [32]	Secondary	Acute coronary syndrome	54.9 ± 10.2	1. On awakening (n=30) 2. At bedtime (n=30)	100mg OD	5-7 days	PFT (WBA)
Suomela et al., 2015, Finland [34]	Primary	Treated essential hypertension	64.9 ± 7.6	1. On awakening (n=32-34) 2. At bedtime (n=32-34)	50 – 250mg OD	3 months – 1 year	Clinic, home and ambulatory BP

b. Once- versus twice-daily (or more) aspirin administration

Study/ year/ country	CVD prevention	Main underlying condition	Age, years (mean ± SD)	Study arms (n=)	Duration of therapy	Outcome measure
Parallel RCTs						
UK-TIA study group, 1991, UK [44]	Secondary	Recent TIA or minor ischaemic stroke	60 ± 8.92 (OD study arm) and 59.9 ± 9.16 (BID study arm)	1. 300mg OD (n=806) 2. 600mg BID (n=815)	Mean 4 years (1-7)	Mortality, ischemic/haemorrhagic stroke, MI, MACE, bleeding
Zaslavskaia et al., 2002, Russia and Kazakstan [45]	Assume primary	IDDM	23.3 ± 7.7	1. 125mg OD (n=15) 2. 125mg TID (n=15)	16 days	24hr profile of hemocoagulation
Rocca et al., 2012, Italy [15]	Primary and secondary	T2DM	Median (IQR) 64.6 (60.7-69.0)	1. 100mg OD (n=11) 2. 200mg OD (n=11) 3. 100mg BID (n=11) Subgroup without T2DM	29 days	PFT (VerifyNow, serum/urinary TxB_2)
Cross-over RCTs						
Spectre et al., 2011, Sweden [43]	Primary	T2DM with micro- or macro-vascular complications	Median (range) 64 (51-75)	1. 75mg OD (n=24) 2. 75mg BID (n=25) 3. 320mg OD (n=24)	2 weeks	PFT (WBA, IMPACT-R, LTA), urinary TxB_2, clinic BP
Pascale et al., 2012, Italy [41]	Mainly primary	ET with aspirin insensitive-platelet $TxB_2 \geq 4$ ng/ml	Median (IQR) 51 (29-67)	1. EC 200mg OD (n=15-21) 2. EC 100mg BID (n=15-21) 3. Plain 100mg OD (n=15-21) 4. EC 100mg OD (usual practice) (n=15-21)	7 days	PFT (LTA, VerifyNow, serum/urinary TxB_2)
Dillinger et al., 2012 (a), France [39]	Secondary	T2DM with CAD	64 ± 10	1. 150mg OD (n=92) 2. 75mg BID (n=92)	10 ± 2 days (mean ± SD)	PFT (LTA, PFA-100)
Bethel et al., 2016, UK [36]	Primary	T2DM without CVD	51 ± 7	1. 100mg OD (n=24) 2. 200mg OD (n=24) 3. 100mg BID (n=24)	2 weeks	PFT (VerifyNow, WBA, LTA, PFA-100, serum/urinary TxB_2)
Cross-over non-randomised controlled trials						
DiMinno et al., 1986, U.S.A. [40]	Mainly secondary	Diabetes	39-51 (range)	1. 100mg OD (n=10) 2. 25mg QID (n=10) 3. 330mg OD (n=10) 4. 100mg QID (n=10)	4 weeks	PFT (LTA, serum TxB_2)
Santos et al., 2006, Spain [42]	Secondary	Ischemic heart disease (IHD) or ischemic cerebrovascular disease (ICD)	IHD: 63.79 ± 10.00; ICD: 63.92 ± 10.34	500mg 2-week intervals plus: 1. 50mg OD (n=31) 2. 100mg OD (n=33) 3. 50mg BID (n=78) 4. 100mg BID (n=95) Or 200-300mg OD (usual practice) (n=206)	≥2 months	PFT (WBA, serum TxB_2)
Addad et al., 2010, Tunisia [35]	Secondary	CAD with diabetes	58.4 ± 7.7	1. 100mg OD (n=25) 2. 100mg BID (n=17)	10 days	PFT (PFA-100)
Capodanno et al., 2011, U.S.A. [37]	Secondary	T2DM with CAD	59 ± 7	1. 81mg OD (usual practice) (n=20) 2. 81mg BID (n=20) 3. 162mg OD (n=20) 4. 162mg BID (n=20) 5. 325mg OD (n=20)	1 week	PFT (LTA, VerifyNow, serum TxB_2)
Dillinger et al., 2012 (b), France [38]	Secondary	ET	62 ± 17	1. 100mg OD (n=32) 2. 250mg OD (n=32) 3. 100mg BID (n=32)	15 ± 5 days (mean ± SD)	PFT (LTA)

c. Once-daily versus alternate-day (or less frequent) aspirin administration

Study/ year/ country	Design	Main underlying condition	Age, years (mean ± SD)	Reason for aspirin administration	Aspirin dose /frequency	Duration of therapy/ follow up	Outcome measure
Lejeune et al., 1988, France [46]	Series of sequential interventions given to one group of patients; non-randomised	Cerebrovascular accident of atheromatous ischemic origin	65.0	Secondary prevention of CVD	0.3g, 0.5g and 1-3g daily or every second day (n=14-17)	2 weeks/8 months after last visit	Bleeding time (Ivy method) & PFT (Salzman's method, LTA)
Posada et al., 1999, Spain [48]	Parallel RCT	Primary atrial fibrillation	62.0	Primary & secondary prevention of CVD	125mg daily (n=104) or on alternate-days (n=90)	Long-term treatment /550 days (mean)	Death, CVA, MACE and compliance
Temperilli et al., 2015, Italy [49]	Retrospective comparison of two non-concurrent treatment groups	HAPS patients defined by serum TxB_2 >3.1 ng/ml	68.3 ± 11.6	Primary & secondary prevention of CVD	100-160mg daily (n=132) or on alternate-days (n=48)	For more than one month /retrospective analysis	Serum TxB_2
Lin et al., 2015, Taiwan [47]	Population-based retrospective cohort study	Diabetes	63.47 ± 12.11	Mainly secondary	Cumulative dosage from < 300 to ≥ 2100mg (n=26,494) • ≤ 2 times/ week • 3-5 times/week • > 5 times/week	≤ 1 year to > 5 years /retrospective analysis	Incidence of colorectal cancer

BID, twice-daily; BP, blood pressure; CAD, coronary artery disease; CV, cardiovascular; CVA, Cerebrovascular accident; CVD, cardiovascular disease; EC, enteric coated aspirin; ET, essential thrombocythaemia; HAPS, high on-aspirin treatment platelet reactivity; HDR, non-pharmacological hygienic-dietary recommendations; IDDM, insulin-dependent diabetes mellitus; IQR, interquartile range; LTA, light transmission aggregometry; MACE, major adverse cardiac event; MEA, multiple electrode platelet aggregometry; MI, myocardial infarction; OD, once-daily; PFT, platelet function test; QID, four times a day; RCT, randomised controlled trial; SD, standard deviation; T2DM, Type 2 diabetes mellitus; TIA, transient ischaemic attack; TID, three times a day; TxB_2, thromboxane B_2; WBA, whole blood aggregometry.