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Research Paper

Aspirin for primary prevention of ST segment elevation myocardial infarction in persons with diabetes and multiple risk factors

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ABSTRACT

Background: Controversy exists as to whether low-dose aspirin use may give benefit in primary prevention of cardiovascular (CV) events. We hypothesized that the benefits of aspirin are underevaluated.
Methods: We investigated 12,123 Caucasian patients presenting to hospital with acute coronary syndromes as first manifestation of CV disease from 2010 to 2019 in the ISACS-TC multicenter registry (ClinicalTrials.gov, NCT01218776). Individual risk of ST segment elevation myocardial infarction (STEMI) and its association with 30-day mortality was quantified using inverse probability of treatment weighting models matching for concomitant medications. Estimates were compared by test of interaction on the log scale.
Findings: The risk of STEMI was lower in the aspirin users (absolute reduction: 6·8%; OR: 0·73; 95%CI: 0·65−0·82) regardless of sex (p for interaction=0·1962) or age (p for interaction=0·1209). Benefits of aspirin were seen in patients with hypertension, hypercholesterolemia, and in smokers. In contrast, aspirin failed to demonstrate a significant risk reduction in STEMI among diabetic patients (OR:1·10;95%CI:0·89−1·35) with a significant interaction (p: <0·0001) when compared with controls (OR:0·64,95%CI:0·56−0·73). Stratification of diabetes in risk categories revealed benefits (p interaction=0·0864) only in patients with concomitant hypertension and hypercholesterolemia (OR:0·87, 95% CI:0·65−1·15), but not in smokers. STEMI was strongly related to 30-day mortality (OR:1·93; 95%CI:1·59−2·35)
Interpretation: Low-dose aspirin reduces the risk of STEMI as initial manifestation of CV disease with potential benefit in mortality. Patients with diabetes derive substantial benefit from aspirin only in the presence of multiple risk factors. In the era of precision medicine, a more tailored strategy is required.
Funding: None.

1. Introduction

Aspirin is still on the medication list of a new patient with hypertension and hypercholesterolemia and no history of clinically evident cardiovascular (CV) disease. According to the National Health Interview Survey, about 29 million patients who do not have CV disease take aspirin daily for prevention—and 6.6 million do so without a health care provider recommendation [1]. All of these patients endorse the aspirin use despite the announcement in August 2018 about two clinical trials that found aspirin offered few benefits for healthy adults and might even raise their risk of bleeding, namely the ARRIVE (Aspirin to Reduce Risk of Initial Vascular Events) [2] and the ASCEND (A Study of Cardiovascular Events in Diabetes) [3]. Notably, a large proportion of patients in both studies were taking statins and antihypertensive drugs, and only a small proportion were current smokers.

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Research in context

Evidence before this study

The role of low-dose aspirin in the general population without CV disease remains controversial. The USPSTF 2016 guidelines recommended low-dose aspirin to high-risk individuals aged 50−69 years with 10−year cardiovascular risk ≥10%, regardless of the presence or absence of diabetes. The 2019 Scientific Statement by the American Diabetes Association recommended aspirin therapy for primary prevention for diabetic patients with 10-year CV risk ≥10% provided that these patients are aged more than 50 and less than 70 years and have at least one additional major risk factor. No previous study has estimated the effects of low-dose aspirin on prevention of ST segment elevation myocardial infarction as initial clinical presentation of coronary heart disease in individuals with diabetes alone or diabetes in combination with other conventional risk factors.

Added value of this study

To our knowledge, this study provides the first estimate of benefit of aspirin prophylaxis in patients with diabetes depending on the number and type of associated risk factors. No beneficial effect of aspirin was seen in individuals with diabetes alone and in those who had one additional major conventional risk factor. Low-dose aspirin was insufficient to confer protection in diabetic patients who were smokers. Conversely, prevention of ST segment elevation myocardial infarction through aspirin use was consistent in diabetic individuals who were concomitantly affected by hypertension and hypercholesterolemia.

Implications of all the available evidence

Implications of all the available evidence: According to the National Health Interview Survey, about 29 million patients who do not have clinically CV disease take aspirin daily for prevention, and 6·6 million do so without a health care provider recommendation.

In 2016, about 1·7 million hospital discharges for major cardiovascular diseases were reported with diabetes as any listed diagnosis among US adults including 438,000 for ischemic heart disease.

Clinical and public health efforts should focus on identifying optimal preventive measures for the whole diabetic population and individual patients.

These new studies push the pendulum away from aspirin prophylaxis for primary prevention. However, in our view, differences between benefits and harms are likely to be a razor thin, as balancing one adverse CV event against one bleeding event is not straightforward. For example, an episode of ST segment elevation myocardial infarction (STEMI), is more lethal than a minimally symptomatic gastrointestinal bleeding, whereas some bleeding events, such as severe intracranial haemorrhages, are more lethal than some ischemic CV events, such as episodes of stable or unstable angina. Prospectively, we cannot predict which of those outcomes would apply to any given patient and to any given sex. The role of aspirin in primary prevention has, therefore, become increasingly uncertain and many important questions remain unsolved.

The first question is whether aspirin is reducing the incidence of STEMI, which is the most serious and catastrophic clinical manifestation of coronary heart disease (CHD). Relying on the generic outcome of “myocardial infarction” to ascertain a benefit from aspirin use might be challenging, as reduction in short-term case fatality rates for myocardial infarction in US appears to be driven by a decreased incidence of STEMI [4]. Shifting the mode of initial presentations of CHD from STEMI to non-ST elevation acute coronary syndromes (NSTE-ACS) or to stable angina, may reduce the overall patient risk and ultimately CV mortality.

The second question is whether concomitant preventive medications may blunt the cardio-protective effect of aspirin. Currently, many patients are intensively treated with beta-blockers, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) and statins. Widespread statin use is the norm in clinical practice. Their role in prevention therapy is still unsettled. Concomitant medications may be tracked as both a history item, as well as, during the study. Lack of consistency on concomitant medications reporting makes it difficult to correlate the data with the primary endpoints of the study. While this issue is clearly important, there is no or little information on concomitant medications in prior aspirin prevention trials.

A further question is whether aspirin may have a margin of benefit in specific populations such as healthy older adults [5] or in presence of specific comorbid conditions such as diabetes [3]. In addition, some trials have documented sex-specific heterogeneity of treatment effects [6]. Careful analysis of the data does not allow one to draw firm conclusions on these points. Heterogeneity in the predicted outcome risk deserves further investigations.

The most commonly used method of examining whether treatment effects vary in a trial population is to serially divide patients into subgroups based on potentially relevant characteristics. In the current era, the main problem with this conventional approach is that better management of risk factors has lowered the risk of developing disease and disease severity. Hence, the number of patients that must be enrolled in a trial becomes much greater as lower risk patients are included. Thus, subgroup analyses may be underpowered. On the other hand, a randomized trial cannot be done for every subgroup. One approach that can help to counter this problem is to carry out an aspirin prevention study using a register-based cohort data in a case-control or match weighted design. In such type of studies, groups are defined by the outcome. Researchers then look back to ascertain each person's exposure status to aspirin, and to compare the frequency of outcome in the aspirin group with that in the control group. Investigators would correctly address confounding due to concomitant medications and evaluate the potential incremental value of preventing cardiovascular outcomes by using aspirin on the top of these medications.

The current investigation reflects this approach. All patients had a first clinical presentation of CHD documented by a diagnosis of acute coronary syndromes. The outcome of interest was STEMI, since it is a marker of poorer short-term prognosis. A requirement of the study was that the outcomes of patients exposed to aspirin could not be influenced by other potential prevention therapies. This task was achieved by matching concomitant medications using inverse probability of treatment weighting. Aspirin users versus nonusers had a similar pattern of exposure to concomitant medications.

2. Methods

2.1. Derivation cohort

The International Survey of Acute Coronary Syndromes in Transitional Countries (ISACS-TC; NCT01218776) is a large observational and multinational registry. Methods have been previously described [7,8]. Data were collected from 41 centres in 12 countries. The University of Bologna is the data-coordinating centre and is responsible for the budget and the quality of data delivered to the ISACS-TC. Since information was collected anonymously, institutional review boards waived the need for individual informed consent.

2.2. Patient population

The initial population consisted of 20,189 patients with ACS enrolled between January 2010 and January 2019. Patients

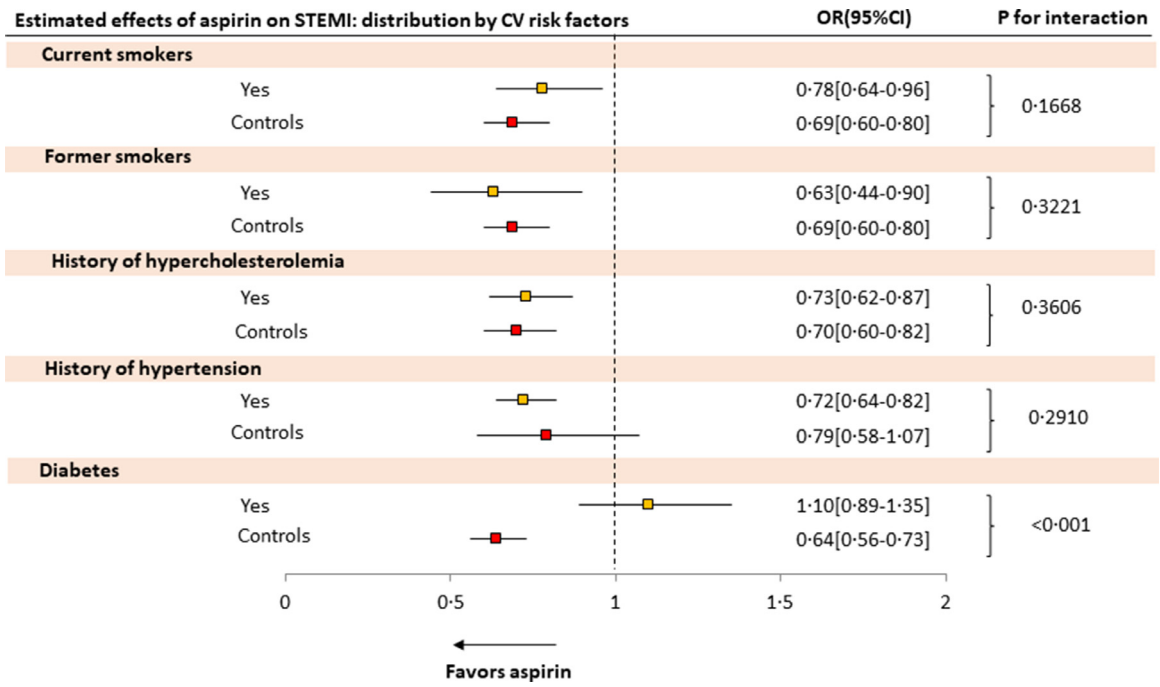

Fig. 1. Estimated effects of aspirin on STEMI: distribution by CV risk factors. Association between use of aspirin before index event and incidence of ST elevation myocardial infarction sorted by the presence of one traditional risk factor. Abbreviations: OR, Odds Ratio; CI, Confidence Interval; CV, cardiovascular; STEMI, ST elevation myocardial infarction.

presenting with a history of a vascular events were excluded. Patients using thienopyridine before index admission were also excluded. The final population consisted of 12,123 patients (60.0% of the overall study population) (Fig. 1 in the Supplement).

2.3. Main outcome measures and definitions

The primary outcome measure was the rate of STEMI at hospital presentation. STEMI is a predictor of heart failure and 30-day mortality. In the current study, heart failure was defined as Killip class≥ 2. We defined prior low-dose (75/150 mg/day) aspirin, statins, ACE inhibitors, ARBs and beta-blockers users as those patients who had taken these medications on a regular basis at least for two weeks before the onset of the qualifying event. Medications received immediately before hospitalization or in the emergency department were not considered prior medication use. We defined multivessel disease as at least two main branches of the epicardial coronary artery with 70% or more stenotic lesions, or a 50% or greater stenosis in the left main coronary artery. Smoking habits, weight and height were self-reported. The specific risks of STEMI were estimated for current and former smokers compared with never smokers. For simplicity, the categories of current and former smoking were collapsed in some analyses, specifically on the effect of smoking in association with other risk factors. Hypertension, hypercholesterolemia and diabetes were assessed by designation of medical history prior to admission in the database. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). (Supplemental Methods, page 4). The 10-year CV risk for each patient was calculated by using the Pooled Cohort Equations. We set the cut-off for increased level of CV disease risk at 10% according to the 2016 final recommendation statement of the USPS Task Force [9].

2.4. Statistical analysis

Baseline characteristics were reported as percentages for categorical variables and means with standard deviation (SD) for continuous variables. Comparisons between groups were made either by Pearson $\chi 2$ test for baseline categorical variables or two-sample *t*-test for continuous variables. A 2-sided *p* value of <0·05 was considered statistically significant. Variables describing demographic characteristics, medical history, cardiovascular risk factors, and clinical features at hospital presentation are reported in Supplemental Table 1. We used inverse probability of treatment weighting and logistic regression models to assess the effect of variables on the associations of interest (Supplemental Methods, page 4) [10]. Standardized differences after weighting were calculated to ensure balanced treatment groups with respect to baseline characteristics. Groups were considered balanced when the standardized difference was less than 20% (Supplemental Methods, page 4). We calculated ORs with their 95% CIs from these models. Fixed covariates included demographic information and baseline clinical characteristics (Table 1). We had complete data on aspirin use, sex, age and index event. Some patients had missing data on other variables. We used k-nearest neighbour (KNN) algorithms as imputation method to treat missing data [11,12]. (Supplemental Methods, page 5). Separate analyses were done to quantify the specific impact of aspirin use on STEMI rate for each of the traditional risk factors. For these analyses, we divided the risk factors into dichotomous variables and grouped aspirin users and nonusers in those with and without the risk factor under consideration. We estimated the odds ratios (ORs) and 95% confidence intervals (CI) in patients with and without the risk factor under consideration. Estimates were compared by test of interaction on the log scale (Supplemental Methods, page 6) [13].

2.5. Role of the funding source

No sponsor had any role in the design of the study or in the collection, analysis, interpretation of data, in the writing of the report, and/or in the decision to submit the paper for publication. The Principal Investigator had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

3. Results

Overall, 12,123 patients entered into the study. (Supplemental Fig. 1). Patient baseline characteristics are listed in Supplemental Table 1.

Table 1
Inverse probability of treatment weighting: outcomes sorted by aspirin use before index event.

Characteristics	Overall population		
	Aspirin users*N* = 1506	Aspirin nonusers*N* = 10,617	Standardized difference
Age, y	62·7 ± 12·5	61·4 ± 12·2	0·1046
Cardiovascular risk factors			
Diabetes	25·4	22·6	0·0661
History of hypertension	69·8	65·4	0·0955
History of hypercholesterolemia	43·0	38·8	0·0870
Current smokers	43·6	44·5	−0·0164
Former smokers	8·4	7·4	0·0372
Clinical history			
COPD	6·0	5·2	0·0367
Chronic kidney disease	6·4	5·1	0·0599
Medications before admission			
Statins	10·5	9·3	0·0413
ACE inhibitors/ ARBs	38·8	33·2	0·1174
Beta blockers	24·1	20·1	0·0962
Angiographic findings			
Multivessel disease	44·2	42·5	0·0338
Outcome			
STEMI	65·4	72·2	−0·1459*
Odds Ratio (95%CI)	0·73 (0·65 − 0·82)		−0·1459**

Data are percentages or means ± Standard deviation (SD) unless stated otherwise.
*P-value for STEMI for aspirin users versus non-users <0·0001; ** P-value <0·0001.
Abbreviations: ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; COPD, chronic obstructive pulmonary disease; STEMI, ST elevation myocardial infarction.

The distribution of diabetic patients in those taking oral antidiabetics, insulin or controlling the disease with diet is shown in Supplemental Fig. 2. Slightly more than 12% (n = 1506) of patients reported use of aspirin. Aspirin users were older. They also had more frequently diabetes, hypertension, hypercholesterolemia, and chronic kidney disease compared with aspirin nonusers. Aspirin users were also more likely to take concomitant evidence-based medications. A detailed description of the sociodemographic and clinical characteristics associated with 10-year CV risk categories is presented in Supplemental Table 2. Aspirin users showed higher predicted 10-year CV risk compared with nonusers. Clinical presentation with STEMI as index event was strongly related to 30-day mortality (OR: 1·93; 95%CI: 1·59–2·35) and heart failure on hospital admission (Supplemental Figs. 3 and 4).

3.1. Balancing covariates and aspirin use in the overall population

Inverse probability of treatment weighting eliminated most of the differences between covariates of aspirin users versus nonusers (Table 1). Baseline characteristics were balanced between the two study groups for patient demographics, disease risk factors and prior evidence-based medication use. Prior aspirin use was associated with a significantly decreased rate of STEMI as compared with no prior aspirin use (absolute difference 6·8%; OR, 0·73; 95%CI 0·65–0·82). Next, we investigated the association of prior aspirin use with outcomes separately in women versus men, in patients aged 50 but less than 60 versus 60–69 years, and in patients with BMI <25 kg/m^2 versus those with BMI ≥25 kg/m^2. Effects were consistent for all subgroups (Tables 2–4), with no interaction effects by age, sex or weight. (Supplemental Tables 3–5). Benefits of aspirin were also observed when the analysis was restricted to the higher CV risk group (absolute difference 9·0%; OR, 0·67; 95%CI 0·59–0·75), but were not seen in the lower risk group (absolute difference 2·4%; OR ratio, 0·88; 95%CI 0·66–1·19). (Table 5). The relative risks from the CV risk subgroups significantly differed from each other (Supplemental Table 6)

3.2. Subgroup analyses sorted by specific risk factors

To explore potential sources of heterogeneity, we evaluated the primary end point of STEMI in subgroups of patients defined by the presence or absence of the four conventional risk factors (hypertension, hypercholesterolemia, diabetes mellitus, and smoking) (Fig. 1). In all subgroups apart from diabetes, the benefits of aspirin were approximately similar (Supplemental Tables 7–10) to those seen in the overall patient population. Aspirin use was associated with a substantially lower risk of presenting with STEMI in patients who were current (OR: 0·78; 95% CI 0·64–0·96) or former smokers (OR: 0·63; 95% CI 0·44 to 0·90), for those with hypercholesterolemia (OR: 0·73; 95% CI 0·62–0·87), and for those with hypertension (OR: 0·72; 95% CI 0·64–0·82). Conversely, in diabetic patients (Supplemental Table 11), aspirin treatment was associated with a nonsignificant increase in the main end point of STEMI (OR: 1·10, 95% CI: 0·89–1·35). Analysis of the overall population of patients excluding patients with diabetes showed opposite results: (OR: 0·64;95% CI: 0·56–0·73). The interaction between the two subgroups was highly significant (p < 0·0001) (Fig. 1).

3.3. Diabetes and one more risk factor

We compared outcomes in aspirin users versus nonusers in patients having two conventional risk factors of which one was diabetes (Fig. 2). For these analyses we combined current and former smokers in a single category. In patients who were smokers, (Supplemental Table 12), aspirin was associated with a nonsignificant 25% increase in STEMI (OR: 1·25; 95% CI, 0·87–1·80). There was power to detect interaction with controls (interaction: p = 0·0010). In patients with history of hypercholesterolemia (Supplemental Table 13), aspirin was associated with a nonsignificant 8% reduction of STEMI (OR: 0·92; 95% CI, 0·70–1·22). The relative risk compared with that of controls was significantly different (interaction: p = 0·039). In patients with history of hypertension (Supplemental Table 14), aspirin use resulted in a not statistically significant 4% increase of STEMI (OR ratio: 1·04; 95% CI, 0·83–1·29). Again, there was power to detect interaction with controls (p = 0·0004).

3.4. Diabetes and multiple risk factors

Diabetic patients were separated into strata based on the type of association with two or more conventional risk factors (Fig. 3). Compared with controls, diabetic patients with two more risk factors did not benefit of aspirin when diabetes was accompanied by smoking,

Table 2
Inverse probability of treatment weighting: outcomes sorted by sex and aspirin use before index event.

Characteristics	Women			Men		
	Aspirin users *N* = 563	Aspirin nonusers *N* = 3112	Standardized difference	Aspirin users *N* = 943	Aspirin nonusers *N* = 7505	Standardized difference
Age, y	66·6 ± 11·6	65·6 ± 12·2	0·0918	60·8 ± 12·5	59·6 ± 11·8	0·0996
Cardiovascular risk factors						
Diabetes	28·6	27·6	0·0242	24·1	20·5	0·0875
History of hypertension	74·6	73·9	0·0158	67·2	61·7	0·1152
History of hypercholesterolemia	44·1	40·4	0·0758	42·3	38·1	0·0856
Current smokers	34·4	31·7	0·0556	47·7	50·0	−0·0454
Former smokers	4·4	3·9	0·0271	10·5	9·0	0·0511
Clinical history						
COPD	5·7	5·7	0·0022	6·2	5·0	0·0556
Chronic kidney disease	7·4	6·0	0·0572	6·0	4·7	0·0576
Medications before admission						
Statins	12·4	11·2	0·0380	9·4	8·4	0·0344
ACE inhibitors/ ARBs	46·7	42·1	0·0930	35·1	29·3	0·1241
Beta blockers	31·0	26·8	0·0920	20·9	17·3	0·0923
Angiographic findings						
Multivessel disease	44·3	41·6	0·0545	44·0	43·0	0·0205
Outcome						
STEMI	64·5	69·7	−0·1103*	66·0	73·3	−0·1587§
Odds Ratio (95% CI)	0·79 (0·65 − 0·96)		−0·1103**	0·71 (0·61 − 0·82)		−0·1587§§

Data are percentages or means ± Standard deviation (SD) unless stated otherwise.
*P-value for STEMI for aspirin users versus non-users 0·0179; ** P-value 0·0148.
§ P-value for STEMI for aspirin users versus non-users <0·0001.
§§ P-value <0·0001.
Abbreviations: ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; COPD, chronic obstructive pulmonary disease; STEMI, ST elevation myocardial infarction.

Table 3
Inverse probability of treatment weighting: outcomes sorted by age and aspirin use before index event.

Characteristics	50–59 years			60–69 years		
	Aspirin users *N* = 297	Aspirin nonusers *N* = 3066	Standardized difference	Aspirin users *N* = 487	Aspirin nonusers *N* = 3047	Standardized difference
Age, y	54·9 ± 2·7	54·9 ± 2·9	0·0042	64·0 ± 3·0	64·0 ± 2·9	−0·0133
Cardiovascular risk factors						
Diabetes	15·9	18·9	−0·0798	29·0	26·9	0·0454
History of hypertension	64·6	61·5	0·0641	74·9	70·2	0·1051
History of hypercholesterolemia	50·3	41·3	0·1810	42·6	41·0	0·0340
Current smokers	59·4	57·6	0·0347	44·6	42·5	0·0423
Former smokers	8·1	6·7	0·0536	10·4	9·0	0·0454
Clinical history						
COPD	3·2	3·4	−0·0103	7·8	6·0	0·0707
Chronic kidney disease	3·4	2·9	0·0293	6·0	5·2	0·0354
Medications before admission						
Statins	9·9	8·1	0·0630	11·9	10·7	0·0389
ACE inhibitors/ ARBs	35·3	27·3	0·1958	42·8	38·6	0·0858
Beta blockers	21·1	16·9	0·1081	25·7	22·7	0·0699
Angiographic findings						
Multivessel disease	38·5	38·0	0·0108	47·7	44·2	0·0688
Outcome						
STEMI	66·3	72·3	−0·1303*	64·4	74·4	−0·2200§
Odds Ratio (95% CI)	0·75 (0·59 − 0·97)		−0·1303**	0·62 (0·51 − 0·76)		−0·2200§§

Data are percentages or means ± Standard deviation (SD) unless stated otherwise.
*P-value for STEMI for aspirin users versus non-users 0·0370; ** P-value 0·0288.
§ P-value for STEMI for aspirin users versus non-users <0·0001.
§§ P-value <0·0001.
Abbreviations: ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; COPD, chronic obstructive pulmonary disease; STEMI, ST elevation myocardial infarction.

either in patients who additionally had history of hypercholesterolemia (OR ratio: 1·25; 95% CI, 0·80–1·94; interaction test: $p = 0·0067$) or in those who had history of hypertension (OR ratio: 1·11; 95% CI, 0·77–1·62; interaction test: $p = 0·0104$) (Supplemental Tables 15 and 16). Diabetic patients did not benefit of aspirin even combining all four conventional risk factors into the model (OR: 1·26; 95% CI, 0·80–1·99; interaction test: $p = 0·0073$) (Supplemental Table 17). The only cluster of risk factors that improved ischemic outcomes with aspirin was the association of diabetes with history of hypercholesterolemia and hypertension (Supplemental Table 18). In this high–risk population, the estimated treatment benefit was a 13% reduction in risk of STEMI, which was statistically insignificant (OR: 0·87; 95% CI, 0·65–1·15) compared with a 30% reduction in controls, which was significant (OR: 0·70; 95% CI, 0·62–0·80). But the relative

Table 4
Inverse probability of treatment weighting: outcomes sorted by BMI and aspirin use before index event.

Characteristics	BMI <25			BMI ≥25		
	Aspirin users *N* = 430	Aspirin nonusers*N* = 3381	Standardized difference	Aspirin users *N* = 1076	Aspirin nonusers *N* = 7236	Standardized difference
Age, y	63·3 ± 13·2	63·1 ± 12·5	0·0163	62·3 ± 12·2	60·6 ± 12·0	0·1445
Cardiovascular risk factors						
Diabetes	19·7	16·7	0·0792	28·0	25·3	0·0610
History of hypertension	60·7	56·9	0·0772	74·3	69·3	0·1133
History of hypercholesterolemia	33·6	30·6	0·0636	47·2	42·5	0·0943
Current smokers	47·2	44·8	0·0480	42·2	44·3	−0·0418
Former smokers	7·1	6·0	0·0430	9·1	8·0	0·0394
Clinical history						
COPD	5·2	4·9	0·0159	6·2	5·3	0·0401
Chronic kidney disease	7·4	5·4	0·0797	5·7	4·9	0·0389
Medications before admission						
Statins	8·0	7·1	0·0352	11·6	10·3	0·0443
ACE inhibitors/ ARBs	34·2	28·8	0·1166	41·0	35·2	0·1196
Beta blockers	20·7	17·4	0·0843	25·8	21·4	0·1033
Angiographic findings						
Multivessel disease	38·4	41·5	−0·0630	46·3	43·0	0·0670
Outcome						
STEMI	65·3	74·7	−0·2061*	65·2	71·0	−0·1255§
Odds Ratio (95% CI)	0·64 (0·52−0·79)		−0·2061**	0·76 (0·67−0·87)		−0·1255§§

Data are percentages or means ± Standard deviation (SD) unless stated otherwise.
**P*-value for STEMI for aspirin users versus non-users 0·0001; ** *P*-value 0·0001.
§ *P*-value for STEMI for aspirin users versus non-users 0·0002.
§§ *P*-value 0·0001.
Abbreviations: ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; BMI, Body Mass Index; COPD, chronic obstructive pulmonary disease; STEMI, ST elevation myocardial infarction·.

Table 5
Inverse probability of treatment weighting: outcomes sorted by 10-year CVD risk* and aspirin use before index event.

Characteristics	<10% of risk			≥10% of risk		
	Aspirin users *N* = 245	Aspirin nonusers *N* = 2735	Standardized difference	Aspirin users *N* = 1261	Aspirin nonusers *N* = 7882	Standardized difference
Cardiovascular risk factors						
History of hypertension	55·4	51·6	0·0776	73·4	69·8	0·0802
History of hypercholesterolemia	41·1	34·2	0·1424	43·0	40·2	0·0558
Former smokers	9·9	8·0	0·0680	8·0	7·2	0·0277
Clinical history						
COPD	4·4	2·9	0·0811	6·7	5·8	0·0370
Chronic kidney disease	4·1	3·3	0·0405	6·4	5·7	0·0309
Medications before admission						
Statins	9·7	7·6	0·0760	10·5	9·7	0·0262
ACE inhibitors/ ARBs	31·4	24·1	0·1858	40·1	36·0	0·0852
Beta blockers	20·3	16·0	0·1113	24·4	21·3	0·0723
Angiographic findings						
Multivessel disease	32·4	32·3	0·0025	47·7	45·9	0·0371
Outcome						
STEMI	72·7	75·1	−0·0540*	62·3	71·3	−0·1919§
Odds Ratio (95 CI)	0·88 (0·66−1·19)		−0·0540**	0·67 (0·59−0·75)		−0·1919§§

Data are percentages or means ± Standard deviation (SD) unless stated otherwise. Age, diabetes and current smokers were not included in the model as they were represented in the Pooled Cohort Equation·.
**P*-value for STEMI for aspirin users versus non-users 0·4255; ** *P*-value 0·4128.
§ *P*-value for STEMI for aspirin users versus non-users <0·0001.
§§ *P*-value <0·0001.
Abbreviations: ACE, angiotensin converting enzyme; ARBs, angiotensin receptor blockers; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; STEMI, ST elevation myocardial infarction·.
*10-year CVD risk calculated using the simplified Pooled Cohort Equations·.

risks from the two subgroups were not significantly different from each other (interaction tests: p = 0·0864). There was thus good evidence to support a similar treatment effect between the two subgroups, which means that aspirin may be effective in those diabetic patients who concomitantly present with history of hypercholesterolemia and hypertension. Calculations of the interaction tests reported in Figs. 1−3 are showed in Supplemental Tables 19−30. Representation of the main results of the study in diabetic patients is illustrated in Fig. 4.

4. Discussion

The current study examined the use of low-dose aspirin and its association with the incidence of severe clinical manifestation of CHD

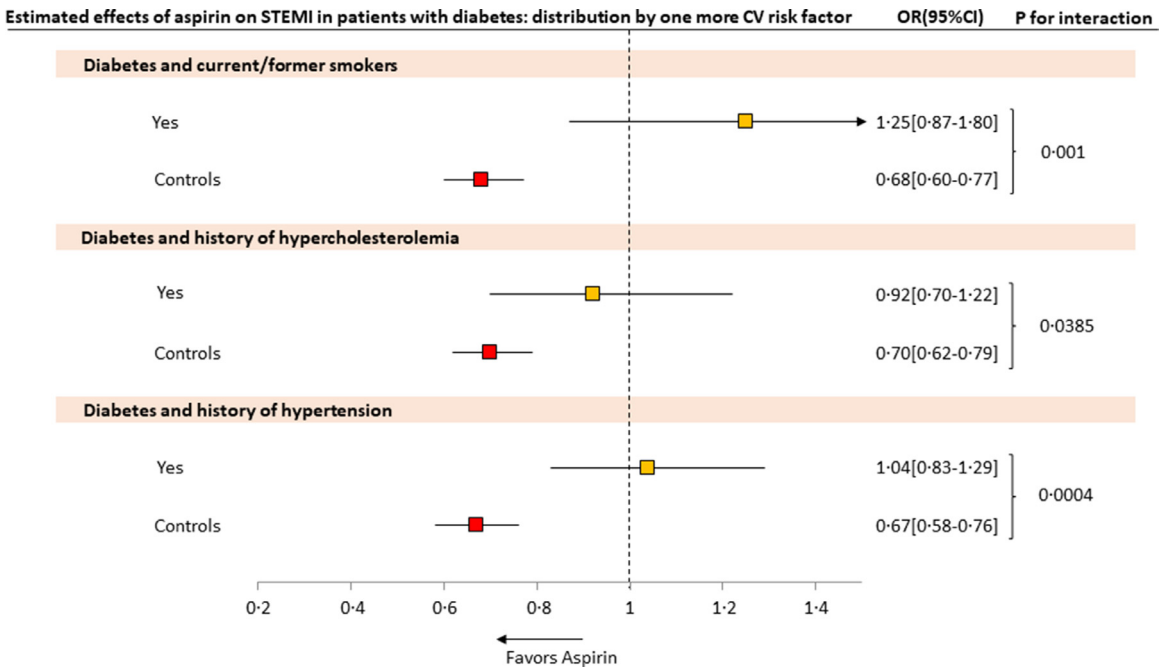

Fig. 2. Estimated effects of aspirin on STEMI in patients with diabetes: distribution by one more CV risk factor. Association between use of aspirin before index event and incidence of ST elevation myocardial infarction in patients with diabetes sorted by its combination with one more traditional risk factor. Abbreviations: OR, Odds Ratio; CI, Confidence Interval; CV, cardiovascular; STEMI, ST elevation myocardial infarction.

in a large cohort of Caucasian adults without evidence of CV before the qualifying event. The main average treatment effect in the overall population was that prior aspirin users were less likely to present with STEMI regardless of age and sex with an absolute event reduction of 6·8% (OR: 0·73; 95% CI, 0·65–0·82). The results of our work do not conflict with USPSTF guidelines as we demonstrated that aspirin treatment reduced the risk of STEMI in the higher CV risk group (absolute difference 9·0%; OR, 0·67; 95%CI 0·59–0·75), but not in the lower risk group (absolute difference 2·4%; OR ratio, 0·88; 95%CI 0·66–1·19). Nevertheless, some groups of subjects varied in their response to aspirin. In other words, there was a strong heterogeneity of treatment effects. When data were sorted by categories of risk, the beneficial effect of aspirin was found to be significant in individuals who were smokers and in those with history of hypertension and hypercholesterolemia, but not in adults with diabetes for whom aspirin is still at present recommended in patients with 10% or more 10-year CV risk [9,14]. Notably, a large proportion of subjects in our study were taking statins and antihypertensive drugs. Medications

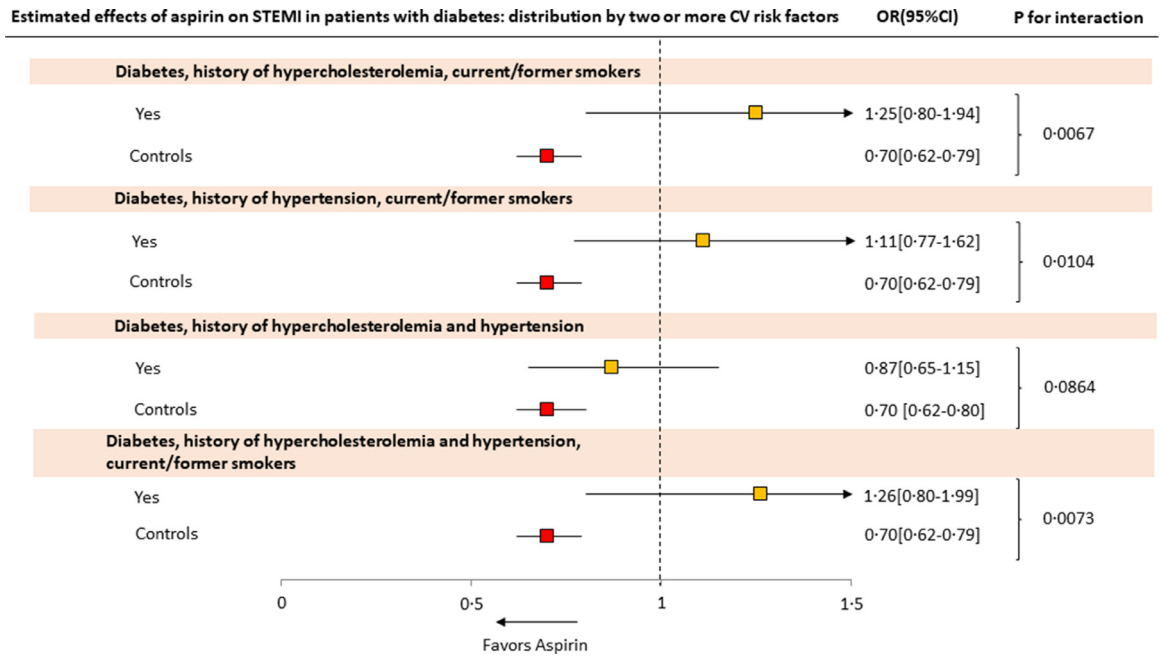

Fig. 3. Estimated effects of aspirin on STEMI in patients with diabetes: distribution by two or more CV risk factors. Association between use of aspirin before index event and incidence of ST elevation myocardial infarction in patients with diabetes sorted its combination with two or more traditional risk factors. Abbreviations: OR, Odds Ratio; CI, Confidence Interval; CV, cardiovascular; STEMI, ST elevation myocardial infarction.

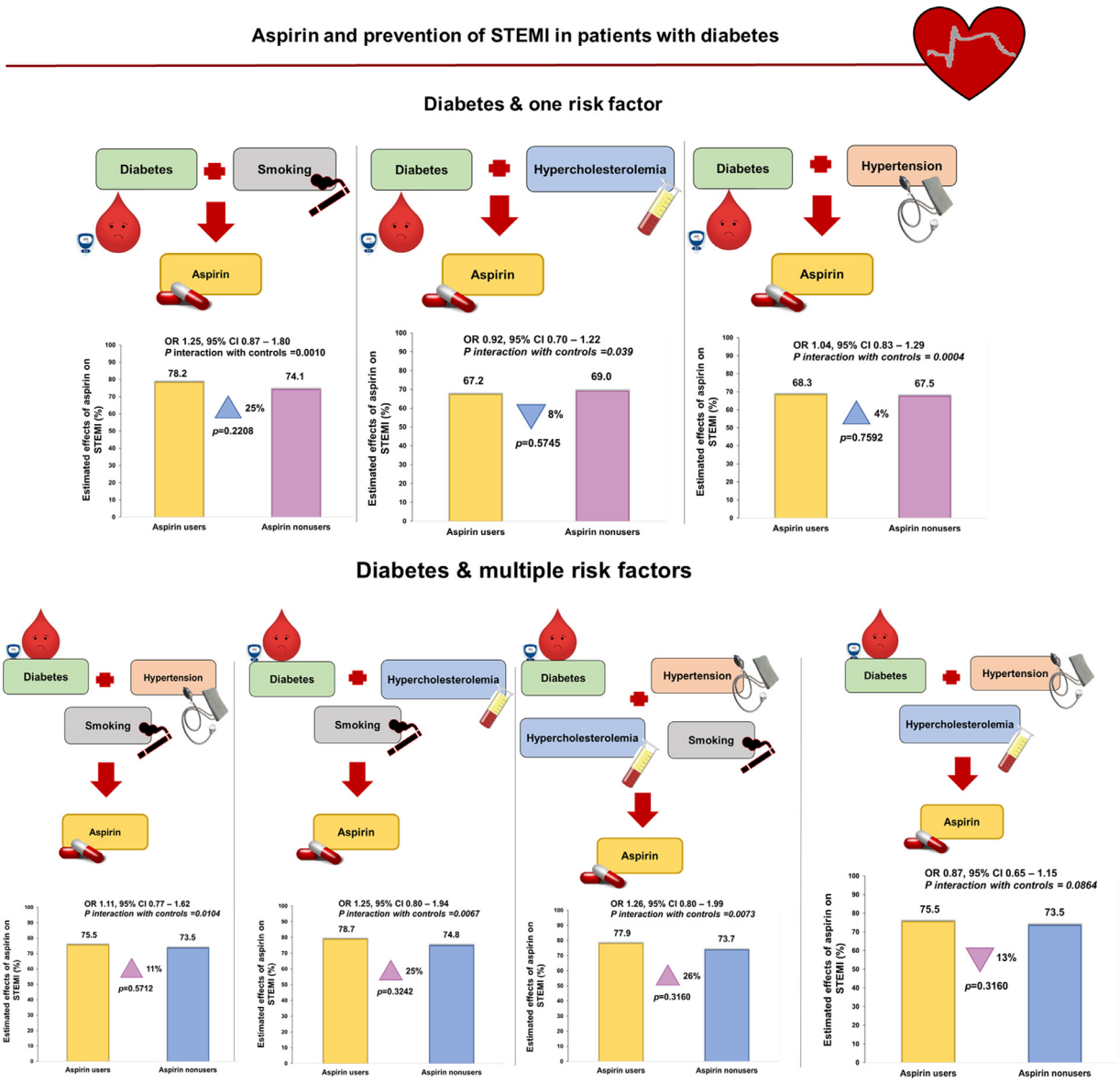

Fig. 4. Aspirin and prevention of STEMI in diabetes. Abbreviations: STEMI, ST elevation myocardial infarction.

were well balanced between aspirin users and nonusers by inverse probability of treatment weighting analyses. Thus, one could reasonably conclude that our study examined the incremental benefit of aspirin, added to other standard preventive interventions. These findings deserve some more considerations.

STEMI remains the most significant contributor to morbidity and mortality worldwide, despite a declining incidence and better survival rates [15]. It is not possible, however, to know exactly why one person dies from STEMI and another does not, as mortality from STEMI can be related to multiple factors including age, Killip class, time delay to treatment, renal failure, number of diseased coronary arteries, and left ventricular ejection fraction. Mortality data has to be interpreted cautiously at individual level. Yet, STEMI denotes a high risk of mortality at population-level. The mortality rate of STEMI in our cohort was approximately twice the mortality of NSTE-ACS (OR: 1·93; 95%CI: 1·59 – 2·35). Thus, our data support the high-risk nature of STEMI and its potential role in risk-stratification algorithms.

Early randomized evidence, suggested differences in response to aspirin for primary prevention between diabetic and nondiabetic subjects [16–18]. As well, the evidence from the most recent primary prevention trial in diabetes has been unpromising [3]. In the ASCEND, over 15,000 middle-aged or older diabetic patients were randomized to daily aspirin or placebo. During an average follow-up of approximately 7 years, the incidence of serious vascular events was modest with one percentage point lower in the aspirin group than in the placebo group. The risk difference was seen mainly in the rate of transient ischemic attacks. On the opposite, the trial showed no significant effect of aspirin use, as compared with placebo, on the rates of myocardial infarction and vascular death. These data are consistent with our findings on aspirin use for prevention of STEMI. Non-diabetic patients demonstrated large benefits from aspirin (OR: 0·64; 95%CI: 0·56–0·73), yet diabetic patients failed to do so (OR, 1·10; 95% CI, 0·89–1·35). So, what is the role of aspirin in primary prevention of CV disease in patients with diabetes?

The scene is familiar in primary care practice. Most diabetic patients have at least one or two more risk factors for CHD. What to do, therefore, with a 65-year-old man with diabetes and hypertension treated with metformin and ACE inhibitors? Whether aspirin may have a margin of benefit in such a patient is still unknown. To address this concern, we performed a sequential switchover design [19]. Diabetes served as its own control balancing the effects of the

other risk factors by inverse probability of treatment weighting. The same modeling approach was used to estimate the effect of diabetes in combination with one or multiple risk factors. The first model consisted of two linked risk factors, of which one was diabetes. The second model comprised diabetes and two of the remaining three risk factors under scrutiny. Finally, a single model structure, which includes all four conventional risk factors was performed. Different answers were derived in diabetic patients depending on whether the associated risk factor was hypertension, hypercholesterolemia, smoking or a combination of such factors. No differential effect of aspirin was seen in diabetic patients who just had one additional major risk factor. Low-dose aspirin was insufficient to confer protection from STEMI in diabetic patients who were smokers. Conversely, prevention of STEMI through aspirin use was effective in diabetics when these patients were concomitantly affected by hypertension and hypercholesterolemia, in the absence of smoking. In sum, our study suggests that when making individual treatment decisions in diabetes, clinicians and patients should consider not only the 10-year risk of CV disease, but also the number and the quality of CV risk factors. The coexistence of hypertension and hypercholesterolemia with diabetes is particularly pernicious because of the strong linkage of these conditions with all CV diseases [20].

One more source of uncertainty merits attention. The USPSTF recommends to calculate 10-year risk models for estimating life-years gained per 10,000 patients taking aspirin, stratified by age and CV risk factors [9]. However, the 10-year CV risk model accuracy is controversial [21,22] as it incorporates decision modeling that might not reflect the incremental benefit of aspirin in contemporary patients who already are addressing CV risk through pharmacologic and lifestyle measures. The contemporary approach in risk factor control might potentially reduce the benefits of aspirin. The ASCEND trial did not provide sufficient information to address this question. In the ASCEND, hypercholesterolemia was managed according to the current standard of care, and as so, a large proportion of the participants were randomized with statins [3]. The trial, instead, did not notice the use of antihypertensive agents, which may as well be of relevance for outcomes. Differences between antihypertensive drugs exist with respect to target-organ damage and prevention of CV events. In a subgroup analysis of diabetic patients in the LIFE (Losartan Intervention For Endpoint Reduction) study [23], treatment with a strategy based on an ARB significantly reduced CV morbidity and mortality compared with treatment with beta-blockers. More recently, the use of ACE inhibitors was associated with a significant reduction in CV-related mortality in a broad spectrum of patients with diabetes and no CV disease [24]. This issue is further compounded by the fact that beta-blocker use in diabetes may be associated with an increased risk for cardiovascular events [25]. Although FDA guidelines [26] have always called for an analysis of the impact of concomitant medications in clinical studies, this has not always been pursued. Our study did so. In the matched population, the magnitude of the effects of statins, ACE inhibitors, ARBs and beta-blockers on the outcome was comparable in aspirin users versus non-users. Thus, our results are unlikely to be caused by one drug interfering with the effects of the other.

To date, there is no objective estimate about the threshold of risk to warrant aspirin prophylaxis in diabetes. The entire coagulation cascade is dysfunctional in diabetes. Increased levels of fibrinogen and plasminogen activator inhibitor 1 favor both thrombosis and defective dissolution of clots once formed [27]. Platelets adhere to vascular endothelium and aggregate more readily than those in healthy individuals [28]. Aspirin mainly inhibits platelet aggregation by irreversible acetylation of the cyclo-oxygenase-1 (COX-1) enzyme, resulting in almost complete inhibition of thromboxane production [29]. Thus, all of these afore mentioned abnormalities in diabetes cannot be counteracted by aspirin alone. To some extent, the effects of smoking and diabetes are similar. According to the 2014 Surgeon General's Report [30], smoking may decrease insulin sensitivity, which may contribute to potentiate the extra-platelet COX-1 -related metabolic pathways of CV risk. So, it is not surprising that smoking may contribute to exceed the threshold of risk at which aspirin is assumed to become beneficial in terms of cardiovascular disease prevention.

New findings by our study include the potential of aspirin to reduce the risk of STEMI in diabetic patients who concurrently present with hypertension and hypercholesterolemia. Hypertension and hypercholesterolemia are important risk factors for the development of new CHD events in patients with diabetes [31]. Hypertension and hypercholesterolemia are also known to increase platelet activation. An increased level of circulating monocyte-platelet aggregates represents one of the most robust markers of platelet activation in hypertension [32]. Hyperlipidemia is associated with shortened platelet survival and high platelet production [33]. Under these circumstances, inhibition by aspirin of COX-1 may avoid continued production of new uninhibited platelets, and circulating monocyte-platelet aggregates. All of these are assumptions. Further investigations are required to better define a clearer understanding of the mechanistic effects of aspirin.

Subgroup analyses are generally not considered to provide definitive evidence for several reasons, including the need of a specific prior suspicion of the existence of a particular interaction, the statistical methods used to identify interactions, and the spurious associations that may arise as a result of multiple testing [34]. Our analysis addressed each of these issues. There were sufficient clinical data concerning aspirin-based heterogeneity of treatment effects in diabetics [3,16–18]. We identified the interaction between diabetes and low aspirin therapy using tests of interaction on the log scale [13]. We addressed concern about multiple testing by matching patients using inverse probability of treatment weighting [10]. The magnitude of the interaction between diabetes and aspirin prophylaxis and the precision in its estimation in all clinical settings lead us to believe that the interaction we identified is clinically relevant and probably not a statistical artefact.

Our findings are particularly important for practice. It is common occurrence that diabetic patients come into a physician's office for a routine visit. Aspirin is still often on their medication list. Patients should, therefore, make an informed decision to discontinue or retain their aspirin. Physicians pull up the 2019 ACC/AHA guidelines [35], the 2016 USPSTF recommendations [9] and the 2019 American Diabetes Association (ADA) statement [14] on aspirin online, to discuss these indications together. The USPSTF guidelines suggested that the presence of diabetes did not alter the effectiveness of aspirin therapy in reducing CV disease events, and recommended low-dose aspirin for adults aged 50 to 69 years whose 10-year CV risk exceeded 10%. The ACC/AHA guidelines removed the specific 10-year CV risk threshold as an inclusion criterion for aspirin consideration and recommended that low-dose aspirin should be used infrequently in the primary prevention of CV disease. The ADA Scientific Statement narrowed the recommendations in diabetic patients to those with a 10−year CV risk $\geq$10%, provided that these patients are aged more than 50 and less than 70 years and have at least one additional major risk factor. In this conundrum of scientific opinions, almost all patients would prefer to defer decisions to their physicians. Yet, physicians may fail to provide patients with basic information about the risk benefit ratio of such intervention. Assessing bleeding risk remains a challenge. Better estimation of future ischemic risk is therefore essential to better inform shared decision making with patients. In the present study, we take a step in helping to account for patient-specific factors. The amount of benefit of preventing STEMI may clearly outweighs the amount of bleeding risk. Diabetes should be endorsed as a significant part of risk estimates. Modest average effects in recent studies may reflect a mixture of considerable benefits for some subjects or little benefit, or any, for others.

There are a number of strengths to this study. The study tackles an important question to clinicians: the appropriateness and potential

benefit of aspirin for primary prevention of cardiovascular disease. The dataset is large with good ascertainment of cardiovascular risk factors covering a contemporary period of time. The methods are reasonable for addressing measured confounding. A further strength was the use of subgroups related to diabetes and other conditions. The study thoroughly took different variations into account alongside having diabetes, which is relevant to the community and patients.

Our study has some potential limitations. First, some of the risk factors were ascertained by the general practitioner, which might have led to errors in the dataset. This was the best way to handle this issue. Blood pressure value after an ACS is potentially confounded because it might have fallen in some subjects as a result of heart failure. As well, stress hyperglycemia may frequently occur and is not an indication of disease. Although we acknowledge some misclassifications, it is unlikely that these misclassifications differentially affect diabetic over nondiabetic patients and, thus, are unlikely to modify the main difference that we found. Another limitation is that we were unable to assess the exact duration of aspirin therapy. Outcomes may be dependent on frequency of aspirin exposure. We relied on what the patients reported at hospital admission. This could result in possible misclassification effects of unknown size and direction. Further, a general problem of the approach chosen in the present study is that analyses of bleeding events of low-dose aspirin cannot be ascertained. Many questions remain regarding the decision as to which patients must weigh the benefits of chronic aspirin therapy against the possible risks associated with its use, including the risk of intracerebral and subarachnoid hemorrhage. In the ASCEND trial the incidence of major bleeding events was 1 percentage point higher with aspirin than with placebo (4.1% vs. 3.2%). The incidence of hemorrhagic stroke was similar among persons in the aspirin group and among those in the placebo group (0.3.vs 0.3%) [3]. Based on the rarity of hemorrhagic stroke risk, concerns about this risk should not discourage appropriate patients from using low-dose aspirin. Accordingly, our study demonstrates that aspirin has a high margin of benefit as it substantially reduces the rates of STEMI in specific higher-risk comorbid conditions such as in hypertension, hypercholesterolemia and smoking. The presence of diabetes in combination with other risk factors should not necessarily be viewed as a guarantee of a failure of aspirin prophylaxis.

In conclusion, our study underscores the importance of investigations on the heterogeneity of treatment efficacy. The observed interactions between presence of diabetes and its combination with traditional risk factors and aspirin prophylaxis can be confirmed only by a very large number of risk -stratified, randomized, controlled trials of low dose aspirin therapy. In the absence of definitive evidence from trials, we believe that our data provide sufficient grounds for a reexamination of the use of aspirin therapy for primary prevention of CV disease.

Author contributions

RB contributed to study design, data interpretation, literature search and writing and editing of the manuscript. SP contributed to study design and editing of the manuscript. JY and MvdS contributed to data analysis, and data interpretation. SK, MV, ZV contributed to data collection and editing of the manuscript. MB contributed to the literature search and editing of the manuscript. DM contributed to data collection and editing of the manuscript. OM contributed to data interpretation and editing of the manuscript. EC contributed to study design, data interpretation and critical revision of the manuscript. LB contributed to data interpretation and editing of the manuscript.

Data sharing statement

The source codes of the study are uploaded on https://github.com/jsyoon0823/Treatment_Phenotype. They will be available to anyone who wishes to access these data.

Declaration of Competing Interest

Professor Badimon reports other from Bayer, personal fees and other from International Aspirin Foundation, UK, during the conduct of the study; other from SANOFI, personal fees from LILLY, grants from ASTRAZENECA, personal fees from ASTRAZENECA, other from Glycardial, personal fees from BMS/Pfizer, personal fees from PACE, personal fees and other from FICYE (FORUM TO STUDY BEER & LIFESTYLE), outside the submitted work; In addition, Professor Badimon has a patent APOj-Gly licensed, a patent IV_STATIN pending, and a patent DJ1-F pending. All other authors have nothing to report.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2020.100548.

References

[1] O'Brien CW, Juraschek SP, Wee CC. Prevalence of aspirin use for primary prevention of cardiovascular disease in the United States: results from the 2017 National Health Interview Survey. Ann Intern Med 2019;171(8):596–8.
[2] Gaziano JM, Brotons C, Coppolecchia R, et al. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. Lancet 2018;392(10152):1036–46.
[3] Bowman L, Mafham M, Wallendszus K, et al. Effects of aspirin for primary prevention in persons with diabetes mellitus. N Engl J Med 2018;379(16):1529–39.
[4] Yeh RW, Sidney S, Chandra M, Sorel M, Selby JV, Go AS. Population trends in the incidence and outcomes of acute myocardial infarction. N Engl J Med 2010;362(23):2155–65.
[5] McNeil JJ, Wolfe R, Woods RL, et al. Effect of aspirin on cardiovascular events and bleeding in the healthy elderly. New England Journal of Medicine 2018;379(16):1509–18.
[6] Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352(13):1293–304.
[7] Cenko E, Yoon J, Kedev S, et al. Sex differences in outcomes after STEMI: effect modification by treatment strategy and age. JAMA Intern Med 2018;178(5):632–9.
[8] Cenko E, van der Schaar M, Yoon J, et al. Sex-related differences in heart failure after ST-segment elevation myocardial infarction. J Am Coll Cardiol 2019;74(19):2379–89.
[9] Bibbins-Domingo K. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: U.S. preventive services task force recommendation statement. Ann Intern Med 2016;164(12):836–45.
[10] Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med 2015;34(28):3661–79.
[11] Beretta L, Santaniello A. Nearest neighbor imputation algorithms: a critical evaluation. BMC Med Inform Decis Mak 2016;16 Suppl 3:74.
[12] Troyanskaya O, Cantor M, Sherlock G, et al. Missing value estimation methods for DNA microarrays. Bioinformatics 2001;17(6):520–5.
[13] Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ 2003;326(7382):219.
[14] Association American Diabetes. Cardiovascular disease and risk management: standards of medical care in diabetes-2019. Diabetes Care 2019:S103–S23.
[15] As Orlandini, R Díaz, Wojdyla D, et al. Outcomes of patients in clinical trials with ST-segment elevation myocardial infarction among countries with different gross national incomes. Eur. Heart J. 2006;27(5):527–33.
[16] Kassoff A, Buzney SM, McMeel JW, et al. Aspirin effects on mortality and morbidity in patients with diabetes mellitus: early treatment diabetic retinopathy study report 14. JAMA 1992;268(10):1292–300.
[17] Belch J, MacCuish A, Campbell I, et al. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. BMJ 2008;337 a1840-a.
[18] Ogawa H, Nakayama M, Morimoto T, et al. Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial. JAMA 2008;300(18):2134–41.
[19] Maclure M, Mittleman MA. Should we use a case-crossover design. Annu Rev Public Health 2000;21:193–221.
[20] Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB. Clustering of metabolic factors and coronary heart disease. Arch Intern Med 1999;159(10):1104–9.

[21] Brett A.S. Accuracy of the ACC/AHA cardiovascular risk calculator is challenged. 2015. https://www.jwatch.org/na37408/2015/03/25/accuracy-acc-aha-cardiovascular-risk-calculator-challenged2020).
[22] DeFilippis AP, Young R, Carrubba CJ, et al. An analysis of calibration and discrimination among multiple cardiovascular risk scores in a modern multiethnic cohort. Ann Intern Med 2015;162(4):266–75.
[23] Lindholm LH, Ibsen H, Dahlöf B, et al. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002;359(9311):1004–10.
[24] Eurich DT, Majumdar SR, Tsuyuki RT, Johnson JA. Reduced mortality associated with the use of ACE inhibitors in patients with type 2 diabetes. Diabetes Care 2004;27(6):1330–4.
[25] Tsujimoto T, Sugiyama T, Shapiro MF, Noda M, Kajio H. Risk of cardiovascular events in patients with diabetes mellitus on β-blockers. Hypertension 2017;70(1):103–10.
[26] FDA. E3 structure and content of clinical study reports. 1996. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/e3-structure-and-content-clinical-study-reports.
[27] Festa A, D'Agostino R, Mykkänen L, et al. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance. Arterioscler Thromb Vasc Biol 1999;19(3):562–8.
[28] Harrison HE, Reece AH, Johnson M. Effect of insulin treatment on prostacyclin in experimental diabetes. Diabetologia 1980;18(1):65–8.
[29] Patrono C. The multifaceted clinical readouts of platelet inhibition by low-dose aspirin. J Am Coll Cardiol 2015;66(1):74.
[30] U.S. Department of Health and Human Services (USDHHS). The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014
[31] Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK prospective diabetes study group. BMJ 1998;317(7160):703–13.
[32] Gkaliagkousi E, Ritter J, Ferro A. Platelet-derived nitric oxide signaling and regulation. Circ Res 2007;101(7):654–62.
[33] Harker LA, Hazzard W. Platelet kinetic studies in patients with hyperlipoproteinemia: effects of clofibrate therapy. Circulation 1979;60(3):492–6.
[34] Oxman AD, Guyatt GH. A consumer's guide to subgroup analyses. Ann Intern Med 1992;116(1):78–84.
[35] Arnett Donna K, Blumenthal Roger S, Albert Michelle A, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation 2019;140(11):e596–646.