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Prevention and Management of Cardiovascular Disease in Metabolic Disorders

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9 Luglio 2020

Education is the most powerful weapon which you can use to change the world
[Nelson Mandela]

Ilaria Cavallari

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Abstract

Background. A close link exists between metabolic disorders and cardiovascular disease which is the most prevalent cause of morbidity and mortality in western countries. The contemporary epidemiological investigation of cardiovascular complications in metabolic disorders allows better risk stratification in order to optimize treatment strategies.

Hypothesis. We hypothesized that clinical features and risk of cardiovascular disease may vary within subjects affected by different metabolic disorders.

Aims and methods. The overall aim of this Ph.D. project was to investigate the prognostic impact of metabolic disorders and their related therapies on cardiovascular health in the current era. More specifically we aimed to:

1. investigate causes of death and their associated risk factors in type 2 diabetes patients with or without established cardiovascular disease.

To this aim, we performed a cause-of-death analysis among patients enrolled in SAVOR-TIMI 53 and used the competing-risk methodology to identify independent predictors of cardiovascular death and non-cardiovascular death. In addition, we aimed to describe features associated specifically with sudden cardiac death in diabetes.

2. investigate the impact of metabolic disease on cardiovascular outcomes in a particular subset of patients, a contemporary cohort of subjects after an acute coronary syndrome (ACS).

To this aim, we aimed to assess long-term cardiovascular risk associated with the presence of the metabolic syndrome or diabetes mellitus among patients recently hospitalized with an ACS using data from the SOLID-TIMI 52 trial.

3. investigate the risk of thromboembolic complications in patients with atrial fibrillation and diabetes.

To this aim, we explored the differential prognostic weight of diabetes on insulin therapy versus no insulin therapy on thromboembolic events in patients with AF using data from a multicenter, European AF registry called PREFER in AF.

4. assess cardiovascular safety (a composite of cardiovascular death, myocardial infarction, or stroke, and new-onset atrial fibrillation or flutter) of odanacatib, a cathepsin K inhibitor for the treatment of postmenopausal osteoporosis.

To this aim, the anti-fracture efficacy and safety of odanacatib in postmenopausal women with osteoporosis was assessed in the Long-term Odanacatib Fracture Trial (LOFT) and its extension study (LOFT Extension).

Results.

Specific aim 1. This study involving more than 16,000 patients with T2D found that cardiovascular disease remains the leading cause of death in a contemporary cohort of patients with or at high risk for atherosclerotic cardiovascular disease. Approximately 1/3 of all deaths observed in the study met criteria for sudden death, regardless of whether patients did or did not have established atherosclerotic cardiovascular disease. While excess mortality is associated with older age, worse glycemic control, kidney complications including albuminuria, prior heart failure, peripheral artery disease, prior cardiovascular events (myocardial infarction or ischemic stroke) and elevated heart rate, biomarkers, especially elevated levels of NT-proBNP and hs-TnT measured in a stable population, are strongly associated with many causes of death, including non-cardiovascular death.

Specific aim 2. This study demonstrates that, in patients who have recently suffered an ACS, the presence of metabolic syndrome is associated with the risk of adverse cardiovascular events but this risk appears to be primarily driven by the presence of diabetes mellitus. In contrast, a diagnosis of metabolic syndrome on its own did not provide incremental information for risk stratification in this population once diabetes history was considered; however, in patients without diabetes at baseline, the presence of metabolic syndrome was associated with nearly a 3-fold higher risk of developing new-onset diabetes mellitus during follow-up.

Specific aim 3. In this analysis of individual patient data from the prospective PREFER in AF registry, patients with diabetes on insulin therapy had a significantly higher risk of stroke/systemic embolism at 1 year versus both patients without diabetes and patients with noninsulin-requiring diabetes; yet, for people with diabetes not treated with insulin, there was no significantly increased risk. These results may have implications in the assessment of thromboembolic risk in the AF population with diabetes and might have therapeutic implications.

Specific aim 4. In a trial of more than 16,000 postmenopausal women with osteoporosis followed for up to 5 years, treatment with the cathepsin K inhibitor odanacatib was associated with progressive increases in bone mineral density and reductions in the incidences of vertebral, hip, and non-vertebral fractures. However, treatment also increased the risk of stroke with most events ischemic in aetiology. Further development of odanacatib as a potential treatment for patients with osteoporosis was stopped based on the overall balance between benefit and risk.

Conclusions. This contemporary epidemiological investigation of cardiovascular complications in people with metabolic disorders indicates that cardiovascular disease remains the leading cause of death in this population. However, we also highlighted that subjects with metabolic disorders represent an heterogeneous group in terms of cardiovascular risk, with individuals affected by diabetes showing the highest risk, especially those treated with insulin, compared to subjects with other endocrinological disorders such as metabolic syndrome or post-menopausal osteoporosis.

1. State of the art

1.1. Atherosclerotic cardiovascular disease in diabetes

Diabetes mellitus (DM) is an important risk factor for a first cardiovascular event and for worse outcomes once a cardiovascular event has occurred. Cardiovascular disease in DM is a progressive process characterized by early endothelial dysfunction, oxidative stress and vascular inflammation leading to monocyte recruitment and formation of foam cells and fatty streaks, that cause development of atherosclerotic plaques over years.[1] Compared to individuals without DM, atherosclerotic plaques from patients with DM are more vulnerable (rupture-prone), and therefore at higher risk of developing over-imposed thrombosis, because of increased amounts of soft extracellular lipids, inflammation and prothrombotic milieu; this predisposes diabetic patients to acute cardiovascular events.[1]

Platelet function in diabetic patients

Platelets of individuals with DM, compared to those of healthy controls, have a dysregulation both at the receptor and at the intracellular signal transduction level, leading to hyperreactive adhesion, activation, degranulation and aggregation.[2] Reduced insulin sensitivity causes increased signaling of $P2Y_{12}$ receptors, the main platelet receptor for adenosine diphosphate (ADP) (Figure 1).[3]

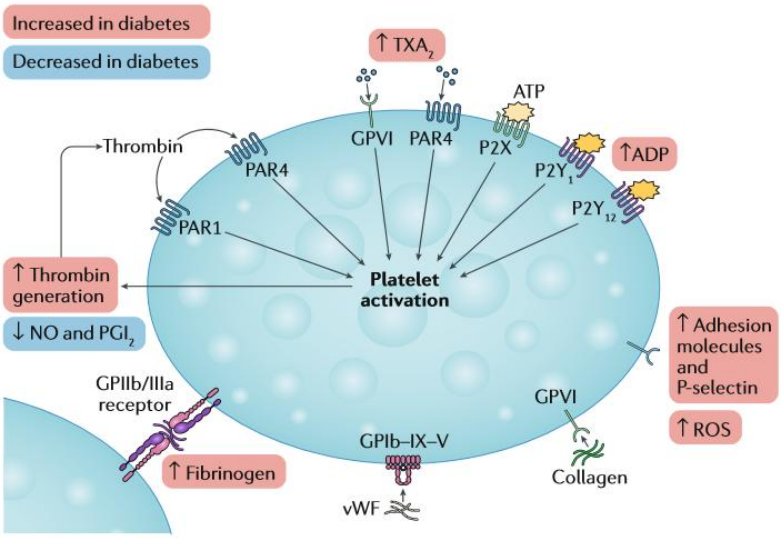

Figure 1. Pathways leading to increased platelet aggregability in diabetes mellitus. Increased platelet reactivity in diabetes involves higher levels of thrombin generation, increased production of thromboxane A2 (TXA2), hyperresponsiveness of proteinase- activated receptor 4 (PAR4) to thrombin and TXA2, and increased platelet membrane expression of P-selectin, adhesion molecules, and glycoprotein (GP) IIb/IIIa. Signalling of P2Y purinoceptor 12 (P2Y12 receptor) — the main platelet receptor for ADP — is also increased. Vascular synthesis of nitric oxide (NO) and prostaglandin I2 (PGI2; also known as prostacyclin) is decreased, and the production of reactive oxygen species (ROS) is increased. P2X, P2X purinoceptor ; P2Y1, P2Y purinoceptor 1; vWF, von Willebrand factor.

Hyperglycemia and associated conditions, such as obesity, dyslipidemia and inflammation, modulate this phenotype.[2] *In-vitro* studies from diabetic patients have shown increased platelet membrane expression of P-selectin and glycoprotein (GP) IIb/IIIa, as well as enhanced platelet response to epinephrine and thrombin.[5] Impaired arachidonic acid pathway accounts for platelet dysfunction *in-vivo*, that can be improved through suppression of platelet cyclo-oxygenase-1 (COX-1).[4]

Diabetic platelets show increased turnover, resulting in a higher number of reticulated platelets, and enhanced adhesion to endothelial cells.[5] A reduction of platelet fluidity occurs through changes in membrane lipid structure or glycation of membrane proteins.[2] Furthermore, diabetic platelets have an increased expression of adhesion molecules, such as CD63, CD62p, CD49b, CD36 and CD31, as documented by flow cytometry.[6]

Another important determinant of platelet dysfunction in diabetes is the reduced vascular production of nitric oxide (NO) and prostaglandin (PG) I_2, that physiologically inhibit platelet aggregation.[5] Other reactive oxygen species (ROS) may also contribute to *in-vivo* platelet hyperactivation in diabetes.[5]

Coagulation activity in diabetic patients

Multiple pathophysiological mechanisms may contribute to the prothrombotic environment associated with type 2 DM (Figure 2), a condition characterized by hyperglycemia, hyperinsulinemia, low-grade inflammation, and raised plasma triglycerides.

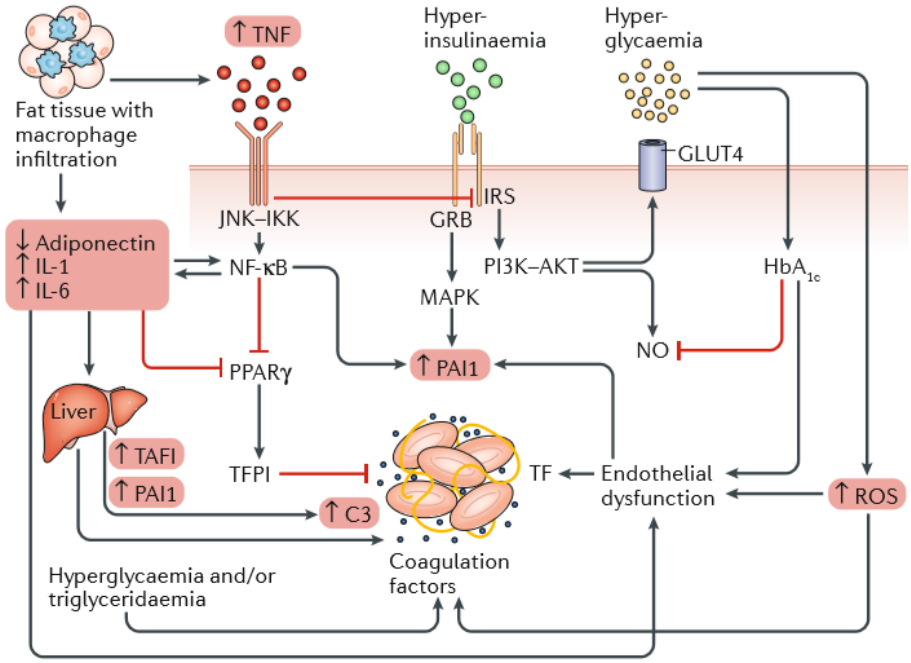

Figure 2. Intracellular pathways underlying procoagulant patterns in diabetes mellitus. Fat tissue produces less adiponectin and is infiltrated by macrophages that release tumour necrosis factor (TNF), IL-1, and IL-6. This inflammatory state increases the synthesis of plasminogen activator inhibitor 1 (PAI1) and tissue factor (TF) by endothelial cells, as well as coagulation factors, carboxypeptidase B2 (also known as thrombin- activable fibrinolysis inhibitor ; TAFI), PAI1, and acute phase proteins, such as complement C3, by the liver. TNF blocks the vasculoprotective insulin pathway involving insulin receptor substrate (IRS)–phosphoinositide 3-kinase (PI3K)–RACα serine/threonine- protein kinase (AKT) and activates inflammation through the signalling pathway involving c-Jun N-terminal kinase (JNK)–inhibitor of nuclear factor-κB kinase (IKK)–nuclear factor-κB (NF-κB). Impaired IRS–PI3K–AKT transduction alters nitric oxide (NO) and insulin-responsive glucose transporter type 4 (GLUT4; also known as SLC2A4) function, whereas the prothrombotic insulin pathway involving growth factor receptor- bound protein (GRB)–mitogen-activated protein kinase (MAPK) remains effective. Inflammation also blunts peroxisome proliferator-activated receptor-γ (PPARγ)- mediated synthesis of the anticoagulant tissue factor pathway inhibitor (TFPI). Hyperglycaemia increases production of reactive oxygen species (ROS) and contributes to endothelial dysfunction. Increased levels of glycated haemoglobin (HbA1c) alter the physiological transport and release of NO. Hyperglycaemia and triglyceridaemia favour the synthesis of coagulation factors and PAI1.

Hyperglycemia has direct effects on gene transcription of coagulation factors, and hyperglycemia-induced oxidative stress alters the natural vasculoprotective endothelial glycocalyx.[7,8] Insulin and proinsulin-like molecules in experimental systems promote the expression and secretion of plasminogen activator inhibitor type 1 (PAI-1) by hepatocytes and endothelial cells.[8,9]

Quantitative changes of coagulation factors in patients with DM involve higher plasma levels of fibrinogen (the soluble precursor of solid fibrin) and of coagulation factors VII (FVII) and FXII, increased endothelial expression of tissue factor (TF) and tissue factor/FVIIa complex activity, and a reduction in the anticoagulant protein, tissue factor pathway inhibitors (TFPI).[7,8] These changes culminate in enhanced thrombin generation and fibrin formation. Quantitative changes of fibrinolytic factors include increased plasma levels of PAI-1 and thrombin activatable fibrinolysis inhibitor (TAFI).[8,9] Levels of tissue-type plasminogen activator (t-PA) are also altered, with raised antigen concentrations and reduced t-PA activity.[8,9]

Qualitative changes of hemostatic factors include the glycation/oxidation of fibrinogen and plasminogen, as well as the incorporation of antiplasmin, PAI-1, TAFI and C3 into the fibrin mesh, resulting in a denser clot structure and in delayed spontaneous clot lysis.[7,8] Enhanced thrombin generation activates coagulation FXIII; this cross-links and further stabilizes the fibrin network.[8] Moreover, glycation of hemoglobin (Hb) in patients with DM alters the physiological transport and release of NO by Hb itself, with impaired peripheral vasodilatation, enhanced insulin resistance, and proinflammatory/prothrombotic effects.[10] An overall hypercoagulable state is demonstrated by the significant shortening of activated partial thromboplastin time (aPTT) in patients with DM compared to non-diabetic subjects.[8]

Thus, our current understanding of the pathophysiological steps implicated in the increased thrombotic risk of patients with DM includes increased thrombin and fibrin generation, delayed clot lysis and reduced NO bioavailability. Future therapies targeting the coagulation, fibrinolysis and NO systems, rather than platelets alone, may open new frontiers in the prevention and treatment of cardiovascular diseases in these patients.

1.2. Antithrombotic therapy for secondary prevention in diabetic patients with coronary artery disease

Stable Coronary Artery Disease

The presence of DM impairs clinical outcomes in patients with cardiovascular disease, in whom meta-analyses of randomized trials indicate an overall 40% increase of major adverse cardiovascular events [MACE, i.e., myocardial infarction (MI), stroke or cardiovascular death], compared to patients without diabetes.[11,12] In the setting of secondary cardiovascular prevention in patients with DM and stable CAD, data from the ATT Collaboration on approximately 4,500 patients showed a 4.2% absolute reduction of cardiovascular events with aspirin versus placebo at 60-months' follow-up, similar to that observed in non-diabetic patients.[11] Clopidogrel has been extensively investigated in patients with stable CAD undergoing PCI or treated conservatively. Table 4 reports the main results of randomized trials evaluating antiplatelet therapy in patients with stable CAD for primary efficacy endpoints in the overall population and in the diabetic subgroup. In the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial, patients with established cardiovascular disease were randomized to clopidogrel (75 mg daily) or aspirin (325 mg daily).[13] The subgroup analysis including 3,866 diabetic patients demonstrated that the use of clopidogrel instead of aspirin prevented 21 adverse events (vascular death, MI, stroke, hospitalization for ischemic or bleeding complications) per 1000 patients per year (versus 9/1000 patients per year in non-diabetic individuals).[14] Of note, the cardiovascular protection provided by clopidogrel was even higher in the subgroup of patients with DM on insulin therapy (38 events prevented/1000 patients per year).[14]

The addition of clopidogrel on top of aspirin in stable patients was evaluated in the secondary prevention cohort (i.e., patients with previous MI, stroke or PAD) of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) trial.[15] Here, at a 28-month follow-up, in the subgroup with DM there was no greater protection from cardiovascular events with DAPT versus patients without DM, but higher bleeding risk .[16] Of note the use of aspirin plus clopidogrel versus aspirin alone led to a significant reduction of the

combined endpoint including CV death, MI, or stroke only in patients without diabetic nephropathy (6.7% versus 7.7%; p=0.048), whereas no sizable benefit was observed in those with diabetic nephropathy (11.4% versus 12.0%, p=0.84). Thus, current evidence indicates that the net clinical benefit of routinely adding clopidogrel to aspirin in diabetic patients with stable CAD treated medically is questionable, as the modest ischemic protection is outweighed by the increased bleeding risk. The Clopidogrel for the Reduction of Events during Observation (CREDO) trial investigated the effects of pre-procedural clopidogrel load on top of aspirin in patients undergoing elective PCI.[17] Interestingly, both in the overall population and in the diabetic subgroup, pretreatment with 300 mg clopidogrel led to significant clinical benefit compared to downstream clopidogrel but only when the loading dose was given at least 6 hours prior to the intervention. This essentially reflects the fact that several hours are needed to achieve maximal platelet inhibition after a 300 mg clopidogrel load.[18,19] Interestingly, a meta-analysis showed no benefit for MACE incidence with 12-month versus 6-month DAPT among diabetic patients undergoing PCI with drug-eluting stent implantation for a variety of coronary syndromes.[20]

The Cardiovascular Outcomes for People Using Anticoagulation Strategies (COMPASS) trial recently explored the safety and efficacy of inhibition of thrombin generation, as provided by rivaroxaban (2.5 mg twice daily) on the background of aspirin therapy, or by rivaroxaban (5.0 mg twice daily) alone in patients with stable coronary or peripheral atherosclerotic disease (91% with CAD, 62% with previous MI); this study was prematurely stopped for superiority of the rivaroxaban-plus-aspirin versus aspirin alone arm after a mean follow-up of 23 months in terms of MACE (cardiovascular death, MI, stroke), all-cause death and cardiovascular death.[21] Consistent with the overall study results, in patients with DM (N=6,922) the addition of rivaroxaban to aspirin resulted in a significantly lower incidence of MACE (HR 0.74, 95% CI 0.61-0.90) with higher rates of major bleeding (HR 1.70, 95% CI 1.25-2.31); net clinical benefit was significantly in favor of combination therapy, without increased rates of intracranial bleeding.

Acute Coronary Syndromes

The clinical benefit provided by antiplatelet therapy in diabetic patients with ACS has been clearly demonstrated. Table 1 reports the main results of randomized trials evaluating antiplatelet therapy in patients with ACS for primary efficacy endpoints in the overall population and in the diabetic subgroup. Meta-analytic data have shown that the reduction of MACE by aspirin versus placebo after MI is similar in patients with and without DM (from 22% to 18% incidence in those with DM, and from 16% to 13% in those without DM).[11,12] The above data also indicate that, despite aspirin therapy, the recurrence of ischemic events in diabetic patients with ACS is approximately 20% over a mean of approximately 2.5 years.[36] Although a persistently higher platelet reactivity while on aspirin may at least in part explain this elevated recurrence rate[22], the randomized Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events−Seventh Organization to Assess Strategies in Ischemic Syndromes (CURRENT-OASIS 7) trial demonstrated no outcome improvement with higher-dose aspirin (300-325 mg daily versus 75-100 mg daily for 30 days) in a mixed population of both diabetic and non-diabetic patients admitted for ACS, with no significant interaction between primary outcome and diabetic status.[23]

Additional inhibition of the P_2Y_{12} platelet receptor on the background of aspirin therapy, aimed at further reducing adverse events in patients with ACS, was first evaluated in the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, whereby patients with non-ST elevation (NSTE)-ACS already receiving aspirin were randomized to clopidogrel, 300 mg load, followed by 75 mg/day maintenance, or placebo for a mean duration of 9 months.[24] The lower rate of MACE observed by adding clopidogrel to aspirin occurred regardless of diabetic status; although no significant interaction was present, the relative reduction of adverse cardiovascular events at one year with clopidogrel in the 2,840 patients with DM was numerically lower than in those without (14% versus 20% relative risk reduction, respectively). This may be due to the more pronounced inter-individual variability of clopidogrel responsiveness in diabetes, resulting in a higher prevalence of HPR.[25–27]

Table 1. Main results of randomized trials evaluating antiplatelet therapy in patients with stable CAD or ACS for primary efficacy endpoints in the overall population and in the diabetic subgroup.

Study	N. overall pts	Scenario	Primary endpoint	% events in the overall population	N. diabetic pts	% events in diabetic population
CAPRIE: ASA vs Clopidogrel	19,185	Patients at risk of ischemic events	Vascular death, MI, stroke, hospitalization for ischemic or bleeding complications at 36 months.	5.8 vs 5.3 RRR=8.7% (0.3-16.5%)	3,866	17.7 vs 15.6 RRR=21% (NA)
CHARISMA: ASA + Clopidogrel vs ASA	15,603	Stable CAD with high atherothrombotic risk	Cardiovascular death, MI, or stroke at 28 months	6.8 vs 7.3 RR=0.93 (0.83-1.05)	6,555	6.7% vs 7.7% in patients without nephropathy 11.4% vs 12% in patients with nephropathy RR not reported
CREDO: ASA + Clopidogrel vs ASA	2,116	Elective PCI	Death, MI or stroke at 1 yr	8.3 vs 11.5 RRR=27% (3.9-44.4%)	560	Not reported
CURE: Asa + Clopidogrel vs ASA	12,562	UA/NSTEMI	Cardiovascular death, MI or stroke at 1 yr	9.3 vs 11.4 RR=0.80 (0.72-0.90)	2,840	14.2 vs 16.7 RR=0.84 (0.70-1.02)
TRITON-TIMI 38: ASA + Prasugrel vs ASA + Clopidogrel	13,608	ACS with scheduled PCI	Cardiovascular death, MI or stroke at 1 yr	9.9 vs 12.1 HR=0.81 (0.73-0.90)	3,146	12.2 vs 17.0 HR=0.70 (0.58-0.85)
PLATO: ASA + Ticagrelor vs ASA + Clopidogrel	18,624	ACS	Cardiovascular death, MI or stroke at 1 yr	9.8 vs 11.7 HR=0.84 (0.77-0.92)	4,662	14.1 vs 16.9 HR=0.88 (0.76-1.03)
TRILOGY-ACS: ASA + Prasugrel vs ASA + Clopidogrel	9,326	ACS patients medically managed	Cardiovascular death, MI or stroke at 30 months	13.9 vs 16.0 HR=0.91 (0.79-1.05)	3,539	24.0 vs 25.6 HR=0.95 (0.81-1.11)

DAPT: ASA + Clopidogrel or Prasugrel vs ASA	9,961	Stable or ACS patients treated with DES implantation	Stent thrombosis, death, MI or stroke at 30 months	4.3 vs 5.9 HR=0.71 (0.59-0.85)	3,391	6.6 vs 7.0 HR=0.92 (0.71-1.20)
PEGASUS: ASA + Ticagrelor vs ASA	21,162	Patients with a prior (1-3 years) history of MI	Cardiovascular death, MI or stroke at 36 months	7.8 vs 9.0 HR=0.84 (0.74-0.95)	6,806	10.0 vs 11.6 HR 0.83 (0.69-1.0)

ACS= Acute coronary syndrome; ASA= Aspirin; CAD= Coronary artery disease; DES= Drug-eluting stent; HR=Hazard ratio (95% confidence interval); MI= Myocardial infarction; NSTEMI= Non-ST segment elevation myocardial infarction; PCI= Percutaneous coronary intervention; RR=Risk ratio (95% confidence interval); RRR=Relative risk reduction (95% confidence interval); UA= Unstable angina

As discussed above, the mechanisms behind poor clopidogrel-induced P_2Y_{12} inhibition in patients with DM may include impaired drug metabolism, leading to reduced active metabolite generation as well as dysregulation of the P_2Y_{12} signaling pathway.[28,29] The Optimizing anti-Platelet Therapy In diabetes MellitUS-3 (OPTIMUS-3) study found that prasugrel in patients with DM achieved faster and greater platelet inhibition, as well as lesser inter-individual variability, than clopidogrel, even when the latter was given at high doses (600 mg load plus 150 mg/day).[30] Similar pharmacodynamic results were obtained with ticagrelor.[31] Given the pharmacodynamic drawbacks of clopidogrel in patients with DM, potentially reversed by the newer, more potent $P2Y_{12}$ inhibitors, the subgroup analyses of diabetic populations from phase III studies comparing prasugrel and ticagrelor versus clopidogrel in the setting of ACS are of particular interest.

In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel–Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38 trial), the use of prasugrel instead of standard-dose clopidogrel after PCI for ACS led to a higher relative risk reduction of MACE (cardiovascular death, stroke and MI) at 15 months in diabetic patients than in non-diabetic patients (30% versus 14%, respectively; p for interaction = 0.009).[32] Importantly, in the former group prasugrel did not increase the rates of major bleeding. In TRITON-TIMI 38, the greatest benefit in terms of adverse event reduction with prasugrel was observed in patients on insulin therapy (37% relative risk reduction, versus 26% in diabetic patients not receiving insulin).[32] The TRILOGY-ACS (Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes) study included patients with non-ST-segment elevation ACS managed without revascularization and randomized to receive prasugrel or clopidogrel for up to 30 months on top of aspirin.[33] A sub-analysis by diabetes status showed no difference in the incidence of MACE (cardiovascular death, MI, or stroke) with either antiplatelet strategy in both patient subgroups, with (N=3,539) and without DM.[34] In the PLATelet inhibition and patient Outcomes (PLATO) trial, patients with ACS were randomized to receive ticagrelor (180 mg loading, then 90 mg twice daily) or clopidogrel (300 or 600 mg load, then 75 mg daily), irrespective of the

subsequent therapeutic strategy.[35] A lower rate of MACE at one year by ticagrelor was observed in both patients with and without DM (12% and 17% relative reductions, respectively). This ischemic protection largely outweighed the elevation in non-bypass surgery-related major bleeding observed in the ticagrelor arm. Of note, the use of ticagrelor had a strong impact in decreasing MACE, all-cause death and stent thrombosis in diabetic patients with HbA1c ≥6%. Notably, a pharmacodynamic comparison of prasugrel versus ticagrelor in diabetic patients with CAD showed that the latter drug exerts similar or greater inhibition of ADP-induced platelet reactivity than prasugrel both in the acute and chronic phase of treatment.[36] Based on the available evidence, it is reasonable to recommend consistent and highly-effective platelet inhibition with the newer, more potent $P2Y_{12}$ inhibitors for patients with ACS and concomitant DM, given their high baseline risk profile; thus, DAPT with aspirin plus prasugrel/ticagrelor should represent the first-line antiplatelet strategy up to one year in this setting, especially in patients without high bleeding risk (Table 3). Moreover, given the poorer long-term cardiovascular outcome of diabetic versus non-diabetic patients suffering a MI, DAPT prolongation is a particularly relevant issue in this setting. The Dual Antiplatelet Therapy (DAPT) study explored the efficacy and safety of prolonging DAPT beyond one year with aspirin plus a thienopyridine (prasugrel or clopidogrel) versus aspirin alone for 18 more months in patients with stable CAD or ACS who had undergone drug-eluting stent implantation.[38] In the sub-analysis by diabetes status (N=3,391 with and N=8,257 without) there was a significant interaction between MI risk reduction and prolonged DAPT in favor of patients without DM (58% relative reduction versus 28% in patients with DM; p for interaction 0.02).[39] Regardless of diabetes status, the rates of stent thrombosis were lower and of bleeding events higher in the DAPT group (stent thrombosis: 75% relative reduction in non-diabetic patients versus 53% in diabetic patients, p for interaction 0.21; moderate/severe bleeding: 71% relative increase in non-diabetic patients versus 47% in diabetic patients, p for interaction 0.61).[39] Of note, withdrawal of thienopyridine in both arms resulted in a consistent numerical increase of early ischemic events in patients with and without DM.[39] The Prevention of Cardiovascular Events in Patients with Prior

Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin-Thrombolysis in Myocardial Infarction 54 (PEGASUS-TIMI 54) trial explored the efficacy and safety of DAPT prolongation (with ticagrelor 90 or 60 mg twice daily plus aspirin versus aspirin alone) in patients with a prior (1-3 year) history of MI.[40] The reduction of MACE with either ticagrelor dosing was consistent in patients with and without DM. Given their higher risk of events, diabetic patients achieved a greater absolute benefit from ticagrelor, with a 3-year number needed to treat for MACE, pooling results of both ticagrelor doses, of 67 versus 91 in those without diabetes. Additionally, in patients with DM, ticagrelor reduced cardiovascular death by 22%. Similarly to non-diabetic patients, in patients with DM ticagrelor significantly increased the rates of non-fatal major bleeding (HR 2.56, p = 0.0004). Regardless of diabetic status, the net clinical outcome was better with the 60 mg versus the 90 mg ticagrelor dosing, i.e., similar ischemic protection, but lower bleeding risk.

1.3. Thromboembolic risk and prevention of thromboembolism in diabetic patients with atrial fibrillation

DM increases the chances of having AF and worsens the prognosis of patients with AF.[41] New-onset AF patients with DM had an increased risk of all-cause death (HR 2.65), MI (HR 2.1) and heart failure (HR 3.8) compared to those without DM.[42] The presence of DM raises the incidence of thromboembolic events (stroke or systemic embolism) in patients with AF, explaining the inclusion of DM in contemporary scores for stroke prediction.[43] In a meta-analysis of 7 studies including >12,000 AF patients, an overall 70% relative increase in the risk of thromboembolic complications was observed in diabetic versus non-diabetic patients, with a yearly incidence ranging from 3.6% to 8.6%.[44] This variability reflects differences in study designs, definitions of outcome measures, patients' baseline risk profile, concomitant therapies, and types of diabetic populations included. Recent data have identified a duration of DM >3 years as an independent predictor of ischemic

stroke, stronger than glycemic control.[45] A recent sub-analysis from the European Prevention of thromboembolic events-European Registry in Atrial Fibrillation (PREFER in AF) registry, involving >5,000 patients with AF, showed that diabetic patients on insulin therapy had a >2-fold higher risk of stroke/systemic embolism at 1 year versus non-diabetic patients, whereas diabetes not treated with insulin did not entail a significantly increased risk.[46]

Oral anticoagulant therapy (OAC), historically with warfarin, is the cornerstone of treatment to reduce thromboembolic risk of patients with AF, including those with diabetes.[47] In the last decade, NOACs have signed a revolution in the antithrombotic treatment to prevent AF-related thromboembolic events, because of their favorable characteristics: predictable dose-response, rapid offset and onset, fixed doses, no interaction with food, limited interactions with other drugs, no need for routine monitoring. A recent meta-analysis of 4 phase III trials comparing NOACs versus warfarin in patients with AF showed NOACs to be associated with an overall statistically significant 19% relative reduction of the combined endpoint including any stroke or systemic embolism ($p<0.0001$) and a 14% relative reduction of major bleeding ($p<0.06$), compared to well-managed warfarin;[48] among 71,683 patients on NOACs, the prevalence of DM ranged from 23.3% (in the Randomized Evaluation of Long-Term Anticoagulation Therapy - RE-LY study) to 40% (in the Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation – ROCKET AF-study). Of note, in those trials blood glucose levels and HbA1 values were not routinely available and patients with creatinine clearance <30 mL/min were excluded. Therefore, patients with severe diabetic nephropathy, who are at even higher risk of cardiovascular complications, were excluded.[49] In these phase III studies there was no interaction between diabetes status and clinical efficacy of NOACs versus warfarin, although safety superiority of apixaban versus warfarin was lost among AF patients with DM (p for interaction 0.003).[49] Moreover, a recent study-level meta-analysis of those trials added more robust evidence on the topic (Figure 3), indicating that, compared to warfarin: a) the use of NOACs decreased the rates of intracranial bleeding regardless of diabetic

status (43% relative reduction versus warfarin in patients with DM and 38% in those without; p for interaction 0.47); b) in patients with DM, NOACs significantly reduced cardiovascular death versus warfarin use (Risk Ratio 0.83, 95% CI 0.72-0.96; p = 0.01).[50]

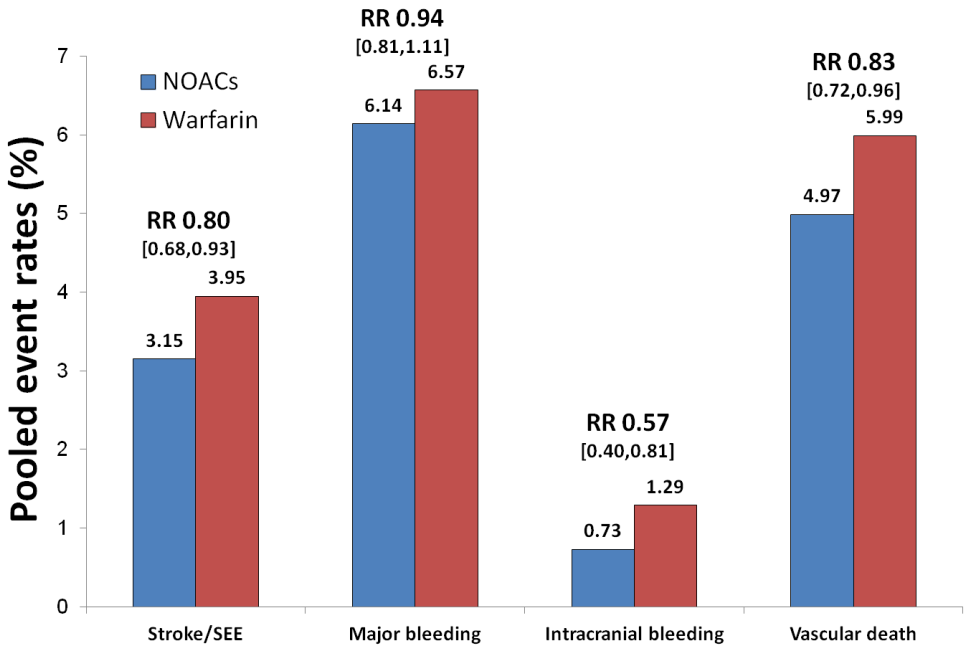

Figure 3. Anticoagulation in patients with diabetes mellitus and atrial fibrillation. Pooled event rates of the various outcome measures from phase III trials comparing non-vitamin K antagonist oral anticoagulants (blue) versus warfarin (red) for the treatment of patients with diabetes and atrial fibrillation. RR , risk ratio.

To date, no data on a direct comparison among different NOACs in diabetic patients are available; therefore, the choice of NOAC type in patients with DM is not supported by specific evidence, but should refer to general principles and take into account the co-morbidities associated with DM.

According to the above-mentioned data, in Table 3 we suggest antithrombotic strategies for AF patients with DM.

2. Hypothesis and Aims

2.1. Hypothesis

We hypothesized that clinical features and risk of cardiovascular disease may vary within subjects affected by different metabolic disorders. The contemporary epidemiological investigation of cardiovascular complications in metabolic disorders allows better risk stratification in order to optimize treatment strategies.

2.2. Overall aim

The overall aim of this Ph.D. project was to investigate the prognostic impact of metabolic disorders and their related therapies on cardiovascular health in the current era.

2.3. Specific aims

4. **to investigate causes of death and their associated risk factors in type 2 diabetes patients with or without established cardiovascular disease**

To this aim, we performed a cause-of-death analysis among patients enrolled in SAVOR-TIMI 53 and used the competing-risk methodology to identify independent predictors of cardiovascular death and non-cardiovascular death. In addition, we aimed to describe features associated specifically with sudden cardiac death in diabetes.

5. **to investigate the impact of metabolic disease on cardiovascular outcomes in a particular subset of patients, a contemporary cohort of subjects after an acute coronary syndrome**

To this aim, we aimed to assess long-term cardiovascular risk associated with the presence of the metabolic syndrome or diabetes mellitus among patients recently hospitalized with an acute coronary syndrome using data from the SOLID-TIMI 52 trial.

6. **to investigate the risk of thromboembolic complications in patients with atrial fibrillation and diabetes**

To this aim, we explored the differential prognostic weight of diabetes on insulin therapy versus no insulin therapy on thromboembolic events in patients with AF using data from a multicenter, European AF registry called PREFER in AF.

4. to assess cardiovascular safety (a composite of cardiovascular death, myocardial infarction, or stroke, and new-onset atrial fibrillation or flutter) of odanacatib, a cathepsin K inhibitor for the treatment of postmenopausal osteoporosis

To this aim, the anti-fracture efficacy and safety of odanacatib in postmenopausal women with osteoporosis was assessed in the Long-term Odanacatib Fracture Trial (LOFT) and its extension study (LOFT Extension).

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3. Specific aim 1: to investigate causes of death and their associated risk factors in type 2 diabetes patients with or without established cardiovascular disease

Cavallari I, Patel RB, Bhatt DL, Steg PG, Leiter LA, McGuire DK, Mosenzon O, Kanevsky E, Im K, Raz I, Braunwald E, Scirica BM. Causes of Death and Their Associated Risk Factors in Type 2 Diabetes Patients With or Without Established Atherosclerotic Cardiovascular Disease: A Competing-Risk Analysis from the SAVOR-TIMI 53 Trial. Submitted for publication.

3.1. Background

The prevalence of type 2 diabetes (T2D) continues to increase throughout the world. The International Diabetes Federation estimates that 425 million people have diabetes and are thus at risk for its complications, including disability, reduced quality of life, and premature death.[51] The rate of death among patients with T2D is approximately twice as high as that among people without T2D.[52–54] More than half the mortality and a substantial proportion of the associated morbidities in T2D is due to cardiovascular (CV) complications.[55]

Data from a large cohort study showed that the excess risk of death, myocardial infarction or stroke among patients with T2D has a graded association with five CV risk-factors, such that when all five are within the clinical target range, the excess risk associated with T2DM is no longer evident.[56] Given the benefit of aggressive and comprehensive CV risk factor management, and the potential to change the natural history of T2D through newer glucose lowering agents that reduce cardiovascular risk, an assessment of the causes of death and clinical factors associated with specific categories of death in the contemporary era may aid the management of patients with T2D and identify a high-risk subgroup that could benefit from close monitoring and targeted treatment strategies.[57,58] To date, specific causes of death and their actual risk factors in a contemporary cohort of patients with T2D with or without established atherosclerotic cardiovascular disease (ASCVD) have not been well described.

The Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction 53 (SAVOR-TIMI 53) trial, which investigated cardiovascular efficacy and safety of saxagliptin, a DPP-4 inhibitor, when added to standard of care in >16,000 patients with T2D, represents a unique opportunity to analyze causes of mortality in T2D.[59]

In the present study, we performed a cause-of-death analysis among patients enrolled in SAVOR-TIMI 53 and used the competing-risk methodology to identify independent predictors of CV death and non-CV death. In addition, we aimed to describe features associated specifically with sudden cardiac death (SCD) in diabetes.

3.2. Methods

Study Design and Population

SAVOR-TIMI 53 was a randomized, double-blind, placebo-controlled trial to assess the cardiovascular efficacy and safety of saxagliptin, a DPP-4 inhibitor, when added to standard of care in patients with T2D.[59] The trial was designed by the TIMI Study Group and Hadassah Medical Organization in conjunction with AstraZeneca and Bristol-Myers Squibb.

The study population of the SAVOR-TIMI 53 trial included 16,492 patients with T2D (HbA1c between 6.5% and 12.0%) and either established ASCVD (N=12,825, 78%) or multiple risk factors for ASCVD (N=3,667, 22%). To be included in the established ASCVD disease cohort, patients had to be at least 40 years old and have a history of a clinical event secondary to atherosclerosis involving the coronary, cerebrovascular, or peripheral artery systems. Patients in the multiple risk factor cohort had to be at least 55 years old (men) or 60 years old (women) and have at least one of the following risk factors for atherosclerosis: dyslipidemia, hypertension, or active smoking. The full eligibility criteria of the trial have been reported previously.[60] The median follow-up was 2.1 years. The primary safety and efficacy outcome was a composite of CV death, myocardial infarction (MI), or ischemic stroke.

Blood samples for biomarkers measurements were collected at baseline in approximately 12,500 participants. Plasma and serum were carefully separated on site and stored at -20°C or colder until shipped to the central laboratory on dry ice, where they were stored at -70°C or colder until thawed for analysis. Serum N-terminal pro brain natriuretic peptide (NT-proBNP) concentrations were measured using a sandwich immunoassay (proBNP II, Roche Diagnostics, Indianapolis, IN). Plasma high-sensitivity troponin T (hsTnT) was measured with an electrochemiluminescent immunoassay assay (Roche Diagnostics). C-reactive protein (CRP) was measured with an enhanced immuno-turbidimetric assay (Roche Diagnostics).

Death Events

A Clinical Events Committee comprising specialists in cardiovascular medicine, unaware of the study group assignments, adjudicated the cause for all deaths and classified them as CV death and non-CV death, based on the Standardized Definitions for End Point Events in Cardiovascular Trials.[59,61] SCD refers to a death that occurs unexpectedly in a previously stable patient and includes the following deaths: witnessed death, without symptoms or within 60 minutes of the onset of new or worsening symptoms; death attributed to an identified arrhythmia; death after unsuccessful resuscitation from cardiac arrest or after successful resuscitation without identification of a non-cardiac etiology; unwitnessed death without other evident cause of death. For the purpose of the present analyses, two physicians (I.C. and R.B.P.) further reviewed source documents of all adjudication-confirmed SCD events to abstract additional clinical data.

Statistical Analysis

Baseline characteristics of patients who died during follow-up were compared with those alive using a χ^2 test for categorical variables or Kruskal Wallis test for continuous variables. Two-year cumulative incidence rates were used to estimate the incidence of each type of death.[8] The

crude associations between mortality and different baseline variables were first quantified by univariable Cox regression. All covariates that reached a significance level of P <0.10 in univariable regression or the risk factors traditionally known to be associated with mortality regardless of initial significance level (i.e., sex, body mass index, duration of diagnosed diabetes, fasting serum glucose, HbA1c, retinopathy, estimated glomerular filtration rate [eGFR] ≤50 ml/min/1.73m^2 and insulin treatment) were then included in a multivariable regression model. A backwards selection method was applied to obtain a final model (P for retention in the model <0.05). A Cox proportional hazards regression analysis was used to identify variables independently associated with overall mortality. We performed a competing risk analysis based on the proportional hazards model for the subdistribution of competing risks.[62,63] Competing risks occur frequently in the analysis of survival data; indeed, in a study examining time to death attributable to cardiovascular causes, death attributable to non-cardiovascular causes is a competing risk as the occurrence of non cardiovascular death will preclude the occurrence of cardiovascular death. When fitting regression models in the presence of competing risks, there are two different families of models: the Fine and Gray model which allows us to estimate the effect of covariates on the cumulative incidence function for the each competing risk or the cause-specific hazard which models the effect of covariates on the cause-specific hazard of the outcome.[62,63] As the aim of analysis is to make inferences about the association of clinical parameters on the incidence of death, Fine and Gray model would be more appropriate.[64] However, a sensitivity analysis using a cause-specific hazard model was also conducted.[62] Additional sensitivity analyses included available cardiac and inflammatory biomarkers in each model. Statistical analysis was performed using SAS statistical software version 9.4 (SAS Institute Inc) and R software (www.r-project.org).

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3.3. Results

Among 16,492 patients with T2D followed for a median of 2.1 years, there were 798 deaths with 66.3% (N=529) classified as CV death and 33.7% (N=269) as non-CV death. Two-year cumulative incidence rates were 3.0% for CV death and 1.5% for non-CV death. SCD accounted for the majority of CV death (N=240; 30.1% of total deaths) followed by presumed CV death or other CV causes not related to cerebrovascular events or acute MI (N=106; 13.3%), and death for heart failure (N=84; 10.5%). Malignancy was the most frequent cause of non-CV death (N=111; 13.9% of total deaths) followed by infections (N=74; 9.3%). Figure 1 reports detailed information about causes of death in the entire population enrolled in the study.

Among 12,825 patients with T2D with established CV disease, there were 671 deaths (two-year cumulative incidence rate 5.10%) distributed as follows: 68.3% CV death (N=458) and 31.7% non-CV death (N=213). Two-year cumulative incidence rates were 3.51% for CV death and 1.59% for non-CV death. SCD accounted for the majority of CV death (N=203; 44.3%) (Supplemental Figure 1A). Among 3,667 patients with T2D without established CV disease but with multiple CV risk factors, there were 127 deaths (two-year cumulative incidence rate 2.61%) distributed as follows: 55.9% CV death (N=71) and 44.1% non-CV death (N=56). Two-year cumulative incidence rates were 1.54% for CV death and 1.07% for non-CV death (Supplemental Figure 1B). SCD accounted for the majority of CV death (N=37; 52.1%). Figure 2 reports the incidence of each cause of death comparing patients with T2D with established CV disease and those with multiple CV risk factors. Among the two cohorts, the incidence of the above-mentioned causes of death was comparable except for acute MI that was more frequently the cause of death in patients with established CV disease, and for malignancy that was more frequently cause of death in patients with multiple CV risk factors.

Patient characteristics according to vital status are summarized in Table 1. Patients who died were more likely to be older, have longer duration of diagnosed T2D, higher baseline HbA1c

and be on insulin therapy (P<0.05 for all comparisons). In addition, they had higher prevalence of macrovascular and microvascular complications.

Independent correlates of overall mortality are reported in Table 2. Older age (hazard ratio [HR] per 5 years 1.26), prior heart failure (HR 2.04), albuminuria defined as albumin to creatinine ratio ≥3.4 mg/mmol (HR 1.84), eGFR≤50 ml/min/1.73m^2 (HR 1.80), peripheral artery disease (PAD) (HR 1.88), higher heart rate (HR per 5 bpm 1.10), prior MI (HR 1.44) and higher HbA1c (HR per 1% 1.11) at baseline were significantly associated with overall mortality (all P values<0.001). On the other hand, BMI, diagnosed duration of diabetes, fasting serum glucose, CAD without prior MI, retinopathy and insulin use were not found to be independent predictors of all-cause death.

Clinical Factors Associated with Specific Causes of Death in Type 2 Diabetes

Clinical factors associated with different causes of death are reported in Tables 3 and 4. The following predictors exhibited relatively large magnitude of association with incidence of CV death: prior heart failure (subdistribution hazard ratio [SHR] 2.49), older age (SHR per 5 years 1.24), albuminuria (SHR 1.98), PAD (SHR 1.94), eGFR ≤50 ml/min (SHR 1.71), prior MI (SHR 1.57), higher HbA1c (SHR per 1% 1.11), higher heart rate (SHR per 5 bpm 1.08), male sex (SHR 1.37) and insulin use (SHR 1.23) (all P values <0.05). SCD and other types of CV death shared the same predictors.

Older age (SHR per 5 years 1.28), higher heart rate (SHR per 5 bpm 1.13), eGFR ≤50 ml/min (SHR 1.78), albuminuria (SHR 1.59), prior ischemic stroke (SHR 1.66) and PAD (SHR 1.61) were found to be independently associated with non-CV death (all P values <0.05).

A sensitivity analysis using cause-specific hazard models was conducted and results were generally consistent (Supplemental Table 1).

Biomarkers

At baseline, abnormal NT-proBNP (≥125 pg/ml if <75 years or ≥450 pg/ml if ≥75 years), hsTnT (>14 pg/ml, the 99th percentile upper reference limit value) and CRP (>3mg/l) concentrations were detected in 74.6%, 73.9% and 74.6% of the participants. Elevated levels of each of these biomarkers were significantly associated with all causes of death, including non-CV death (Supplemental Tables 2 and 3). In particular, elevated levels of NT-proBNP and hsTnT were more significantly associated with CV death than clinical variables and elevated CRP (SHR 2.82; 95% CI, 2.16-3.69 and SHR 2.46; 95% CI, 1.91-3.18, respectively, all P values <0.001; Supplemental Table 2). Elevated levels of NT-proBNP and hsTnT were confirmed significant predictors of all causes of death in both the primary and secondary prevention cohorts.

Features of SCD in Type 2 Diabetes

Review of source documents revealed that the majority of SCD occurred out of hospital (81.2%) and independent of location, medical resuscitation was attempted in 28.7% of patients with SCD. For most of the deaths, detailed information was missing (Table 5). New or worsening symptoms within 24 hours were reported for 28.3% of patients; the most frequent symptoms were chest pain, dyspnea and nausea or vomiting. The presenting rhythm was documented in only 15% of patients. A minority of patients underwent autopsy (N=26, 10.8%) and the most common findings were ischemic heart disease and heart failure. In the primary prevention cohort, the significantly associated independent correlates of SCD were higher HbA1c (SHR per 1% 1.34), prior heart failure (SHR 3.66) and eGFR ≤50 ml/min/1.73m^2 (SHR 2.35). In the secondary prevention cohort, in addition to the above-mentioned factors, albuminuria (SHR 1.87), PAD (SHR 1.85), prior MI (SHR 1.49) and older age (SHR per 5 years 1.11) were additional independent correlates of SCD.

3.4. Conclusions

This post hoc analysis from SAVOR-TIMI 53 involving more than 16,000 patients with T2D found that CVD remains the leading cause of death in a contemporary cohort of patients with or at high risk for ASCVD. Approximately 1/3 of all deaths observed in the present study met criteria for sudden death, regardless of whether patients did or did not have established ASCVD. While excess mortality is associated with older age, worse glycemic control, kidney complications including albuminuria, prior heart failure, PAD, prior CV events (MI or ischemic stroke) and elevated heart rate, biomarkers, especially elevated levels of NT-proBNP and hs-TnT measured in a stable population, are strongly associated with many causes of death, including non-CV death.

Prior studies have proposed specific risk prediction scores for CV disease in diabetes but few have attempted to evaluate the best predictors of all-cause and CV mortality.[65–73] For example, the TIMI Risk Score for Secondary Prevention, previously validated in this dataset, is a simple and well-calibrated risk prediction tool for the composite of CV death, MI, or ischemic stroke in patients with T2D.[74] For analyses of death causes and its prognostication, the principal limitations in many of the prior studies were small sample sizes with relatively few deaths to evaluate, lack of adjudication for causes of death, and the lack of a cause-specific mortality analysis.

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Diabetes is a known risk factor for SCD[75–78] and it is estimated that about one-fifth of SCDs occur in patients with diabetes such that of 400,000 events each year in the United States, approximately 80,000 involve patients with diabetes.[79,80] Our analysis found that SCD accounts for the majority of CV death in patients with diabetes at high ASCVD risk in both the primary and secondary prevention cohorts. This finding is in line with a recent report from another cardiovascular safety trial including only patients with atherosclerotic cardiovascular disease.[81] Our data highlight the importance of early detection of additional modifiable risk factors as well as further investigation regarding specific mechanisms responsible for the increased risk of SCD among those with diabetes. Interestingly, Balkau, et al. reported a graded risk of SCD according to glucose tolerance status (normal, impaired glucose tolerance, and diabetes), advocating that assessing glucose parameters could play a pivotal role in risk stratification.[76]

SAVOR-TIMI 53 included a large cohort of patients (more than 12,000) with baseline biomarkers measured, such as NT-proBNP, high-sensitivity troponin T, and CRP.[82] When included in the models, elevated biomarker concentrations (especially NT-proBNP and high-sensitivity troponin T) were independently associated with all-cause mortality in patients with or without established CV disease. These data are consistent with the post hoc analysis from the BARI 2D (Bypass Angioplasty Revascularization Investigation in Type 2 Diabetes) trial that examined the association between the hs-TnT and a composite outcome of CV death, MI, or stroke in 2,285 patients with both T2D and stable ischemic heart disease.[83]

The present study has several limitations including the fact that some variables potentially associated with mortality were not available for inclusion in the models, such as baseline LDL cholesterol, ECG or echocardiographic data (i.e., QT interval, left ventricular ejection fraction) and history of diabetic neuropathy. The lack of autopsy in the majority of patients who died prevents the exact ascertainment of cause of death, such that sudden cardiac death may include some non-CV causes and thus bias results towards the null. As with most clinical trials, especially given the ASCVD risk enrichment by trial design, the population included in this analysis does not completely reflect the overall diabetes population, therefore, limiting broader generalizability.

In conclusion, the major contributors to the risk of all-cause death in T2D were due to cardiometabolic factors (including heart failure), kidney disease and malignancy. Biomarkers, especially NTproBNP and hsTnT, were strongly associated with overall mortality and therefore did not aid in discriminating between types of death. With the identification of glucose lowering agents that reduce all-cause and CV mortality, better understanding of the strongest correlates of death in T2D may help in identifying patients who are likely to achieve the greatest potential reductions in the risk of death.

3.5. Tables

Table 1. Baseline Characteristics According to Vital Status

Characteristics	Patients Alive at the End of Follow-up (N=15694)	Patients who Died During Follow-up	
		CV Death (N=529)	Non-CV Death (N=269)
Age, yrs, median (IQR)	65 (60-71)	69 (62-75)*	69 (63-75)*
Male Sex, n (%)	10478 (66.8)	380 (71.8)*	179 (66.5)
BMI, kg/m^2, median (IQR)	30.5 (27.3-34.5)	30.1 (26.7-34.5)	30.3 (26.6-33.6)
Hypertension, n (%)	12830 (81.8)	432 (81.7)	230 (85.5)
Dyslipidemia, n (%)	11169 (71.2)	367 (69.4)	203 (75.5)
Current Smoking, n (%)	2106 (13.4)	71 (13.4)	42 (15.6)
Duration of Diabetes, yrs, median (IQR)	10.3 (5.2-16.5)	13.0 (7.5-20.2)*	10.6 (6.4-18.6)*
HbA1c, (%), median (IQR)	7.6 (6.9-8.7)	8.0 (7.1-9.2)*	7.7 (7.0-8.8)
Fasting Serum Glucose, n (%) < 126 mg/dl 126-220 mg/dl ≥ 220 mg/dl	 4768 (31.9) 8399 (56.2) 1785 (11.9)	 163 (33.0) 259 (52.4) 72 (14.6)	 85 (33.9) 126 (50.2) 40 (15.9)
Insulin Use, n (%)	6375 (40.6)	289 (54.6)*	122 (45.4)
Established Atherosclerotic Disease, n (%)	12271 (78.2)	468 (88.5)*	220 (81.8)
Coronary Artery Disease and Prior Myocardial Infarction, n (%)	5878 (37.5)	259 (49.0)*	100 (37.2)
Peripheral Artery Disease, n (%)	1804 (11.5)	105 (19.8)*	49 (18.2)*
Prior Ischemic Stroke, n (%)	1967 (12.5)	73 (13.8)	54 (20.1)*
Prior Heart Failure, n (%)	1900 (12.1)	162 (30.6)*	43 (16.0)
Heart Rate, median (IQR)	70 (63-77)	71 (64-79)*	73 (65-81)*
eGFR ≤ 50 ml/min, n (%)	2316 (14.8)	176 (33.3)*	84 (31.2)*
Albuminuria¶, n (%)	5621 (37.4)	304 (61.5)*	139 (54.7)*
Retinopathy, n (%)	1908 (12.2)	90 (17.0)*	39 (14.5)
hsTnT > 14 pg/ml†, n (%)	4406 (38.0)	310 (76.2)*	124 (63.9)*
NT-proBNP ≥ cutoff‡, n (%)	5414 (46.3)	333 (81.0)*	139 (70.6)*
C-Reactive Protein > 3 mg/l§, n (%)	4749 (40.6)	227 (55.2)*	110 (55.6)*

Values are reported as n (%) or median (interquartile range, IQR).
*P value <0.05 for comparison vs. alive.
¶Albuminuria is defined as Albumin to Creatinine Ratio ≥3.4 mg/mmol. Data were available for 15760 patients.
† 14 pg/ml represents the 99th percentile upper reference limit value.
‡ Cutoff was 125 pg/ml for patients < 75 years and 450 pg/ml for patients ≥ 75 years.
§ 3 mg/l represents the 99th percentile upper reference limit value.

Table 2. Correlates of Overall Mortality in Type 2 Diabetes Ordered by χ^2

(Cox Proportional Hazard Model, Multivariate Analysis)

Variables	HR	95% CI	P Value
Age (per 5 years)	1.26	1.21-1.32	<0.001
Prior heart failure	2.04	1.72-2.41	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.84	1.58-2.14	<0.001
eGFR ≤50 ml/min	1.80	1.53-2.12	<0.001
Peripheral artery disease	1.88	1.56-2.25	<0.001
Heart rate (per 5 bpm)	1.10	1.06-1.13	<0.001
CAD and prior MI	1.44	1.24-1.68	<0.001
HBA1c (per 1%)	1.11	1.05-1.16	<0.001
Male	1.30	1.11-1.53	0.001
Prior ischemic stroke	1.35	1.11-1.65	0.003

Sample size of 15,691 patients.
Candidate variables tested in the model but non-significant: BMI (per 1 kg/m^2), duration of diabetes (≥10 vs. <10 years), fasting serum glucose (≥150 vs. <150 mg/dl), CAD without prior MI, retinopathy and insulin.

Table 3. Correlates of Cardiovascular Mortality in Type 2 Diabetes Ordered by χ^2 (Fine-Gray Subdistribution Hazard Models, Multivariate Analysis)

Variables	SHR	95% CI	P Value
Prior heart failure	2.49	2.03-3.04	<0.001
Age (per 5 years)	1.24	1.17-1.32	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.98	1.63-2.40	<0.001
Peripheral artery disease	1.94	1.55-2.43	<0.001
eGFR ≤50 ml/min	1.71	1.39-2.11	<0.001
CAD and prior MI	1.57	1.30-1.90	<0.001
HBA1c (per 1%)	1.11	1.04-1.19	0.001
Heart rate (per 5 bpm)	1.08	1.03-1.13	0.001
Male	1.37	1.11-1.67	0.003
Insulin	1.23	1.02-1.49	0.030

Sample size of 15,691 patients.
Candidate variables tested in the model but non-significant: BMI (per 1 kg/m^2), duration of diabetes (≥10 vs. <10 years), fasting serum glucose (≥150 vs. <150 mg/dl), CAD and no prior MI, prior ischemic stroke and retinopathy.
SHR=Subdistribution Hazard Ratio.

Table 4. Correlates of Non-Cardiovascular Mortality in Type 2 Diabetes Ordered by χ^2 (Fine-Gray Subdistribution Hazard Models, Multivariate Analysis)

Variables	SHR	95% CI	P Value
Age (per 5 years)	1.28	1.19-1.38	<0.001
Heart rate (per 5 bpm)	1.13	1.07-1.20	<0.001
eGFR ≤50 ml/min	1.78	1.35-2.36	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.59	1.23-2.05	<0.001
Prior ischemic stroke	1.66	1.22-2.27	0.001
Peripheral artery disease	1.61	1.17-2.22	0.004

Sample size of 15,749 patients.
Candidate variables tested in the model but non-significant: sex, BMI (per 1 kg/m^2), duration of diabetes (≥10 vs. <10 years), fasting serum glucose (≥150 vs. <150 mg/dl), HBA1c (per1%), CAD and prior MI, CAD without prior MI, prior heart failure, retinopathy and insulin.
SHR=Subdistribution Hazard Ratio.

Table 5. Features of Sudden Cardiac Death in Type 2 Diabetes

	Yes	**No**	**Unknown**
Out-of-hospital	195 (81.2)	35 (14.6)	10 (4.2)
Witnessed	93 (38.8)	85 (35.4)	62 (25.8)
During sleep	41 (17.1)	111 (46.2)	88 (36.7)
New or worsening symptoms within 24 hours	68 (28.3)	49 (20.4)	123 (51.3)
Attempted resuscitation	69 (28.7)	107 (44.6)	64 (26.7)
Autopsy	26 (10.8)	214 (89.2)	-

Values are reported as n (%).

Table 1 Appendix. Predictors of Cardiovascular and Non-Cardiovascular Mortality in Type 2 Diabetes Ordered by χ^2 (Cause-specific Hazard Models, Multivariate Analysis)

Cardiovascular Mortality (n=15,691)			
Variables	**CSHR**	**95% CI**	**P Value**
Prior heart failure	2.48	2.03-3.02	<0.001
Age (per 5 years)	1.24	1.18-1.31	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	2.01	1.66-2.43	<0.001
Peripheral artery disease	2.05	1.64-2.57	<0.001
eGFR ≤50 ml/min	1.78	1.45-2.18	<0.001
CAD and prior MI	1.78	1.43-2.23	<0.001
HBA1c (per 1%)	1.13	1.06-1.20	<0.001
Heart rate (per 5 bpm)	1.08	1.04-1.13	<0.001
Male	1.34	1.10-1.64	0.004
CAD without prior MI	1.31	1.01-1.70	0.039
Non-Cardiovascular Mortality (n=15,749)			
Age (per 5 years)	1.29	1.19-1.39	<0.001
Heart rate (per 5 bpm)	1.14	1.08-1.20	<0.001
eGFR ≤50 ml/min	1.82	1.38-2.41	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.62	1.26-2.09	<0.001
Prior ischemic stroke	1.68	1.23-2.30	0.001
Peripheral artery disease	1.65	1.19-2.27	0.002

CSHR=Cause Specific Hazard Ratio

Table 2 Appendix. Predictors of Cardiovascular Mortality in Type 2 Diabetes Ordered by χ^2 (Fine-Gray Subdistribution Hazard Models, Multivariate Analysis Including Biomarkers)

Variables	SHR	95% CI	P Value
NT-proBNP ≥cutoff (125 pg/ml if <75 yrs, 450 pg/ml if ≥75 yrs)	2.82	2.16-3.69	<0.001
hsTnT >14 pg/ml	2.46	1.91-3.18	<0.001
Age (per 5 years)	1.22	1.14-1.31	<0.001
Prior heart failure	1.91	1.53-2.39	<0.001
Peripheral artery disease	1.81	1.41-2.34	<0.001
C-Reactive Protein >3 mg/l	1.42	1.16-1.74	<0.001
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.44	1.15-1.80	0.001
CAD and prior MI	1.40	1.13-1.72	0.002
Heart rate (per 5 bpm)	1.08	1.03-1.13	0.003
HBA1c (per 1%)	1.09	1.02-1.17	0.017

Sample size of 11,689 patients.
Candidate variables tested in the model but non-significant: sex, BMI (per 1 kg/m^2), duration of diabetes (≥10 vs. <10 years), fasting serum glucose (≥150 vs. <150 mg/dl), CAD without prior MI, prior ischemic stroke, retinopathy, eGFR ≤50 ml/min and insulin.
SHR=Subdistribution Hazard Ratio.

Table 3 Appendix. Predictors of Non-Cardiovascular Mortality in Type 2 Diabetes Ordered by χ^2

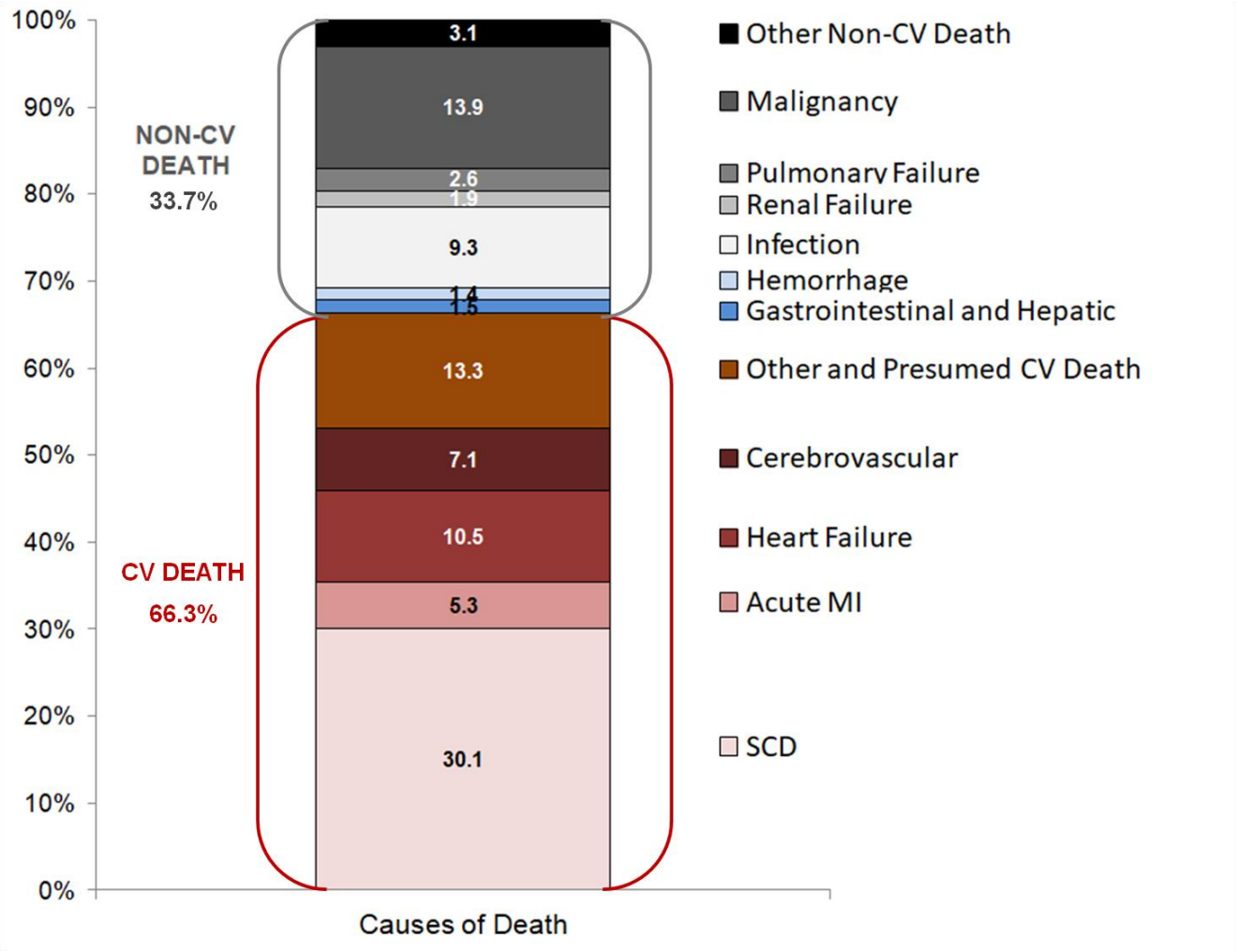
(Fine-Gray Subdistribution Hazard Models, Multivariate Analysis Including Biomarkers)

Variables	SHR	95% CI	P Value
Age (per 5 years)	1.25	1.14-1.36	<0.001
NT-proBNP ≥cutoff (125 pg/ml if <75 yrs, 450 pg/ml if ≥75 yrs)	2.07	1.49-2.88	<0.001
Heart rate (per 5 bpm)	1.13	1.06-1.20	<0.001
eGFR ≤50 ml/min	1.61	1.15-2.25	0.005
hsTnT >14 pg/ml	1.57	1.13-2.19	0.007
C-Reactive Protein >3 mg/l	1.43	1.07-1.92	0.016
Albumin to Creatinine Ratio ≥3.4 mg/mmol	1.42	1.06-1.90	0.02
CAD without prior MI	0.67	0.46-0.98	0.04

Sample size of 11,722 patients.
Candidate variables tested in the model but non-significant: sex, BMI (per 1 kg/m^2), duration of diabetes (≥10 vs. <10 years), fasting serum glucose (≥150 vs. <150 mg/dl), HbA1c (per 1%), prior heart failure, CAD without prior MI, PAD, prior ischemic stroke, retinopathy and insulin.
SHR=Subdistribution Hazard Ratio.

3.6. Figures

Figure 1. Causes of Death in Type 2 Diabetes.



MI=Myocardial Infarction; SCD=Sudden Cardiac Death.

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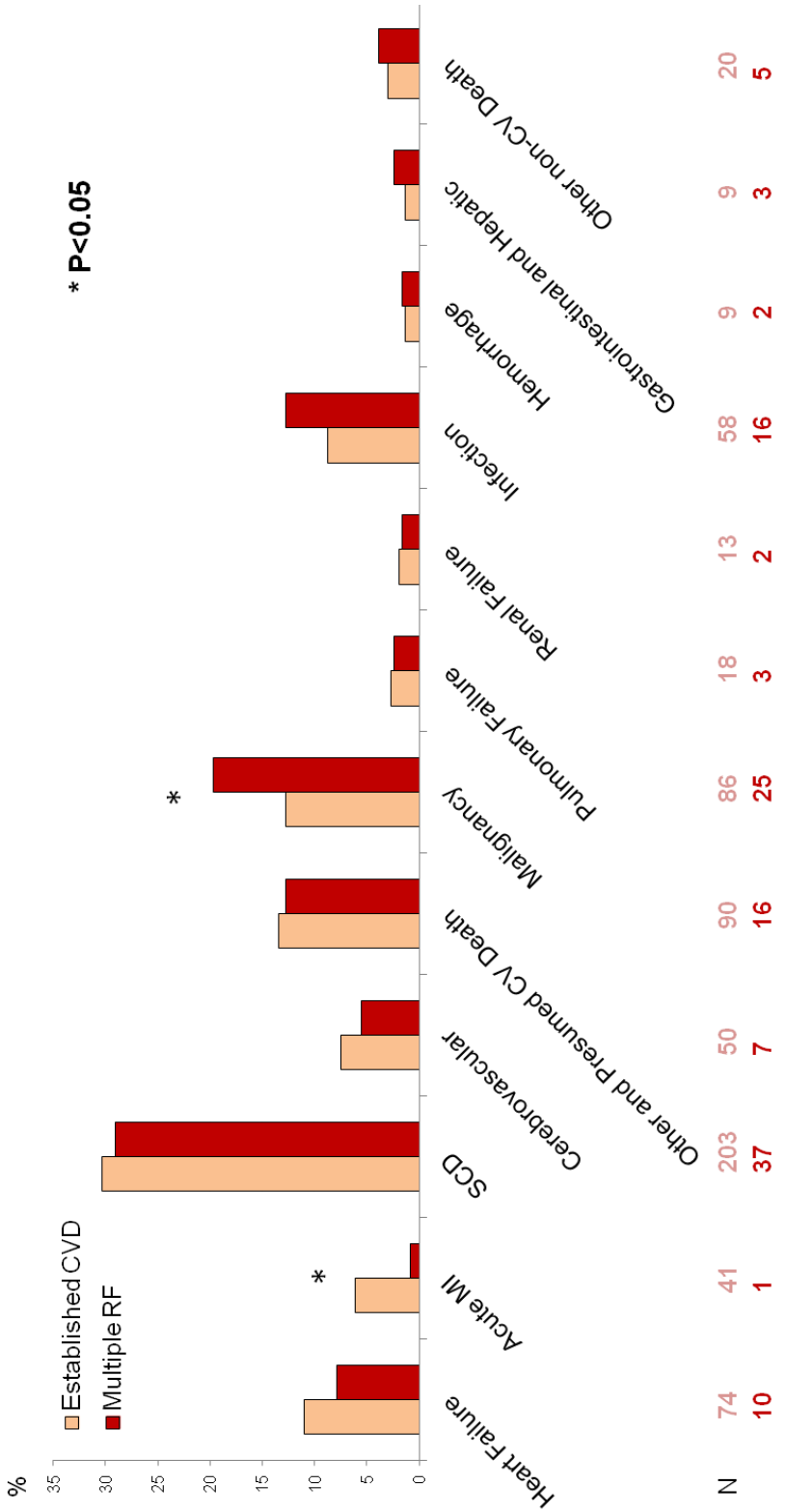
Figure 2. Causes of Death in Type 2 Diabetes Patients With Multiple Cardiovascular Risk Factors and in Those With Established Cardiovascular Disease.



CVD=Cardiovascular Disease; RF=Risk Factors; SCD=Sudden Cardiac Death.

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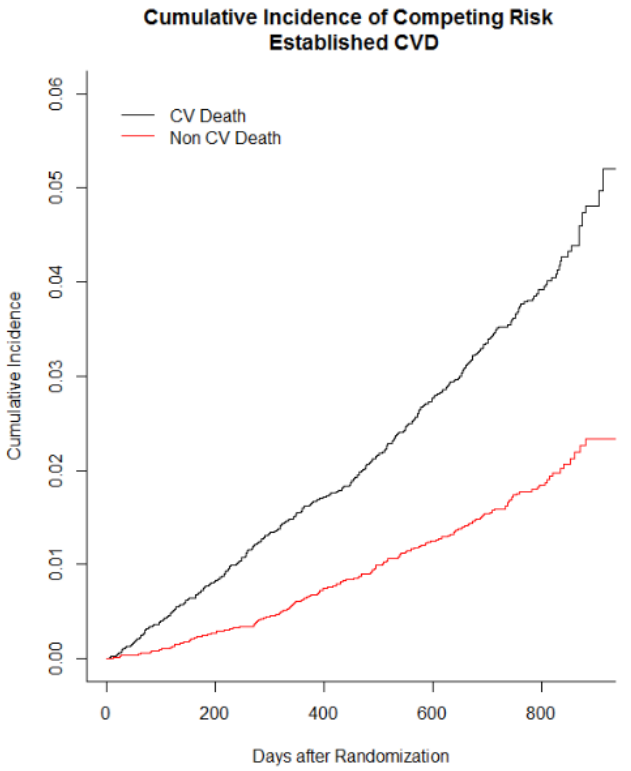
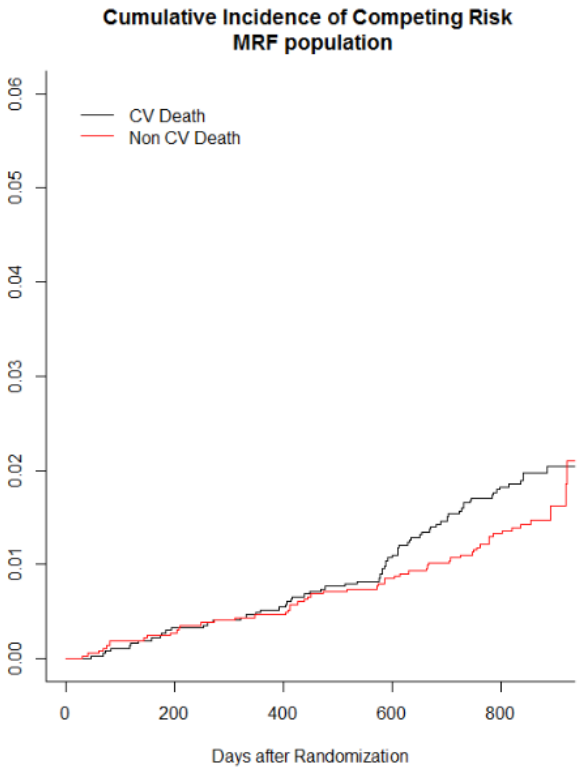
Supplementary Figure 1. Cumulative Incidence of Competing Risk of Mortality in Type 2 Diabetes Patients A) With Established Cardiovascular Disease and in Those With B) Multiple Cardiovascular Risk Factors.

A



B



CVD=Cardiovascular Disease; MRF=Multiple Risk Factors.

4. Specific aim 2: to investigate the impact of metabolic disease on cardiovascular outcomes in a particular subset of patients, a contemporary cohort of subjects after an acute coronary syndrome

Cavallari I, Cannon CP, Braunwald E, Goodrich EL, Im K, Lukas MA, O'Donoghue ML. Metabolic syndrome and the risk of adverse cardiovascular events after an acute coronary syndrome. Eur J Prev Cardiol. 2018 May;25(8):830-838.

4.1 Background

The metabolic syndrome is described as a constellation of physiological risk factors for atherogenesis that occur to a greater degree together than expected by chance and increase an individual's risk of developing heart disease.[84] In the United States, it is estimated that the metabolic syndrome affects approximately 35% of adults and nearly 50% of those aged 60 years or older.[85] Since 2007, the prevalence of the metabolic syndrome overall appears to have remained stable and to have declined in women, which may be a consequence of initiatives designed to optimize modifiable risk factors.[85]

The incremental value of identifying the presence of the metabolic syndrome beyond individual risk factors as a predictor of cardiovascular events has been debated for many years. A large meta-analysis of 87 studies including 951083 patients demonstrated that the metabolic syndrome is associated with a 2-fold increase in risk of cardiovascular mortality, myocardial infarction (MI) and stroke and a 1.5-fold increase in the risk of all-cause mortality.[86] Nevertheless, some have argued that the association between the metabolic syndrome and cardiovascular risk is primarily explained by type 2 diabetes mellitus that is present in a large proportion of individuals who meet the metabolic syndrome definition.[87] To that end, cardiovascular disease remains the most common cause or morbidity and mortality in diabetes.[88,89] To date, no firm conclusion has been made

regarding the incremental prognostic value of assessing the presence of the metabolic syndrome in patients after an acute coronary syndrome (ACS), including those with established diabetes.

In the present study, we aimed to assess long-term cardiovascular risk associated with the presence of the metabolic syndrome or diabetes mellitus among patients recently hospitalized with an ACS.

4.2 Methods

Study Design and Population

SOLID-TIMI 52 (ClinicalTrials.gov identifier: NCT01000727) was a randomized, double-blind, placebo-controlled trial to assess the long-term cardiovascular efficacy and safety of darapladib, a selective inhibitor of the lipoprotein-associated phospholipase A_2 (Lp-PLA_2) enzyme, when added to a background of optimal medical therapy within 30 days of hospitalization for an ACS.[90]

The study population of SOLID-TIMI 52 trial included 13026 patients who had been hospitalized with an ACS event in the 30 days prior to randomization (unstable angina, non–ST-elevation MI, and ST-elevation MI). All participants were required to have at least one additional predictor of cardiovascular risk including age of at least 60 years, history of MI prior to the qualifying event, significant renal dysfunction (estimated glomerular filtration rate [eGFR] 30-59 ml/min/1.73 m^2), diabetes mellitus requiring pharmacotherapy or polyvascular disease (including carotid or peripheral arterial disease). The full eligibility criteria of the trial have been reported previously.[91]

Patients were followed for a median of 2.5 years. The primary endpoint was time to first major coronary event (MCE), defined as the composite of coronary heart disease death, MI or urgent coronary revascularization for myocardial ischemia. All reported deaths, cardiac ischemic events, cerebrovascular events, and heart failure hospitalizations were adjudicated by an independent and blinded clinical events committee. Post-randomization diagnosis of new-onset diabetes was based on one of the five following criteria confirmed by a repeated measurement: fasting plasma glucose

≥126 mg/dl; random plasma glucose ≥200 mg/dl or oral glucose tolerance test 2-hour post load glucose ≥200 mg/dl; glycated hemoglobin >6.5% or initiation of an anti-diabetes medication.

Definition of Metabolic Syndrome

The presence of metabolic syndrome and its 5 components according to 2005 International Diabetes Federation (IDF) definition were assessed in all patients with available data at baseline.[92] In brief, the IDF clinical definition of metabolic syndrome makes the presence of abdominal obesity necessary for diagnosis (waist circumference ≥94 cm in men and ≥80 cm in women for Europids or BMI >30 kg/m^2). When such is present, 2 additional risk factors such as hypertension (≥130 mm Hg systolic or ≥85 mmHg diastolic blood pressure), high triglycerides (≥150 mg/dl), reduced HDL-cholesterol levels (<40 mg/dl in men or <50 mg/dl in women) or elevated fasting glucose (≥100 mg/dl, including diabetes) are sufficient for diagnosis.

A sensitivity analysis was performed using the 2004 revised National Cholesterol Education Program (rNCEP) definition[93] which does not require demonstration of insulin resistance, but the presence of at least 3 out of 5 risk factors for establishing the diagnosis (abdominal obesity, elevated blood pressure, elevated triglycerides, reduced HDL-cholesterol and elevated fasting glucose). Supplementary Table 1 compares the two definitions used in this study. Since statin therapy is routinely recommended in all patients after ACS regardless of lipid profile, the use of lipid-lowering agents was not considered as satisfying the lipids abnormalities components of the metabolic syndrome to avoid overestimation of its prevalence.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation and categorical variables are presented as numbers and percentages. Baseline characteristics were compared using a χ^2 test for categorical variables or Wilcoxon-Mann-Whitney test for continuous variables. Cumulative event

rates at 3 years following randomization were calculated by the complement of Kaplan–Meier survival estimates. Cox proportional-hazards models were adjusted for age, sex, region (North America vs other countries), baseline LDL-cholesterol, current smoking, prior MI, eGFR <60 ml/min/1.73 m^2 and type of index ACS (STEMI vs NSTE-ACS). In order to assess the relative prognostic value of each of the individual 5 metabolic risk factors included in the definition of metabolic syndrome, an additional Cox proportional hazards models adjusted for the aforementioned variables plus the remaining 4 metabolic risk factors. The metabolic syndrome was further examined by categorizing four mutually exclusive groups: presence and absence of metabolic syndrome in patients with and without diabetes mellitus. The significance level was set at α=0.05. All reported p-values are two-sided. Statistical analysis was performed using SAS statistical software version 9.4 (SAS Institute Inc. Cary, NC USA).

4.3 Results

Of the 13026 patients enrolled in the SOLID-TIMI 52, 12238 patients provided sufficient baseline data to ascertain all the components of the metabolic syndrome. At baseline, 61.6% (n=7537) of patients met the definition for metabolic syndrome, 34.7% (n=4247) had diabetes mellitus and 29.3% had both diabetes and metabolic syndrome (n=3584). Patient characteristics according to the presence or absence of the metabolic syndrome are summarized in Table 1. In general, individuals with metabolic syndrome were more likely to be younger, female, have a higher BMI, have hypertension, diabetes, higher baseline concentrations of LDL-cholesterol, impaired renal function, and a history of MI before the index event. In addition, patients with metabolic syndrome were more likely to be at high-risk of sleep apnea according to the Berlin questionnaire (45.9% vs. 24.1%; p<0.0001).[94]

During long-term follow-up, the presence of metabolic syndrome was associated with an increased risk of MCE (adj HR 1.29, 95% CI 1.16-1.43; p<0.0001), including an increased risk of the individual components of coronary heart disease death (adj HR 1.29, 95% CI 1.05-1.60; p=0.017),

MI (adj HR 1.30, 95% CI 1.14-1.49; p<0.0001), urgent coronary revascularization (adj HR 1.23, 95% CI 1.004-1.52; p=0.045), and all-cause death (adj HR 1.23, 95% CI 1.05-1.44; p=0.012). Metabolic syndrome was associated with a directionally consistent increase in the risk of stroke that was not statistically significant (adj HR 1.29, 95% CI 0.99-1.68; p=0.06) (Figure 1). These results were confirmed when the analysis was restricted to patients in the placebo arm of the trial (Supplementary Table 2). In addition, in 7991 patients without diabetes at baseline, the presence of metabolic syndrome was associated with nearly a 3-fold higher risk of developing new-onset diabetes mellitus during follow-up, compared with those without metabolic syndrome (7.7% vs. 3.2% at 3 years; adj HR 2.77, 95% CI 2.20-3.49; p<0.0001).

When the 5 metabolic risk factors included in the IDF definition of metabolic syndrome were analyzed independently in separate models that were adjusted for other risk factors, the presence of fasting plasma glucose ≥110 mg/dl (adj HR 1.21, 95% CI 1.07-1.37; p=0.002) or diabetes mellitus (adj HR 1.48, 95% CI 1.34-1.63; p<0.0001) were associated with a higher risk of MCE. The presence of hypertension was associated with higher risk of MCE (adj HR 1.46, 95% CI 1.27-1.69; p<0.0001; Figure 2). In contrast, abdominal obesity, elevated triglycerides and reduced HDL-C were not independently associated with the risk of MCE in this population (Figure 2).

Metabolic Syndrome and Cardiovascular Risk According to Diabetes Status

Patients were stratified according to the presence or absence of both metabolic syndrome and diabetes mellitus at baseline. Of these, 29.3% (N=3584) had metabolic syndrome and diabetes, 32.3% (N=3953) had metabolic syndrome alone, 5.4% had diabetes without evidence of metabolic syndrome (N=663), and 33.0% (N=4038) were non diabetics without metabolic syndrome. In patients without diabetes, metabolic syndrome was numerically but not significantly associated with a higher risk of MCE (adj HR 1.13, 95% CI 0.99-1.29; p=0.06, C-statistic 0.631; Figure 3) or recurrent MI (adj HR 1.09, 95% CI 0.92-1.28; p=0.32; Figure 4) as compared to those without the metabolic syndrome.

Conversely, diabetes was a strong independent predictor of MCE (adj HR 1.57, 95% CI 1.27-1.95; p<0.0001, C-statistic 0.643; Figure 3) and recurrent MI (adj HR 1.38, 95% CI 1.04-1.83; p=0.026; Figure 4) in the absence of metabolic syndrome. The presence of both diabetes and metabolic syndrome identified patients at highest risk of adverse cardiovascular outcomes (adj HR 1.67, 95% CI 1.47-1.90; p<0.0001, C-statistic 0.634; Figure 3). However, in patients with diabetes, the incremental risk of metabolic syndrome for MCE was not greater than with diabetes alone (adj HR 1.07, 95% CI 0.87-1.31; p=0.54 for MCE). These results were confirmed when the analysis was restricted to patients in the placebo arm of the trial (Supplementary Table 3).

Sensitivity Analysis

According to the rNCEP definition,[10] the prevalence of metabolic syndrome in the study population was similar when compared to the IDF criteria (60.8% vs. 61.6%). The association between metabolic syndrome and cardiovascular risk remained consistent when the rNCEP definition was applied (Supplementary Table 4), although a stronger association was observed between metabolic syndrome and the risk of stroke (adj HR 1.50, 95% CI 1.14-1.96; p=0.003). When patients were stratified according to the presence of rNCEP-defined metabolic syndrome, the stratified results by both metabolic syndrome and diabetes status yielded qualitatively similar results as we observed with the other definition (Supplementary Figure 1).

4.4 Conclusions

The current study demonstrates that, in patients who have recently suffered an ACS, the presence of metabolic syndrome is associated with the risk of adverse cardiovascular events, but this risk appears to be primarily driven by the presence of diabetes mellitus. In contrast, a diagnosis of metabolic syndrome on its own did not provide incremental information for risk stratification in this population once diabetes history was considered.

The term "metabolic syndrome" refers to a cluster of interrelated risk factors of metabolic origin that appear to directly promote the development of atherosclerotic cardiovascular disease.[95] Substantial evidence indicates that insulin resistance and hyperinsulinemia play fundamental roles in risk-factor clustering and may causally contribute to pathways that increase the risk of adverse cardiovascular outcomes. To that end, prolonged insulin resistance is believed to contribute to worsening hypertension through several mechanisms, including an overactive sympathetic nervous system, sodium retention, altered membrane ion transport and proliferation of vascular smooth muscle cells.[96–98] Moreover, hyperinsulinemia increases liver production of VLDL and at the same time reduces high-density lipoprotein production.[99]

Previous studies have shown a significant association between metabolic syndrome and the risk of cardiovascular events, including cardiovascular mortality, in patients without a known history of coronary artery disease.[86,100,101] Additional studies with varying durations of follow-up have not observed an association of metabolic syndrome with adverse cardiovascular events in patients undergoing percutaneous coronary intervention.[102–104] In the setting of ACS, the prognostic value of assessing the presence of the metabolic syndrome as an integrated diagnosis is still uncertain.[103–106] In fact, the individual components of metabolic syndrome were shown to have varying relationships with outcomes, perhaps influencing the observed incremental risk stratification.[102–104] In addition, some experts have argued that the value of including diabetes in the definition of metabolic syndrome is questionable.[87]

Thus far, data regarding the incremental prognostic value of assessing the presence of the metabolic syndrome in patients with and without diabetes have been inconclusive.[107–112] However, only few studies investigated this issue in past ACS or pre-existing coronary artery disease.[102–104,113–116] In the GISSI-Prevenzione Trial including 11323 patients with recent MI, Levantesi et al.[114] showed a highly significant increase in the risk of major cardiovascular events during 3.5 years of follow-up in patients with metabolic syndrome, irrespective of diabetes status, as compared with controls. Of

note, patients with metabolic syndrome had a near two-fold increased risk of developing diabetes during follow-up, slightly lower than the one reported in this study. On the other hand, Stern and colleagues[116] examined a population of approximately 1000 subjects with cardiovascular disease concluding that the effect of metabolic syndrome on cardiovascular mortality is primarily driven by the inclusion of diabetes in the definition. In fact, when the multivariate model was adjusted for diabetes the odds ratios for metabolic syndrome on mortality were substantially reduced and no longer statistically significant, concordant with our results. Moreover, there was no effect of the metabolic syndrome on either all-cause and cardiovascular mortality in diabetic subjects, whereas in non-diabetics the effect was not statistically significant for either outcome. These uncertainties remain in more contemporary studies including patients with coronary artery disease where the presence of metabolic syndrome as an integrated diagnosis did not predict adverse cardiovascular outcomes and results regarding the association between diabetes and subsequent cardiovascular events are conflicting.[102–104] Therefore, the present study adds evidence on this often debated topic taking advantage of a large contemporary cohort of ACS patients enrolled between 2009 and 2014, treated according to current standards (~85% underwent coronary angiography) and followed for a median of 2.5 years.

This study should be evaluated in light of its strengths and limitations. The main strengths include its large sample size, carefully phenotyped population and the duration of follow-up. All together it is based on a total of 29324 patient-years of follow-up. In addition, all events were adjudicated by a clinical events committee. One limitation was that 35.2% of baseline triglyceride assessments were recorded as non-fasting; however, the use of non-fasting triglyceride measurements have been supported by some.[117] Moreover, although statins have only limited effect on HDL cholesterol, one cannot exclude that widespread use of statins attenuated the relationship between components of the metabolic syndrome and CV risk. Finally, these data refer to a post-ACS population; therefore, our findings may not apply to a primary prevention cohort. As well, the study population was enriched

for patients with diabetes mellitus and other cardiovascular risk factors and therefore may not be representative of an ACS population at large.

In conclusion, the presence of the metabolic syndrome is associated with the risk of subsequent cardiovascular events in patients after ACS including all-cause mortality; however, this risk appears to be largely driven by the presence of diabetes mellitus. In this population, the presence of the metabolic syndrome provides only marginal incremental value for risk stratification in patients once the presence or absence of diabetes is established but may potentially be useful for helping to identify patients that could benefit from lifestyle modifications and closer monitoring of glycemic status.[118–120]

4.5 Tables

Table 1. Baseline Characteristics According to the Presence of Metabolic Syndrome as Defined According to the 2005 International Diabetes Federation.

	No Metabolic Syndrome (N=4701)	**Metabolic Syndrome (N=7537)**	**p Value**
Age, yrs, mean (SD)	65.2 (9.2)	63.5 (9.6)	<0.0001
Female Sex, n (%)	831 (17.7)	2294 (30.4)	<0.0001
Region, n (%) North America South America Western Europe Eastern Europe Asia Pacific	 1205 (25.6) 268 (5.7) 1345 (28.6) 1049 (22.3) 834 (17.7)	 1417 (18.8) 644 (8.5) 2012 (26.7) 2602 (34.5) 862 (11.4)	<0.0001
BMI ≥30 kg/m^2, n (%)	557 (11.8)	3280 (43.5)	<0.0001
Waist Circumference, cm, mean (SD)	93.2 (11.8)	105.2 (11.8)	<0.0001
Hypertension, n (%)	2660 (56.6)	6325 (83.9)	<0.0001
Diabetes, n (%)	663 (14.1)	3584 (47.6)	<0.0001
Current Smoking, n (%)	914 (19.5)	1406 (18.7)	0.28
Prior Myocardial Infarction, n (%)	1361 (29)	2434 (32.3)	<0.001
Index Event, n (%) Unstable Angina Non-ST-Elevation Myocardial Infarction ST-Elevation Myocardial Infarction	 453 (9.6) 1927 (41.0) 2321 (49.4)	 1036 (13.7) 3272 (43.4) 3229 (42.8)	<0.0001
High-risk of Sleep Apnea, n (%)	1126 (24.1)	3442 (45.9)	<0.0001
LDL-cholesterol, mean (SD)	78.8 (30.9)	80.8 (34.4)	0.033
HDL-cholesterol, mean (SD)	48.2 (12.1)	40.9 (10.1)	<0.0001
Triglycerides, mean (SD)	123.1 (60.9)	175.0 (105.8)	<0.0001
eGFR <60mL/min/1.73m^2, n (%)	424 (9.0)	1011 (13.4)	<0.0001
Concomitant Medical Therapy, n (%) Aspirin P2Y12 Inhibitor Lipid-lowering agents Beta Blocker ACE-inhibitor or ARB	 4528 (96.3) 4163 (88.6) 4442 (94.5) 3974 (84.5) 3638 (77.4)	 7276 (96.5) 6623 (87.9) 7132 (94.6) 6703 (88.9) 6483 (86.0)	 0.53 0.26 0.75 <0.0001 <0.0001

SD=Standard Deviation; BMI=Body Mass Index; eGFR=estimated Glomerular Filtration Rate; ACE=Angiotensin-Converting Enzyme;ARB=Angiotensin II Receptor Blocker.

Supplementary Table 1. Definitions of Metabolic Syndrome That Were Applied

IDF Definition	**Revised NCEP Definition**
	Any 3 of the following:
Increased waist circumference (population specific cutoffs)* or BMI > 30 kg/m^2 plus any 2 of the following:	Waist circumference ≥102 cm in men and ≥88 cm in women or BMI ≥ 30 kg/m^2;¶
- Triglycerides ≥150 mg/dl;	- Triglycerides ≥150 mg/dl;
- HDL-c <40 mg/dl in men or <50 mg/dl in women;	- HDL-c <40 mg/dl in men or <50 mg/dl in women;
- Blood pressure ≥130/85 mmHg or previously diagnosed hypertension;	- Blood pressure ≥130/85 mmHg or previously diagnosed hypertension;
- Fasting plasma glucose ≥ 100 mg/dl or previously diagnosed diabetes	- Fasting plasma glucose ≥ 100 mg/dl or previously diagnosed diabetes

***** ≥94 cm in men and ≥80 cm in women for Europids; ≥102 cm in men and ≥88 cm in women from United States; ≥90 cm in men and ≥80 cm in women for Asians.

¶ ≥90 cm in men and ≥80 cm in women for Asians.

Supplementary Table 2. The 3-year Kaplan-Meier Event Rates in the Placebo Arm According to the Presence or Absence of the Metabolic Syndrome as Defined According to the 2005 International Diabetes Federation.

Endpoint	No Metabolic Syndrome (N=2386)	Metabolic Syndrome (N=3738)	P Value
Major Coronary Events	13.7%	17.2%	0.001
Coronary Heart Disease Death	3.8%	4.5%	0.19
Myocardial Infarction	8.9%	11.3%	0.009
Urgent Coronary Revascularization	3.2%	4.0%	0.34
Stroke	2.4%	3.0%	0.17
All-Cause Death	7.1%	7.2%	0.28

Supplementary Table 3. The 3-year Kaplan-Meier Event Rates in the Placebo Arm and Adjusted Hazard Ratios for Major Coronary Events According to the Presence or Absence of the Metabolic Syndrome as Defined According to the 2005 International Diabetes Federation and Diabetes Mellitus.

Endpoint	**DM-/MS- (N=2043) Reference**	**DM-/MS+ (N=1987)**	**HR P value**	**DM+/MS- (N=343)**	**HR P value**	**DM+/MS+ (N=1751)**	**HR P value**
Major Coronary Events	13.2%	13.5%	1.05 (0.87-1.26) P=0.60	16.6%	1.43 (1.05-1.94) P=0.022	21.3%	1.67 (1.41-1.99) P<0.0001

Hazard ratios are adjusted for age, sex, region, baseline LDL-cholesterol, current smoking, prior myocardial infarction, eGFR <60 mL/min/1.73 m^2 and type of index acute coronary syndrome.

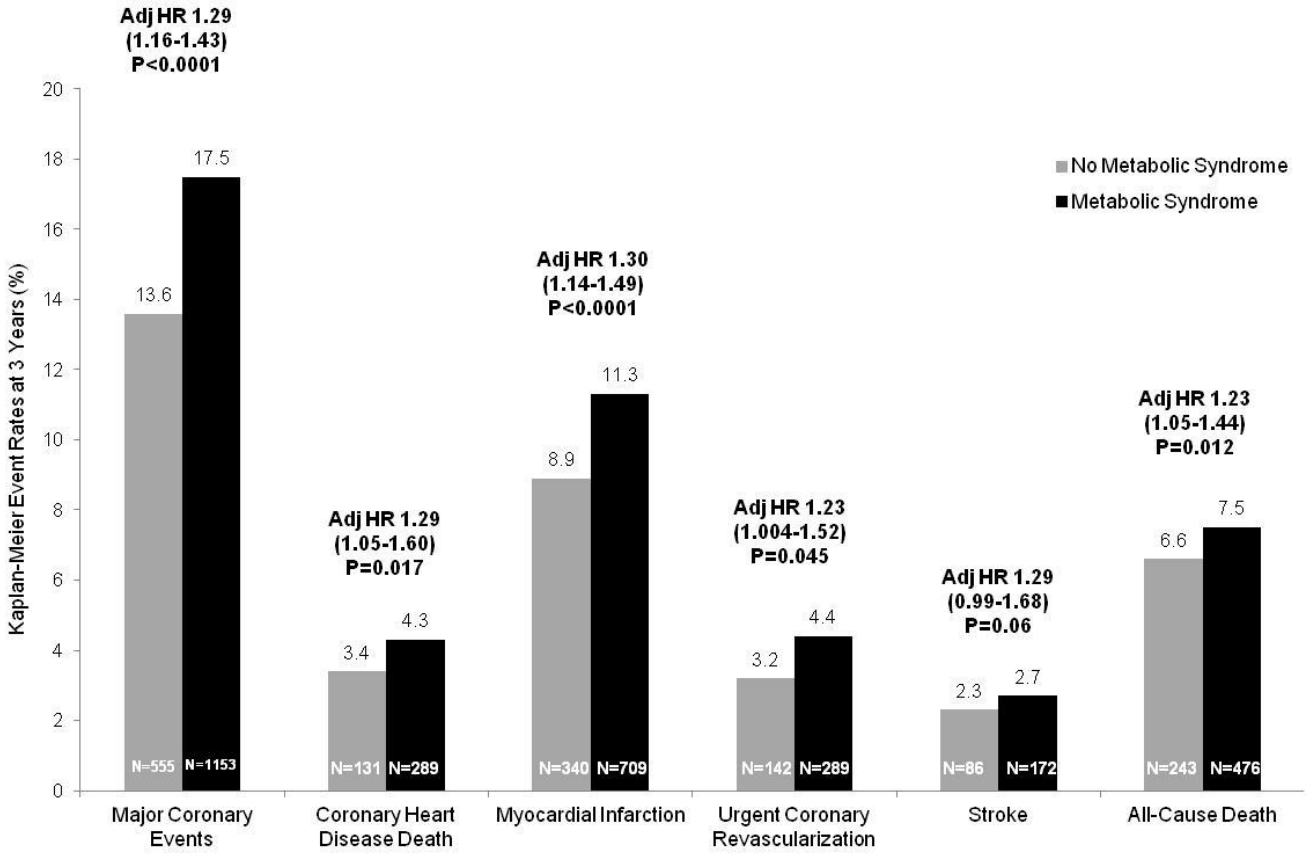
Supplementary Table 4. Cardiovascular Risk Associated with Metabolic Syndrome.

	Adj Hazard Ratio (95% CI) IDF Definition (N=7537/12238)	**p Value**	**Adj Hazard Ratio (95% CI) Revised NCEP Definition (N=7442/12238)**	**p Value**
Major Coronary Events	1.29 (1.16-1.43)	<0.0001	1.28 (1.15-1.41)	<0.0001
Coronary Heart Disease Death	1.29 (1.05-1.60)	0.017	1.40 (1.13-1.73)	0.002
Myocardial Infarction	1.30 (1.14-1.49)	<0.0001	1.30 (1.14-1.49)	<0.0001
Urgent Coronary Revascularization	1.23 (1.004-1.52)	0.045	1.19 (0.97-1.46)	0.10
Stroke	1.29 (0.99-1.68)	0.06	1.50 (1.14-1.96)	0.003
Cardiovascular Death	1.27 (1.04-1.55)	0.018	1.36 (1.11-1.66)	0.002
All-cause Mortality	1.23 (1.05-1.44)	0.012	1.24 (1.06-1.46)	0.007

Major coronary events include coronary heart disease death, MI or urgent coronary revascularization

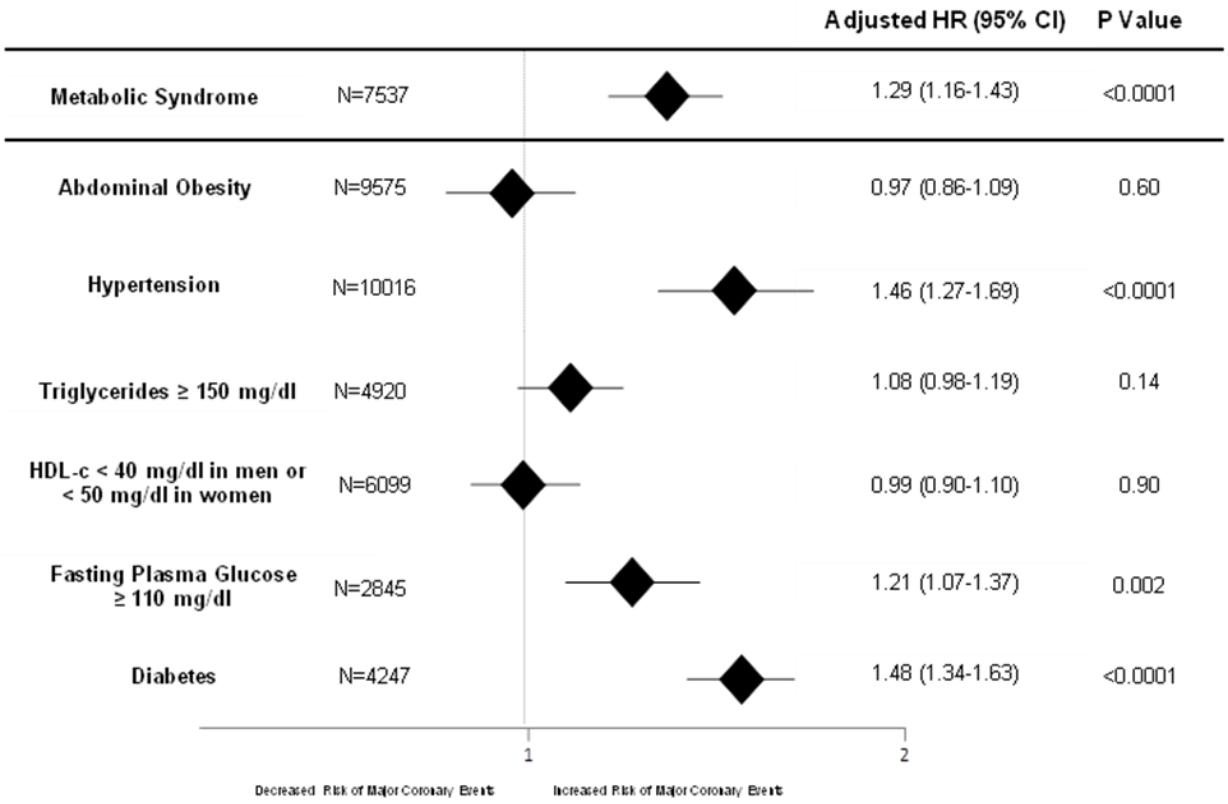
4.6 Figures

Figure 1. The 3-year Kaplan-Meier Event Rates and Adjusted Hazard Ratios for Cardiovascular Events According to the Presence or Absence of the Metabolic Syndrome as Defined According to the 2005 International Diabetes Federation.



Adjusted for age, sex, region, baseline LDL-cholesterol, current smoking, prior MI, eGFR <60 mL/min/1.73 m^2 and type of index ACS.

Figure 2. The Adjusted Risk of Major Coronary Events Based on the Presence or Absence of the Metabolic Syndrome Versus the Individual Components that Contribute to its Definition.



The Cox proportional hazard model included all metabolic risk factors after adjustment for the remaining variables (i.e., abdominal obesity, hypertension, triglycerides ≥150 mg/dl, HDL-cholesterol <40 mg/dl in men or <50 mg/dl in women and fasting plasma glucose ≥100 mg/dl or diabetes).

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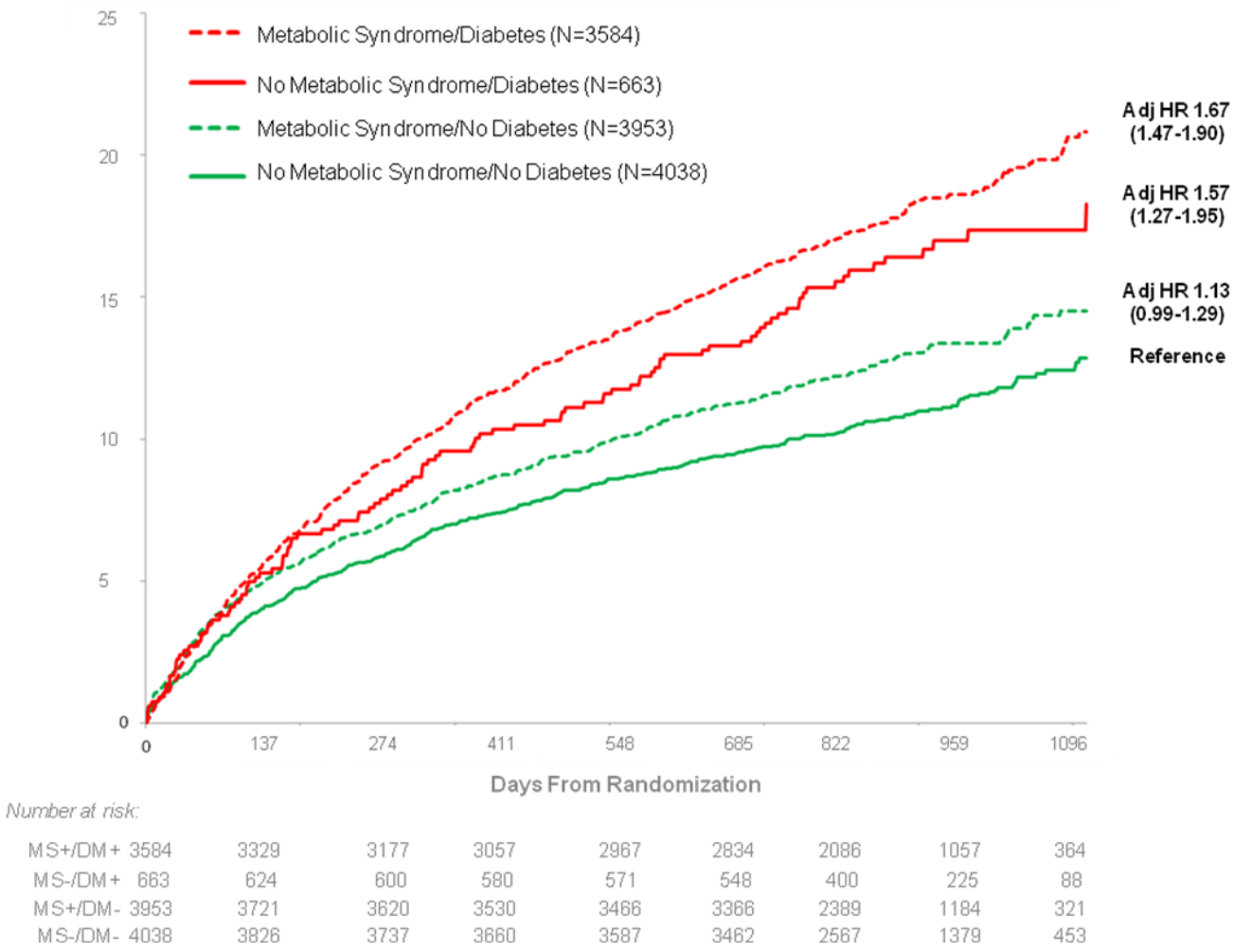
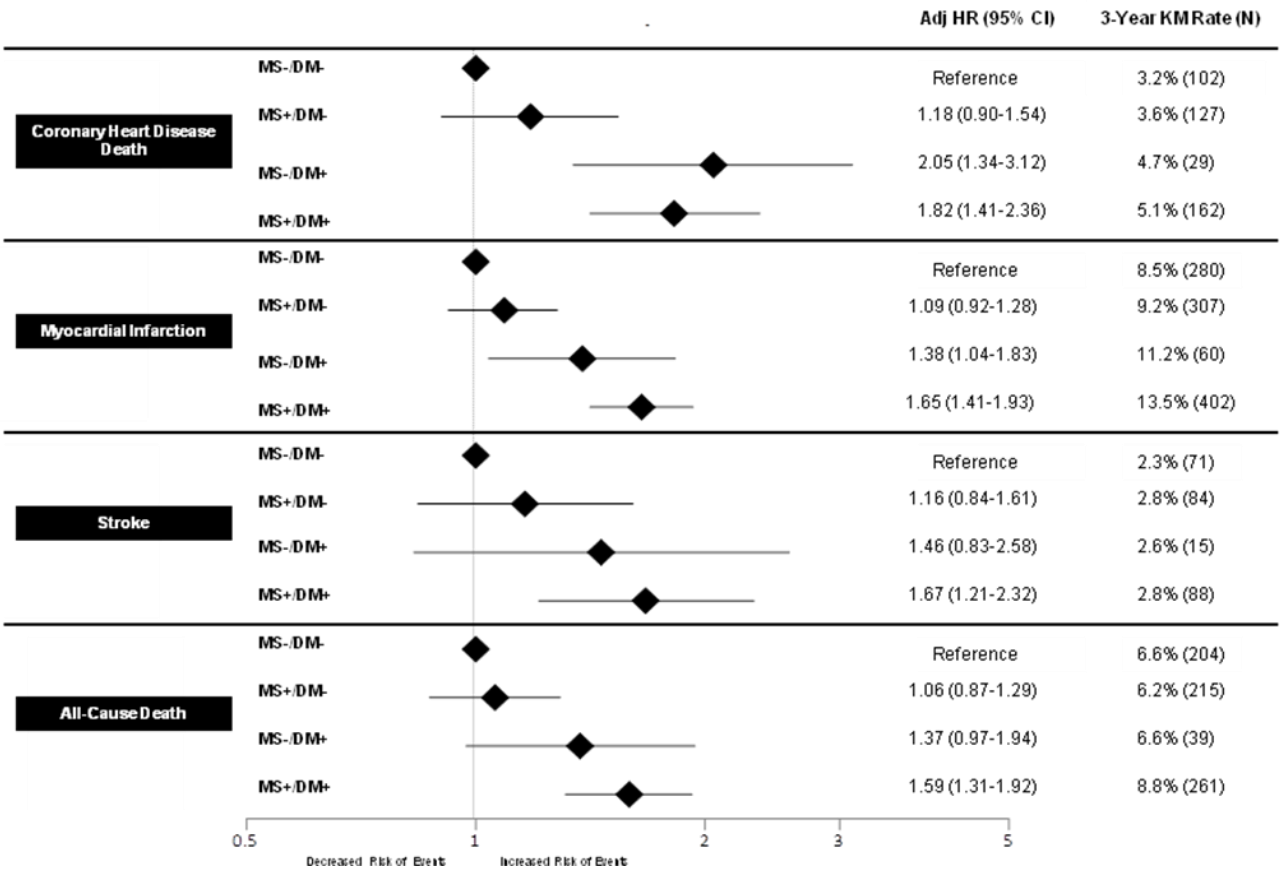
Figure 3. Kaplan-Meier Survival Curves for Major Coronary Events Stratified by the Presence or Absence of the Metabolic Syndrome and Diabetes Mellitus.



Ilaria Cavallari

Figure 4. The Adjusted Risk of Cardiovascular Outcomes According to Presence of Metabolic Syndrome and Diabetes Mellitus.



Adjusted for age, sex, region, baseline LDL-cholesterol, current smoking, prior MI, eGFR <60 mL/min/1.73 m^2 and type of index ACS.

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5. Specific aim 3: to investigate the risk of thromboembolic complications in patients with atrial fibrillation and diabetes

Patti G, Lucerna M, Cavallari I, Ricottini E, Renda G, Pecen L, Romeo F, Le Heuzey JY, Zamorano JL, Kirchhof P, De Caterina R. Insulin-Requiring Versus Noninsulin-Requiring Diabetes and Thromboembolic Risk in Patients With Atrial Fibrillation: PREFER in AF. J Am Coll Cardiol. 2017 Jan 31;69(4):409-419.

5.1 Background

Estimation of thromboembolic risk is crucial in patients with atrial fibrillation (AF) in order to perform an accurate stratification of risk during follow-up and establish optimal therapeutic strategies. Diabetes mellitus has been considered an independent risk factor for thromboembolic events in patients with AF[121] and this has led to inclusion of diabetes in the CHADS2 (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, prior stroke or transient ischemic attack) score[122] and the more recent CHA2DS2-VASc (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, prior stroke, transient ischemic attack, or thromboembolism, vascular disease, age 65–74 years, sex category [female]) score.[123] Patients with diabetes mellitus have a prothrombotic state because of changes in primary (platelet aggregation and vascular function) and secondary (coagulation and fibrinolysis) hemostasis; this is particularly enhanced in those with long-lasting disease receiving insulin therapy.[124] Here, low-grade inflammation, increased levels of coagulation factors, impairment of fibrinolysis, oxidative stress, and reduced expression of protective endothelial factors have been indicated as responsible for these prothrombotic changes.[124]

This is the basis for hypothesizing a stronger predictive role of diabetes requiring insulin therapy compared with less severe forms of diabetes, usually not requiring insulin, on AF-related thromboembolic risk. To date, no study has explored the differential prognostic weight of diabetes on insulin therapy versus no insulin therapy on thromboembolic events in patients with AF. We explored this issue in a recent multicenter, European AF registry.

5.2 Methods

We accessed individual patient-level data from the PREFER in AF registry (Prevention of thromboembolic events-European Registry in Atrial Fibrillation).[125] This prospective, observational, real-world registry enrolled 7,228 patients with AF from 461 hospitals in 7 European countries (Austria, France, Germany, Italy, Spain, Switzerland, and the United Kingdom). The first patient was enrolled in January 2012 and the last follow-up visit occurred in January 2014. Inclusion criteria were age ≥18 years; written informed consent for study participation; and history of AF within the preceding 1 year, as demonstrated by an electrocardiogram or by an implanted pacemaker/defibrillator. Patients were included irrespective of the type of AF. To reduce selection bias, patients were consecutively enrolled at each site, with no explicit exclusion criteria. The study design consisted of a baseline clinical evaluation at the time of enrollment and at 1-year follow-up. Demographic data, clinical characteristics, risk factors, and treatment modalities were collected at baseline; at this time, documentation related to previous AF episodes and use of AF-related antithrombotic therapy within 1 year was also inspected, if needed. The follow-up was performed by office visit at 12 ± 2 months. For the purpose of this study, we only included patients with a complete CHA2DS2-VASc score evaluation and both baseline and 1-year follow-up visits. Only documented stroke or systemic embolism were considered relevant efficacy endpoints, with the date of any event being after the baseline visit. Individual data were entered into an electronic case report form including various plausibility checks for the considered variables. Furthermore, on-site verification of source data was performed in approximately 5% of the centers. The study management was overseen by a scientific Steering Committee; the registry was sponsored by Daiichi-Sankyo Europe GmbH (Munich, Germany) via a contract research organization (SSS International Clinical Research GmbH, Munich, Germany) coordinating various local/national contract research organizations.

Definitions and endpoints.

For the purpose of this study, patients with diabetes were separately considered if they were or were not on insulin therapy.[126] The primary study endpoint was the incidence of stroke/systemic embolism at 1-year follow-up according to diabetes status (no diabetes, noninsulin-requiring diabetes, or insulin-requiring diabetes). Stroke and systemic embolism were defined following the ENGAGE AF–TIMI 48 (Effective Anticoagulation with Factor Xa Next Generation in Atrial Fibrillation–Thrombolysis In Myocardial Infarction 48) definitions[127] as follows:

- Stroke: abrupt onset of a focal neurological deficit, generally distributed in the territory of a single brain artery (including the retinal artery), that is not attributable to an identifiable nonvascular cause (i.e., brain tumor or trauma). The deficit must either be characterized by symptoms lasting >24 h or cause death within 24 h of symptom onset. Stroke definition used in ENGAGE and in our study reflects the Statement for Healthcare Professionals From the American Heart Association/American Stroke Association[128] that incorporates the World Health Organization definition of stroke.[129]

- Systemic embolic event: abrupt episode of arterial insufficiency with clinical or radiological documentation of arterial occlusion in the absence of other likely mechanisms (e.g., atherosclerosis, instrumentation); venous thromboembolism and pulmonary embolism were also included in this outcome measure. Arterial embolic events involving the central nervous system (including the eye) were not considered as systemic embolism.

Statistical analysis.

For categorical variables, absolute and percentage frequencies (n, %) are presented. For continuous variables, mean ± SD is presented. For the analyses of the time-to-stroke/systemic embolism, the Cox proportional hazard regression model was used, with diabetes status as a fixed effect. The hazard ratio (HR), 95% confidence interval (CI), and corresponding p values are presented. These analyses were repeated for different subgroups of patients based on the demographic/clinical characteristics indicated in Table 1. In addition, these characteristics were added as single covariates to the model. Comparisons of all demographic/clinical characteristics for diabetes status were executed by means of a logistic regression model presenting the odds ratio, 95% CI, and corresponding p value. All analyses are not confirmatory, but purely descriptive/exploratory. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

5.3 Results

From the overall PREFER in AF population (N=7,228), a total of 816 patients had no 1-year follow-up visit; therefore, the full analysis set consisted of 6,412 patients, 695 of whom were excluded because of lack of information on stroke/systemic embolic events and/or no availability of CHA2DS2-VASc scores and/or no information on diabetes status (Figure 1). Thus, a total of 5,717 patients were included in this subanalysis. Prevalence of thromboembolic risk factors and different antithrombotic therapies in patients included in this analysis was consistent with the overall PREFER in AF population (data not shown). Among these 5,717 patients, a total of 1,288 (22.5%) had diabetes mellitus. Furthermore, 288 of these 1,288 (22.4%) patients with diabetes were on insulin treatment. Patients with diabetes, irrespective of insulin therapy status, had an increased prevalence of systemic hypertension, congestive heart failure (CHF), prior transient ischemic attack/stroke/thromboembolism, vascular disease, chronic renal impairment, left atrial enlargement, chronic obstructive

pulmonary disease (COPD), and body mass index >30 kg/m2 compared with patients without diabetes (Table 1). Patients receiving insulin showed higher percentages of CHF, vascular disease, chronic renal impairment, COPD, and body mass index >30 kg/m2 versus those with noninsulin-requiring diabetes. Of note, in our study, only 18 patients had type 1 diabetes and only 1 patient experienced a thromboembolic event during follow-up. We also evaluated the prevalence of different antithrombotic strategies in the various subgroups (Table 1). Compared with patients without diabetes, those on insulin treatment had higher use of vitamin K antagonists (VKA) plus antiplatelet therapy (16.7% vs. 9.6%; p=0.0002) at baseline and higher use of VKAs (71.5% vs. 62.9%; p=0.0036) at 1 year. No antithrombotic therapy was less frequent in patients with diabetes on insulin both at baseline and at 1 year (2.4% vs. 6.4% in patients without diabetes, p=0.0093, and 5.6% vs. 9.6%, p=0.0238, respectively). Antithrombotic therapy was similar in patients with diabetes with and without insulin, with the exception of a higher prevalence of VKAs plus an antiplatelet agent at baseline in the former (16.7% vs. 11.1%; p=0.012). In the comparison between patients without diabetes and patients with noninsulinrequiring diabetes, the latter more frequently were given VKAs only at baseline (69.8% vs. 66.2%; p=0.03) and less frequently received antiplatelet treatment and no antithrombotic drug both at baseline and at 1 year. In the overall population, the incidence of stroke/systemic embolism at 1 year was 2.0 per 100 patients/year. Insulin-requiring diabetes was associated with a higher risk of stroke/systemic embolism versus both 100 patients/year; HR: 2.89; 95% CI: 1.67 to 5.02; p=0.0002) and noninsulin-requiring diabetes (5.2 per 100 patients/year vs. 1.8 per 100 patients/year; HR: 2.96; 95% CI: 1.49 to 5.87; p= 0.0019) (Central Illustration). Rates of stroke/systemic embolism were not different in patients with diabetes not receiving insulin and in patients without diabetes (HR: 0.97; 95% CI: 0.58 to 1.61; p=0.90). Adjustment for potential confounders provided similar results (Table 2). After the addition of various risk factors as covariates to the Cox proportional hazard regression model, the correlation between insulin-requiring diabetes and the higher

occurrence of thromboembolic events remained always significant, with HRs ranging from 2.60 to 3.52 (Table 3). In the comparison between insulin-requiring diabetes and noninsulin-requiring diabetes, of 15 tested covariates, 2 had statistically significant interactions with the group; in particular, the relative increase of thromboembolic events related to insulin therapy was higher in patients with CHF (vs. those without) and in patients receiving antithrombotic therapy at baseline. Conversely, the HR for the comparison of patients with no diabetes versus patients with noninsulin-requiring diabetes remained consistently nonsignificant (Table 3).

The prevalence of sustained (persistent or permanent) AF tended to be higher in patients on insulin treatment: 80% on insulin versus 76% in patients with noninsulin-requiring diabetes, compared with 67% of patients without diabetes. However, adjustment for the type of AF did not change the overall study results, in particular regarding stroke/systemic embolism: insulin-requiring diabetes mellitus versus no diabetes (HR: 2.83; 95% CI: 1.60 to 5.03; p=0.0004); stroke/systemic embolism with insulin-requiring diabetes mellitus versus diabetes without insulin therapy (HR: 2.98; 95% CI: 1.48 to 6.02; p=0.0023); and noninsulin-requiring diabetes mellitus versus no diabetes (HR: 0.98; 95% CI: 0.59 to 1.63; p=0.94). We collected additional patient-level data on diabetes duration, daily insulin dose, presence/absence of microvascular complications, and use of oral glucose-lowering agents in a subgroup of 344 patients with diabetes (i.e., 27% of the overall study population with diabetes). The risk profile of these 344 patients was similar to that of the remaining population of patients with diabetes: age 73.3±9.2 years versus 72.7±8.6 years (p=0.31), female sex 36% versus 37% (p= 0.66), and mean CHA2DS2-VASc score 4.7±1.6 versus 4.6±1.6 (p=0.42). The duration of diabetes was higher in patients with diabetes on insulin compared with those not receiving insulin (12.8±8.2 years vs. 9.2±6.7 years; p=0.0003), but the HR of stroke/systemic embolism with insulin therapy, adjusted for duration of diabetes, remained significant (HR: 8.72; 95% CI: 2.89 to 26.33; p=0.0001). The total daily insulin dose was similar in patients with versus without stroke/systemic embolism (37.8±9.9 IU vs. 38.5±26.2 IU; p=0.22), and no

relationship between insulin dose and the occurrence of thromboembolic events was observed (HR: 1.00; 95% CI: 0.98 to 1.02; p=0.94). We found a significantly higher risk of stroke/systemic embolism in patients with at least 1 microvascular complication of diabetes (retinopathy, neuropathy, or nephropathy): HR: 9.27; 95% CI: 2.07 to 41.41; p=0.0036. We attempted an analysis of different therapies in noninsulin-requiring diabetes (e.g., diet vs. oral antidiabetic agents, or among various classes of oral antidiabetic drugs), but these analyses were precluded by the overall low rate of thromboembolic events observed in these subgroups. We further evaluated the risk of stroke/systemic embolism in patients without diabetes, with diabetes not receiving insulin, and in those with insulin-requiring diabetes according to different subgroups, including presence or absence of female sex, age ≥75 years, CHF, systemic hypertension, previous transient ischemic attack/stroke, any vascular disease, COPD, chronic renal impairment, body mass index >30 kg/m2, CHA2DS2-VASc score >1, coronary artery disease, peripheral artery disease, or use of anticoagulant therapy. The highest incidence of thromboembolic events continued to be seen in patients with diabetes on insulin treatment. However, there was no significant difference in thromboembolic events comparing patients with diabetes without insulin treatment with those without diabetes and this absence of any difference for these 2 groups was maintained across various subpopulations (Figure 2). Of note, among patients with diabetes on insulin therapy, the rate of stroke/systemic embolism was even high in those receiving any anticoagulant therapy at baseline (5.1 per 100 patients/year vs. 6.1 per 100 patients/year in those without anticoagulation); the increased incidence of thromboembolic complications in patients with insulin-requiring diabetes was irrespective of the use of anticoagulant therapy (patients receiving any anticoagulant treatment: 5.1 per 100 patients/year in patients with insulin-requiring diabetes vs. 1.6 per 100 patients/year in patients with noninsulin-requiring diabetes and 1.8 per 100 patients/year in patients with diabetes; patients without anticoagulant therapy: 6.1 vs. 3.5 vs. 1.9 per 100 patients/year). A total of 4,354 patients had no diabetes or noninsulin-requiring diabetes and a

CHA2DS2-VASc score >1; the occurrence of stroke/systemic embolism at 1 year in such patients was 2.0%. All patients with diabetes on insulin therapy had a CHA2DS2-VASc score >1 and showed an annual stroke/embolism rate of 5.2 per 100 patients/year (p=0.0005).

5.4 Conclusions

In this analysis of individual patient data from the prospective PREFER in AF registry, patients with diabetes on insulin therapy had a significantly higher risk of stroke/systemic embolism at 1 year versus both patients without diabetes and patients with noninsulin-requiring diabetes; yet, for people with diabetes not treated with insulin, there was no significantly increased risk. The proportion of patients with diabetes in our population was 22.5%, of whom 22.4% were insulin treated; this prevalence is similar to that observed in other contemporary registries of patients with AF.[130] Of note, a 40% relative increase in the risk of AF development and progression has been demonstrated in patients with versus those without diabetes,[41] and has been related to electrical and structural atrial remodeling, changes in the autonomic response, atrial inflammation, and oxidative stress.[131] A wide range (from 3.6% to 8.6%) of annual incidence of thromboembolic events has been reported in patients with diabetes and AF;[41,44] this large variability reflects differences in study designs, definitions of outcome measures, patients' baseline risk profile, concomitant therapies, and types of populations included. Previous large studies have found that patients with AF with coexisting diabetes present a significantly higher risk of thromboembolic events compared with those without. In a previous metaanalysis on the topic, including 7 studies and >12,000 patients, a 70% relative increase in risk has been observed in patients with diabetes (13). To date, however, no study had separately and independently quantified the annual rates of AF-related thromboembolic events in patients with diabetes according to insulin treatment.

The surprising finding of our study was the strikingly similar incidence of thromboembolic events at 1 year in patients with diabetes but without insulin treatment compared with patients

without diabetes. The result was consistent for various analyses performed, even after adjusting for both clinical confounders and concomitant antithrombotic therapy. Of note, the event rate was similar in patients without diabetes and in patients with diabetes not receiving insulin despite the latter having a higher thromboembolic risk profile (i.e., older age, higher prevalence of hypertension, CHF, previous cerebrovascular events, vascular disease, and chronic renal failure). Thus, according to our data, the sole presence of diabetes does not imply an increased thromboembolic risk in patients with AF. Conversely, patients with insulin-requiring diabetes had an approximately 2.5-fold higher risk of stroke or systemic embolism at 1 year compared with patients without diabetes and with patients with noninsulin-requiring diabetes. Of note, this higher risk was more pronounced between 6 months and 1 year of follow-up. A clustering of risk factors likely contributes to this heightened risk, because patients with diabetes on insulin treatment had a longer diabetes duration, and a higher prevalence of cardiovascular risk factors, CHF, COPD, and renal impairment than patients without diabetes or those with diabetes not requiring insulin. The association between insulin-requiring diabetes and thromboembolic events was independent of the type of AF and of other possible confounding factors; this association was also maintained in various subgroups, including those patients receiving anticoagulant therapy. We observed no relationship of daily insulin dose and thromboembolic risk. We cannot exclude a type II error in these results, and it is possible that the daily doses of insulin (marking a diabetes of particular severity) could be related to outcomes in larger cohorts or with a longer follow-up. Of note, patients with diabetes with microvascular complications (retinopathy, neuropathy, or nephropathy) experienced a significantly increased incidence of thromboembolic events. Importantly, the selectively increased thromboembolic risk of patients receiving insulin (with no apparent increase in risk in the other set of patients with diabetes) was independent of all potential confounders from parameters collected in the PREFER in AF Registry, including duration of diabetes.[45] Similar data supporting a

differential prognostic role of diabetes with versus without insulin therapy have been described in at least 1 other setting; in particular, an analysis from SHIFT (Systolic Heart Failure treatment with the If inhibitor ivabradine Trial) demonstrated that patients with chronic systolic heart failure[132] showed no increased incidence of cardiovascular death or hospitalization for worsening heart failure in patients with non insulin-requiring diabetes compared with patients without diabetes, and a significant 33% higher risk of this outcome measure in patients with diabetes on insulin compared with those not on insulin. Therefore, according to our data, it is the need for insulin therapy, rather than the presence of diabetes per se, that seems to be an independent factor affecting the occurrence of AF-related stroke/systemic embolism during follow-up. Results of this study might expand and strengthen observational data from certain investigations suggesting no overall increase of thromboembolic risk in patients with diabetes;[133–137] a different prevalence of patients receiving insulin (generally not reported in most studies) may at least in part explain the important variability in the reported annual rates of thromboembolic events among patients with diabetes and the variable degree of increase in the thromboembolic risk by diabetes mellitus in the various studies. Several pathophysiological mechanisms might explain these findings. In patients with diabetes mellitus there is a hypercoagulable state, and this is particularly evident in those with long-lasting disease receiving insulin therapy. In the latter, an increase in platelet reactivity and platelet turnover has been described, with consequently more pronounced platelet activation.[138] Moreover, a high inflammatory status and oxidative stress cause endothelial dysfunction, with higher expression of adhesion molecules, reduced release of nitric oxide/prostacyclin, and increased production of endothelin-1.[124,139–141] Patients with diabetes on insulin treatment also showed increased levels and/or activity of various coagulation factors, including tissue factor, factor VII, von Willebrand factor, and fibrinogen, as well as enhanced thrombin generation.[138,142,143] Finally, lower tissue-plasminogen activator activity, higher levels of type 1 plasminogen activator inhibitor,[144,145]

and higher levels of incorporation of the C3 complement component in the clot[146] have been demonstrated in such patients, leading to impaired fibrin clot lysis. The presence of insulin treatment is therefore certainly a marker for more advanced disease. However, insulin by itself might trigger some of the disease features, including atherosclerosis.[147] Although the precise mechanisms triggering changes in coagulation in patients with diabetes receiving insulin therapy are not completely known, possible mechanisms include chronic exposure to high glucose levels, increased levels of advanced glycosylation end-products, and direct effects of exogenous insulin, providing pathologically high levels of insulin in the setting of insulin resistance, as occurs in all patients with type 2 diabetes receiving insulin.[147,148]

Study limitations.

This work has strengths in being a prospective analysis of patients with AF who received a complete baseline assessment and underwent a planned follow-up visit at 1 year with accurate evaluation of outcome measures. Limitations are that we could not establish the thromboembolic risk of the untreated population included or the risk associated with specific antithrombotic therapies. However, the crude increase in thromboembolic risk that occurred in the presence of insulin-requiring diabetes is probably even higher than that detected in our investigation. In fact, patients on insulin had a higher prevalence of VKA use and less frequently received no antithrombotic drug than those without diabetes; similarly, they more often were given VKAs plus antiplatelet drugs than those with diabetes without insulin. Thus, it is unlikely that we overestimated the risk of patients with insulin-requiring diabetes in our study. Furthermore, residual confounding cannot be excluded and, because of the size of the population, we could not stratify the thromboembolic risk of patients with diabetes on insulin therapy according to different CHA2DS2-VASc scores (1 vs. >1). The issue of whether the relationship between the type of diabetes and thromboembolic risk was irrespective of the duration of diabetes was evaluated in approximately one-fourth of the population with diabetes within PREFER in AF (n=344 or 27%); in this subset, the HR of stroke/systemic

embolism in patients with insulin therapy, compared with patients not on insulin, remained significant even after adjusting for the duration of diabetes. Therefore, main results of this sensitivity analysis continued to support 1 main conclusion of the paper, that insulin-requiring diabetes is a much worse condition than noninsulin-requiring diabetes. Importantly, the risk profile of those 344 patients providing additional data on diabetes duration was similar to that of the remaining population of patients with diabetes. We can therefore reasonably assume that the results of this further analysis were not affected by the selection of patients, and no bias was introduced in this secondary analysis. Finally, a non-uniform definition of diabetes mellitus might have been used in the study population according to local practices, and more important, we have no data on the specific criteria for initiating insulin therapy, on the type of insulin regimen and on glycemic control during follow-up. However, we consider it unlikely that use of non-uniform definitions of diabetes and criteria for initiating insulin therapy might have affected the study results, inasmuch as the physicians in the Western European countries participating in PREFER in AF are generally accustomed to contemporary, international guidelines for defining diabetes and initiating insulin treatment. Any such limitations should not, however, affect the main finding of our study, which is not only the higher risk of the insulin-requiring diabetes, likely clustering with a higher severity of diabetes, but rather the very low risk of noninsulin-requiring diabetes. This indicated for the first time a quite dichotomous behavior of the population with diabetes and AF as to thromboembolic risk according to the use or lack of use of insulin. Of note, results of our investigation apply essentially to patients with type 2 diabetes, who represented 98.6% of the population with diabetes studied, and it may be that insulin provision in type 1 diabetes, in the absence of insulin resistance, is not associated with increased thromboembolic risk. We cannot completely exclude the possibility that patients with noninsulin-requiring diabetes with AF are at somewhat higher thromboembolic risk than patients without diabetes, and that we could not demonstrate this difference because of a type II statistical error. However, the absence of

any directional trend toward higher risk in patients with noninsulin-requiring diabetes argued against such possibility, but certainly further confirmatory research should address this important issue in studies with a larger sample size.

Our findings robustly indicated that insulin-requiring diabetes, essentially type 2 diabetes, largely contributed to the overall increase of thromboembolic risk in AF, but the mere presence of diabetes without insulin treatment did not apparently convey a negative prognostic value. Our investigation still supported that early diabetes has lower thromboembolic risk than later diabetes and, in our population, the reduced thromboembolic risk in patients without insulin treatment may be caused by the shorter duration of the disease. Our results may have implications in the assessment of thromboembolic risk in the AF population with diabetes and might have therapeutic implications that need to be explored in further dedicated intervention studies.

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5.5 Tables

Table 1. Main demographic/clinical characteristics in the study population according to diabetes status.

Variable	No DM N=4,429	Non-insulin requiring DM N=1,000	Insulin-requiring DM N=288	P value No DM vs non-insulin requiring DM	P value No DM vs insulin-requiring DM	P value Non-insulin requiring DM vs insulin-requiring DM
Age 65-74 yrs	1,431 (32.3)	384 (38.4)	97 (33.7)	0.0002	0.63	0.15
Age ≥75 yrs	1,941 (43.8)	453 (45.3)	137 (47.6)	0.40	0.22	0.50
Female gender	1,782 (40.2)	373 (37.3)	104 (36.1)	0.17	0.09	0.71
BMI >30 kg/m^2	1,089 (24.6)	377 (37.7)	133 (46.2)	<0.0001	<0.0001	0.012
Systemic hypertension	2,998 (67.7)	852 (85.2)	255 (88.5%)	<0.0001	<0.0001	0.15
Congestive heart failure	1,146 (25.9)	342 (34.2)	164 (56.9)	<0.0001	<0.0001	<0.0001
Prior TIA/stroke/thromboembolism	635 (14.3)	192 (19.2)	67 (23.3)	0.0001	<0.0001	0.13
Vascular disease	846 (19.1)	295 (29.5)	133 (46.2)	<0.0001	<0.0001	<0.0001
Chronic renal impairment (Cr Cl <30 mL/min/1.73 m^2)	484 (10.9)	173 (17.3)	100 (34.7)	0.0001	<0.0001	<0.0001
Left atrial enlargement (antero-posterior diameter >40 mm)	2,529 (69.2)	650 (78.2)	190 (79.2)	<0.0001	0.0013	0.7532
Chronic obstructive pulmonary disease	434 (9.8)	131 (13.1)	62 (21.5)	0.0016	<0.0001	0.0005

Antithrombotic therapies at baseline						
NOAC	281 (6.3)	77 (7.7)	13 (4.5)	0.1192	0.2154	0.0648
VKA only	2933 (66.2)	698 (69.8)	194 (67.4)	0.0300	0.6943	0.4295
Antiplatelet only	505 (11.4)	82 (8.2)	26 (9.0)	0.0034	0.2183	0.6553
VKA plus antiplatelet	427 (9.6)	111 (11.1)	48 (16.7)	0.1634	0.0002	0.0120
No therapy	283 (6.4)	32 (3.2)	7 (2.4)	0.0001	0.0093	0.5036
Antithrombotic therapies at one year						
NOAC	576 (13.0)	152 (15.2)	32 (11.1)	0.0661	0.3533	0.0821
VKA only	2788 (62.9)	662 (66.2)	206 (71.5)	0.0538	0.0036	0.0897
Antiplatelet only	384 (8.7)	54 (5.4)	9 (3.1)	0.0007	0.0017	0.1193
VKA plus antiplatelet	255 (5.8)	62 (6.2)	25 (8.7)	0.5900	0.0435	0.1413
No therapy	426 (9.6)	70 (7.0)	16 (5.6)	0.0098	0.0238	0.3881

Values are given as N (%). BMI= Body mass index; DM= Diabetes mellitus; NOAC= non-vitamin K antagonist oral anticoagulants; TIA= Transient ischemic attack; VKA= vitamin K antagonists

Table 2. Adjusted risk of stroke/systemic embolic events at one year *

Comparison	**HR**	**95% CI**	**P value**
Insulin-requiring diabetes vs no diabetes	2.34	1.29-4.25	0.005
Insulin-requiring diabetes vs non-insulin requiring diabetes	2.62	1.26-5.42	0.01
Non-insulin requiring diabetes vs no diabetes	0.88	0.51-1.50	0.64

*Adjusted for congestive heart failure, hypertension, age ≥75 yrs, prior stroke/transient ischemic attack/thromboembolism,
vascular disease, female gender, chronic obstructive pulmonary disease, chronic renal impairment, body mass index >30 kg/m^2,
CHA_2DS_2-VASc score 0-1, vitamin K antagonist therapy, use of non-vitamin K antagonist oral anticoagulants, vitamin K antagonist plus
antiplatelet therapy

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Table 3. COX Proportional Hazard Regression Model

	HR	95% CI	P value	Interaction P value
Insulin-requiring diabetes mellitus: YES vs NO	2.90	1.69-5.00	0.0001	
Model with covariate congestive heart failure	2.27	1.31-3.96	0.0036	<0.0001
Model with covariate systemic hypertension	2.97	1.72-5.14	<0.0001	0.52
Model with covariate age ≥75 yrs	2.86	1.66-4.92	0.0001	0.0105
Model with covariate previous transient ischemic attack/stroke/thromboembolism	2.67	1.55-4.60	0.0004	<0.0001
Model with covariate vascular disease	2.94	1.69-5.11	0.0001	0.83
Model with covariate age 65-74 yrs	2.91	1.69-5.00	0.0001	0.16
Model with covariate female gender	2.95	1.71-5.07	<0.0001	0.0291
Model with covariate left atrial enlargement	2.99	1.66-5.37	0.0003	0.13
Model with covariate chronic obstructive pulmonary disease	2.64	1.53-4.57	0.0005	0.0011
Model with covariate chronic renal impairment	2.59	1.43-4.68	0.0016	0.98
Model with covariate BMI >30 kg/m^2	3.04	1.76-5.27	<0.0001	0.69
Model with covariate no anti-thrombotic therapy at baseline	2.94	1.70-5.05	0.0001	0.47
Model with covariate anticoagulant therapy at baseline	2.90	1.69-5.0	0.0001	0.75
Model with covariate antiplatelet therapy at baseline	2.91	1.69-5.0	0.0001	0.82
Model with covariate VKA + antiplatelet therapy at baseline	2.99	1.74-5.16	<0.0001	0.17
Non-insulin requiring diabetes vs no diabetes	0.97	0.58-1.61	0.90	
Model with covariate congestive heart failure	0.89	0.53-1.48	0.65	<0.0001
Model with covariate systemic hypertension	0.99	0.59-1.66	0.97	0.55
Model with covariate age ≥75 yrs	0.96	0.58-1.60	0.88	0.011
Model with covariate previous transient ischemic attack/stroke/thromboembolism	0.92	0.55-1.54	0.75	0.0002
Model with covariate vascular disease	0.96	0.58-1.60	0.88	0.81
Model with covariate age 65-74 yrs	0.99	0.59-1.64	0.95	0.14
Model with covariate female gender	0.98	0.59-1.63	0.94	0.02
Model with covariate left atrial enlargement	0.82	0.45-1.48	0.50	0.33
Model with covariate chronic obstructive pulmonary disease	0.95	0.57-1.59	0.86	0.07
Model with covariate chronic renal impairment	0.96	0.58-1.60	0.87	0.41
Model with covariate BMI >30 kg/m^2	0.94	0.56-1.60	0.83	0.68

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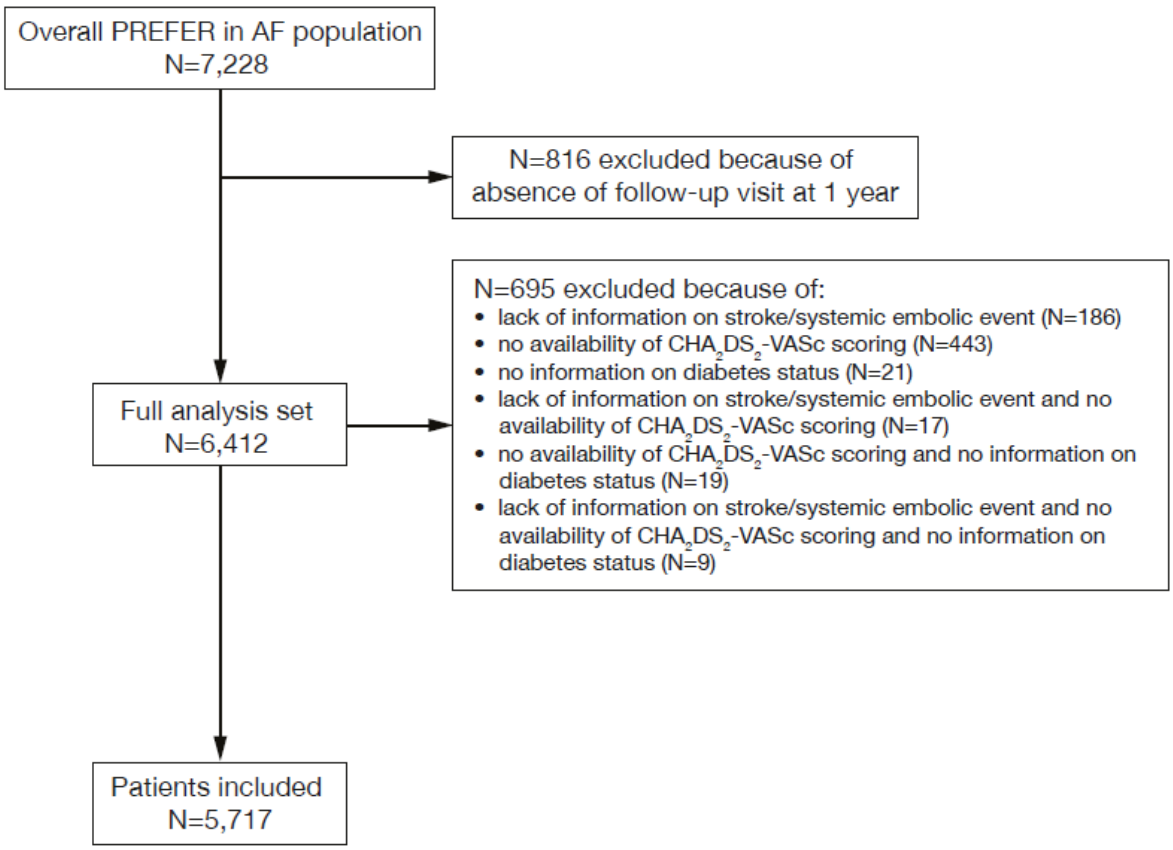
Model with covariate no anti-thrombotic therapy at baseline	0.97	0.58-1.62	0.92	0.65
Model with covariate anticoagulant therapy at baseline	0.97	0.59-1.62	0.92	0.33
Model with covariate antiplatelet therapy at baseline	0.97	0.58-1.62	0.91	0.72
Model with covariate VKA + antiplatelet therapy at baseline	0.97	0.58-1.62	0.91	0.48

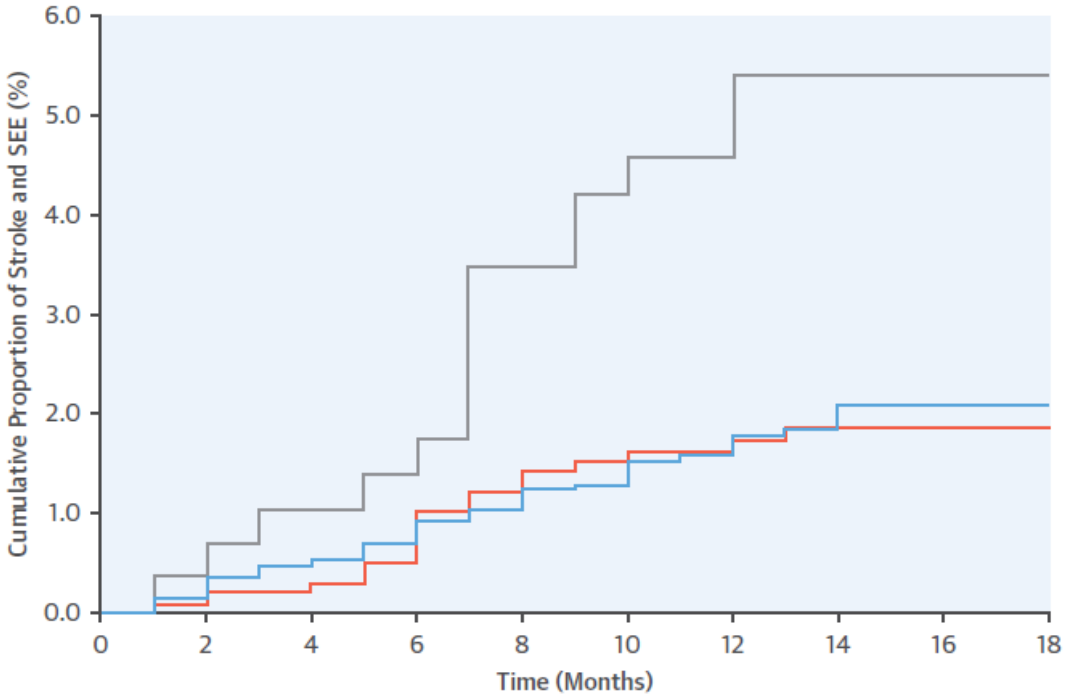
BMI= Body mass index; VKA= Vitamin K antagonist

5.6 Figures

Figure 1. Flow diagram indicating patients' disposition in the present study, leading to the final number of 5,717 patients here included.



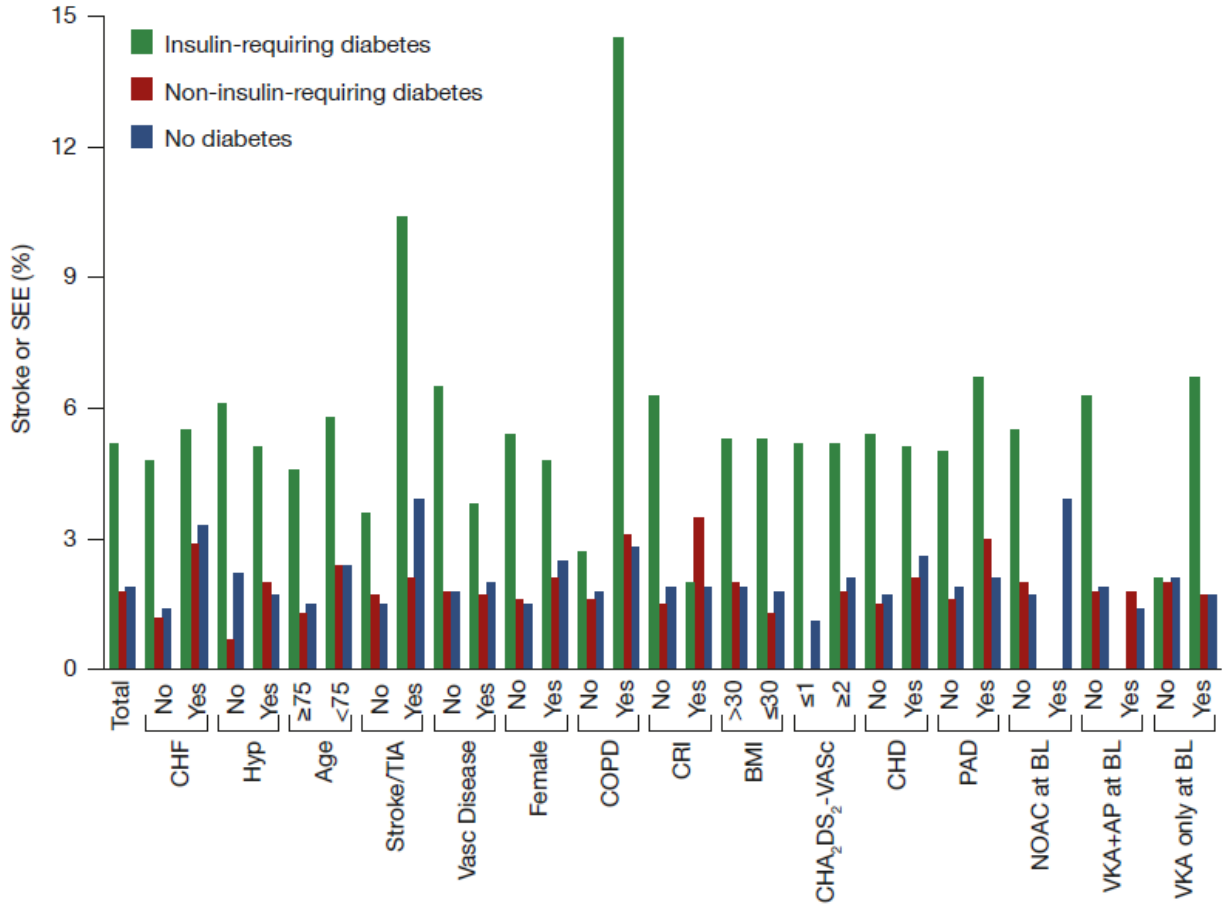
Central illustration. Kaplan-Meier curves for incidence of stroke/systemic embolism according to diabetes status.



No diabetes	4,429	4,409	4,384	4,365	4,326	4,280	3,854	1,642	148	27
Non-insulin DM	1,000	998	994	987	973	963	868	320	33	5
Insulin DM	288	287	285	284	270	260	230	72	5	1

No diabetes — Non-insulin-requiring diabetes — Insulin-requiring diabetes

Figure 3. Stroke or systemic embolism by subpopulations.



AP= Antiplatelet; BL= Baseline; BMI= Body mass index; CHD= Coronary heart disease; CHF= Congestive heart failure; COPD= Chronic obstructive pulmonary disease; CRI= Chronic renal impairment; Hyp= Systemic hypertension; NOAC= Non-vitamin K antagonist oral anticoagulants; PAD= Peripheral artery disease; SEE= Systemic embolic events; TIA= Transient ischemic attack; VKA= vitamin K antagonists

6. Specific aim 4: to assess cardiovascular safety (a composite of cardiovascular death, myocardial infarction, or stroke, and new-onset atrial fibrillation or flutter) of odanacatib, a cathepsin K inhibitor for the treatment of postmenopausal osteoporosis

McClung MR, O'Donoghue ML, Papapoulos SE, Bone H, Langdahl B, Saag KG, Reid IR, Kiel DP, Cavallari I, Bonaca MP, Wiviott SD, de Villiers T, Ling X, Lippuner K, Nakamura T, Reginster JY, Rodriguez-Portales JA, Roux C, Zanchetta J, Zerbini CAF, Park JG, Im K, Cange A, Grip LT, Heyden N, DaSilva C, Cohn D, Massaad R, Scott BB, Verbruggen N, Gurner D, Miller DL, Blair ML, Polis AB, Stoch SA, Santora A, Lombardi A, Leung AT, Kaufman KD, Sabatine MS; LOFT Investigators. Odanacatib for the treatment of postmenopausal osteoporosis: results of the LOFT multicentre, randomised, double-blind, placebo-controlled trial and LOFT Extension study. Lancet Diabetes Endocrinol. 2019 Dec;7(12):899-911..

6.1 Background

Osteoporosis is associated with reduced bone mass, impaired bone strength, and increased risk of fracture.[149] This is a common condition with clinically important consequences. An estimated 54% of the US population aged 50 years and older has osteoporosis or low bone mass1 and around 40% of women will sustain a fracture of their hip, spine, or forearm in their lifetime. Therapies for osteoporosis are available but each has limitations, and most women with osteoporosis remain untreated. Cathepsin K, the primary cysteine protease produced by osteoclasts, is involved in the degradation of type 1 collagen and other bone matrix proteins and is necessary for bone resorption.[150] Inhibition of cathepsin K in animal studies reduced osteoclast-mediated bone resorption without decreasing osteoclast number or inhibiting other osteoclast functions, maintained or produced only a transient decrease in bone formation, maintained normal bone material properties, and increased bone mass and strength.[151,152] In addition to effects on bone, pre-clinical data suggested that inhibition of cathepsin K could have favourable effects on atherothrombotic cardiovascular risk through stabilisation of arterial plaques.[153,154] Odanacatib, an oral, selective inhibitor of cathepsin K, was previously shown to increase bone mineral density over 8 years in postmenopausal women with low bone mass.[155–160] This increase in bone mineral density was the result of inhibition of bone

resorption, which was accompanied by only a transient reduction in bone formation, distinguishing this treatment from other antiresorptive treatments for osteoporosis. We aimed to assess the anti-fracture efficacy and safety of odanacatib in postmenopausal women with osteoporosis14 in the Long-term Odanacatib Fracture Trial (LOFT) and its extension study (LOFT Extension).

6.2 Methods

Study design and participants

We did a multicentre, randomised, double-blind, placebo-controlled, event-driven study at 388 outpatient clinics in 40 countries. The study was approved by local institutional review boards and ethics review committees at all centres. The study design and methods have been published previously.[161] Eligible participants were women aged 65 years or older who were postmenopausal for at least 5 years, with a bone mineral density T-score between -2.5 and -4.0 at the total hip or femoral neck without previous radiographic vertebral fracture, or between -1.5 and -4.0 with a previous radiographic vertebral fracture. Women with a previous hip fracture, more than one vertebral fracture, or a bone mineral density T-score less than -4.0 at the total hip or femoral neck were ineligible unless unable or unwilling to use approved osteoporosis treatment. Other exclusions included clinical fragility fracture in the previous 24 months, metabolic bone disease other than osteoporosis, severe chronic kidney disease, malignancy within 5 years (except treated skin cancer), or previous use of some medications affecting bone metabolism.[161] All participants provided written informed consent. Participants in LOFT Extension were patients in LOFT who did not have excessive bone loss and had completed LOFT study treatment; had not developed a condition, which, based on the investigators' judgment, would interfere with continued participation; were at a study site with approval for participation in LOFT Extension; and had consented to enrol in LOFT Extension. Patients provided written informed consent for the LOFT Extension.

Randomisation and masking

Participants were randomly assigned (1:1) to either odanacatib 50 mg once per week or matching placebo (each as an oral tablet). Randomisation to treatment assignment was based on a computer-generated allocation schedule generated by the sponsor and done using an interactive voice recognition system (IVRS) after stratification for previous radiographic vertebral fracture. Although block sizes were not set, they were anticipated to be 1:3 (previous: no previous radiographic vertebral fracture), but this was flexible with acknowledgment that the study sample size might need to be adjusted based on the emerging observed ratio. Treatment allocation was masked to study participants, investigators and their staff, and sponsor personnel.

Procedures

Participants received odanacatib 50 mg once per week or placebo, vitamin D3 (5600 international units [IU] per week as two 2800 IU tablets), and calcium supplements (eg, calcium carbonate tablets) as needed to ensure intake of approximately 1200 mg/day.

LOFT was planned to continue until the target number of 237 incident osteoporotic hip fractures accrued. Participants who discontinued study drug in LOFT were to be followed up and assessed per protocol. If LOFT completed before 5 years of double-blind treatment, eligible and consenting participants were to continue in LOFT Extension on their LOFT treatment assignment for a total of 5 years from randomisation.14 Bone mineral density was measured at baseline and yearly thereafter. Participants with excessive bone loss, defined as reduction of at least 7% in bone mineral density from baseline at the lumbar spine or total hip, were discontinued from study drug and offered approved therapy for osteoporosis. These participants were to be followed up during LOFT but were ineligible to enrol in LOFT Extension. Participants with excessive bone loss during LOFT Extension were discontinued from study drug and initially discontinued from the study and not followed up; a subsequent protocol amendment on June 19, 2013, instructed investigators to follow up these participants.

For participants who discontinued from LOFT or LOFT Extension and were not being followed up, an observational follow-up protocol was implemented to capture events through visits and telephone contacts. Mortality data were captured whenever possible for all participants. An external data monitoring committee reviewed safety data periodically and did pre-planned interim analyses to determine early stopping for futility or overwhelming efficacy.[161] After the first interim analysis, the committee recommended that LOFT be stopped early on the basis of robust efficacy and a favourable benefit–risk profile, with a plan for the committee to continue to review accrued data from the pre-planned LOFT Extension.[161] Investigators and participants were informed via a protocol amendment on Dec 19, 2012, that the interim analysis showed robust efficacy and a favourable benefit–risk profile, and that the committee would continue to review data from LOFT Extension.

After LOFT was stopped early, a small group of sponsor personnel (not further involved in the undertaking of the trial or screening of data) were unmasked to the results and, after assessment of these data, the decision was made to accrue data from the ongoing LOFT Extension study before submission of regulatory applications for approval. Initial LOFT results were presented at a scientific meeting while LOFT Extension was ongoing;15 further analyses of unblinded information from LOFT and LOFT Extension continued to be limited to a small group of sponsor personnel to maintain the integrity of LOFT Extension.

Outcomes

Primary endpoints were incidence of vertebral fractures in participants for whom evaluable radiograph images were available at baseline and at least one other timepoint and clinical hip and non-vertebral osteoporotic fractures. Secondary endpoints were clinical vertebral osteoporotic fractures; height; bone mineral density at the lumbar spine, total hip, femoral neck, trochanter, and distal forearm; histomorphometry of transilial bone biopsy specimens; major adverse cardiovascular events; and bone turnover markers. Vertebral fractures (new or worsening) were assessed at a central radiology site (BioClinica, San Francisco, CA, USA) by

semi-quantitative and morphometric analysis of lateral spine radiographs at baseline, 6, and 12 months, yearly thereafter, and at each participant's final study visit.14,16 Clinical fractures were adjudicated by clinical history and radiograph. Fractures of the fingers, toes, face and skull, and those adjudicated as caused by high-energy trauma or focal pathology (eg, neoplasm), were not included in efficacy analyses.

Bone mineral density of the lumbar spine and proximal femur was measured by dual energy x-ray absorptiometry at baseline and yearly thereafter (BioClinica, Newark, CA, USA). Bone turnover markers were measured in a randomly selected cohort (by IVRS) of 10% of the participants at baseline, 6 and 12 months, and yearly thereafter (BioClinica Labs, Lyon, France). Urinary N-telopeptide of type I collagen was assessed with the Vitros immunoassay (Ortho Clinical Diagnostics, Rochester, NY, USA), and serum procollagen type I N-terminal propeptide was assessed with the Modular E170 automated analyser immunoassay (Roche Diagnostics, Mannheim, Germany). Safety was assessed by adverse event reports, physical examination, vital signs, haematology, blood chemistry, and urinalysis. Pre-specified adjudicated events included morphea or systemic sclerosis (previously reported with a non-selective cathepsin K inhibitor;[162,163] serious respiratory infections;[164] osteonecrosis of the jaw,[165] atypical femoral fractures[166,167] including subtrochanteric and femoral shaft fractures, and atrial fibrillation[168] (each previously reported in participants receiving existing therapies for osteoporosis); delayed fracture unions; and major adverse cardiovascular events.

After the early stopping of LOFT, initial analyses raised concerns about higher numbers of cardiovascular events in the odanacatib group, but interpretation was confounded by the incompleteness of event adjudication. A cardiovascular academic research organisation called the Thrombolysis In Myocardial Infarction Study Group (TIMI; Brigham and Women's Hospital, Boston, MA, USA), was asked by the sponsor to do an independent adjudication of all potential cardiovascular events reported during LOFT and LOFT Extension. TIMI's Clinical Events Committee had full access to the masked clinical trial database and

adjudicated all reported deaths and potential cardiac ischaemic events (including myocardial infarction and unstable angina), cerebrovascular events, and supraventricular arrhythmias (including atrial fibrillation and atrial flutter). Confirmed cases of atrial fibrillation or flutter were classified as new onset (or presumed new onset) or recurrent, based on participant history.

TIMI pre-specified a statistical analysis plan, and remained masked to previous cardiovascular event adjudication and participant treatment allocation, before the database lock. Primary cardiovascular outcomes were (1) the composite of cardiovascular death, myocardial infarction, or stroke and (2) new onset (or presumed new onset) atrial fibrillation or flutter. Secondary cardiovascular outcomes were the composite of cardiovascular death, myocardial infarction, stroke, or admission to hospital for unstable angina; all-cause death; cardiovascular death; myocardial infarction (fatal or non-fatal); stroke (fatal or non-fatal); admission to hospital for unstable angina; new or presumed new onset of atrial fibrillation or flutter (ECG confirmed only; patients with known history of atrial fibrillation or flutter excluded); and any reported first episode of atrial fibrillation or flutter (ECG confirmation not required; included patients with known history of atrial fibrillation or flutter).

Statistical analysis

For 90% statistical power to show a 50% reduction in relative risk (RR) for radiographic vertebral fracture, 35% reduction in RR for hip fracture, or 20% reduction in RR for non-vertebral fracture, the target numbers of incident events at the final analysis were 114, 237, and 824, respectively. Because hip fractures occur less frequently, a sample size of approximately 16,000 women was required, based on the hip fracture incidence in the alendronate Fracture Intervention Trial.[169] Analysis of clinical hip, non-vertebral, and vertebral osteoporotic fractures included participants who took at least one dose of masked study drug, with follow-up from the start of treatment to study completion. Cumulative incidences were based on Kaplan-Meier estimates for time to first confirmed fracture, with

treatments compared using the Cox proportional hazards model, with terms for treatment, stratum, and geographic region. Analysis of radiographic vertebral fractures was based on life-table estimates from participants with both baseline and at least one post-baseline evaluable spine radiograph. Treatments were compared using a generalised linear model for binary data with the complementary log–log transformation of the probability of an event in an interval, with terms for treatment, stratum, and geographic region.

Bone mineral density endpoints were analysed using a longitudinal model on the percent change from baseline. The log-transformed fraction from baseline in bone turnover markers was analysed using the same model. Because of probable bias created in LOFT Extension from incomplete participation, the primary analysis was restricted to data from LOFT. We also did an analysis of all available follow-up data from both LOFT and LOFT Extension.

Analysis of safety included participants who took at least one dose of masked study drug. Treatment groups were compared using the Miettinen and Nurminen method via TIMI stats for pre-specified adverse events of interest.[170] For analysis of total mortality, data from all sources were used. In addition to the intention-to-treat analysis, an on-treatment analysis was conducted in which participants who did not experience an endpoint event were censored 14 days (approximately four half-lives of odanacatib) following last dose of masked study drug.

All CIs were two-sided, with a p value of less than 0.05 considered significant. No adjustments were made for multiplicity for testing of secondary and exploratory outcomes. Statistical analyses for cardiovascular events were done by TIMI using an independent copy of the clinical trial database (SAS version 9.4) and confirmed by the sponsor. Throughout LOFT and LOFT Extension, data were captured via Electronic Data Capture, using the InForm system package (Oracle Health Sciences, Redwood Shores, CA, USA). Analyses of study data were overseen by the data monitoring committee. Interim analyses were planned when approximately 70% and 85% of the targeted 237 incident hip fractures had accrued.[161]

If the p values for all three primary endpoints (radiographic vertebral, and clinical hip and

non-vertebral fractures) were lower than their corresponding boundaries via the α-spending function (p≤0.008 at the first interim), the committee could recommend early stopping of the trial for efficacy. This trial is registered with ClinicalTrials.gov (number NCT00529373) and European Clinical Trials Database (EudraCT number 2007-002693-66).

Role of the funding source

The funder of the studies, in collaboration with a scientific advisory committee who provided input on the clinical development programme, developed the protocol, and had a role in study design, data collection, data analysis, data interpretation, and reviewing of the report. The lead author drafted the report, had full access to all the data in the study, and had final responsibility for the decision to submit for publication. The TIMI Study Group had an independent copy of the study database and confirmed all cardiovascular outcome analyses.

6.3 Results

Between Sept 14, 2007, and Nov 17, 2009, we randomly assigned 16 713 eligible women to treatment, of whom 16 071 were included in the general safety and clinical fracture efficacy endpoints analyses (8043 odanacatib, 8028 placebo; figure 1). For some analyses, the denominator was less than 16 071, based on the number of participants with data available for the specific endpoint. Database lock occurred on Jan 18, 2013, for LOFT, and July 23, 2016, for LOFT Extension, including completion of adjudication of cardiovascular events by the TIMI study group. Baseline characteristics were similar between treatment groups (table 1). Mean age was 72.8 years (SD 5.3). Mean bone mineral density T-scores were –2.7 (lumbar spine), –2.3 (total hip), and –2.7 (femoral neck). 46% (7470/16 071) had a previous vertebral fracture. A minority of patients had a history of coronary artery disease or previous cerebrovascular event (table 1); however, the prevalent cardiovascular risk factors included hypertension, dyslipidaemia, diabetes, and family history of cardiovascular disease. Approximately 25% were current smokers or had a previous history of smoking. The median

observation period in LOFT was 36.5 months (34.43–40.15) and the median treatment duration was 35.6 months. 8257 participants (4297 receiving odanacatib, 3960 receiving placebo) entered LOFT Extension (figure 1), and 6601 completed the study; ie, completed 5 years' treatment from randomisation. More participants on placebo than on odanacatib discontinued study drug during LOFT (2718 *vs* 2378), many following a fracture, or because they met criteria for excessive bone loss and were ineligible to enter LOFT Extension. Sites in India did not participate in LOFT Extension, which resulted in a smaller proportion of Asian women in LOFT Extension than in LOFT (table 1); sites in Peru also did not participate. Despite these differences, most baseline characteristics were generally similar in the LOFT and LOFT Extension study populations. Cumulatively, the median observation period in LOFT plus LOFT Extension was 47.6 months (35.45–60.06) and the median treatment duration was 42.1 months. In LOFT, the hazard ratio (HR) for odanacatib versus placebo for radiographic vertebral fracture was 0.46 (95% CI 0.40–0.53; p<0.0001), with a cumulative incidence of 3.7% (251/6770) versus 7.8% (542/6910; table 2). Risk reduction was generally similar throughout the study (figure 2). HR for hip fracture was 0.53 (0.39–0.71; p<0.0001), with a cumulative incidence of 0.8% (65/8043) versus 1.6% (125/8028) (table 2, figure 2) and for non-vertebral fracture 0.77 (0.68–0.87; p<0.0001), with a cumulative incidence of 5.1% (412/8043) versus 6.7% (541/8028; table 2).

When interaction with time was tested, efficacy for nonvertebral fracture tended to be greater over time (p-interaction with time=0.017). Risk of clinical vertebral fracture and any clinical osteoporotic fracture were also reduced with odanacatib (table 2).

Results from LOFT plus LOFT Extension showed the anti-fracture efficacy of odanacatib over the 5-year study period was similar to that in LOFT (table 2, figure 2). As in LOFT, the effect of odanacatib on non-vertebral fracture risk tended to be greater over time (p-interaction with time=0.0025). In LOFT, odanacatib increased bone mineral density at the lumbar spine and total hip, whereas with placebo bone mineral density remained generally

stable at the lumbar spine and decreased at the total hip and femoral neck. At month 36, the least-squares (LS) mean percentage increase in bone mineral density from baseline with odanacatib was 8.6% (95% CI 8.4–8.7) at the lumbar spine and 4.8% (4.7–4.9) at the total hip, with between-group differences of 7.8% (7.6–8.1; p<0.0001) and 6.4% (6.3–6.6; p<0.0001), respectively. At month 36, urinary N-telopeptide of type I collagen/creatinine ratio reduced by 44% from baseline and serum procollagen type I N-terminal propeptide (P1NP) reduced by 12% with odanacatib; with placebo, urinary N-telopeptide of type I collagen/creatinine ratio increased by 15% and serum P1NP was stable. Results from LOFT plus LOFT Extension showed progressive increases in bone mineral density in the odanacatib group over the 5-year study period. At month 60, the LS mean between-group difference was 10.9% (95% CI 10.5–11.2; p<0.0001) at the lumbar spine and 10.3% (10.0–10.6; p<0.0001) at the total hip. At month 60, urinary N-telopeptide of type I collagen/creatinine ratio was reduced by 48% from baseline and serum P1NP increased by 12% with odanacatib, whereas with placebo, urinary N-telopeptide of type I collagen/creatinine ratio increased by 2% and serum P1NP increased by 10%. During LOFT and LOFT Extension, there were no meaningful between-group differences in the incidence of adverse events or serious adverse events overall, although selected adjudicated adverse events, including cardiovascular adverse events, were reported more often in patients in the odanacatib group than in the placebo group (tables 3, 4). Femoral shaft and subtrochanteric fractures, with and without atypical radiographic features occurred more often with odanacatib than with placebo (table 3). Femoral shaft fractures classified as atypical were not associated with prodromal pain, did not appear related to duration of treatment, and all but one complete fracture occurred following a fall. One additional atypical femoral fracture was incomplete and healed without progressing to a complete fracture. No untoward effect of odanacatib on delayed fracture union were noted. No confirmed cases of osteonecrosis of the jaw occurred.

During LOFT, adjudicated morphea-like skin lesions were confirmed in more participants in the odanacatib group than in the placebo group (p=0.019); one additional case occurring during the study was reported after the initial database lock (table 3). Autoimmune serology tests in these participants were negative, and none showed systemic involvement. Skin lesions resolved or improved, generally following withdrawal of study drug. Other dermatological adverse events were balanced between treatment groups. Three participants were reported with systemic sclerosis during LOFT, all with positive autoimmune serology at baseline. No additional cases of morphea-like skin lesions or scleroderma were reported during LOFT Extension. During LOFT, 518 patients with events for the composite endpoint of cardiovascular death, myocardial infarction, or stroke, and 208 new onset atrial fibrillation or atrial flutter events were reported. HR for cardiovascular death, myocardial infarction, or stroke (odanacatib *vs* placebo) was 1.12 (95% CI 0.95–1.34; p=0.18) with an incidence of 3.4% (273/8043) versus 3.1% (245/8028; table 4, figure 3). HR for new onset atrial fibrillation or atrial flutter (odanacatib *vs* placebo) was 1.18 (0.90–1.55; p=0.24); incidence 1.4% (112/8043) versus 1.2% (96/8028; table 4). Among secondary outcomes, odanacatib was associated with an increased risk of stroke (table 4, figure 3), of which 78.3% were adjudicated as ischaemic and 9.6% as haemorrhagic. The risk of myocardial infarction or cardiovascular death was not increased with odanacatib. Risk of new or recurrent atrial fibrillation or flutter (table 4, figure 3) tended to be higher with odanacatib. New onset atrial fibrillation was reported in 9.2% (22/240) of participants who had a stroke. When follow-up included LOFT Extension, cardiovascular outcomes were generally similar to those in LOFT, with participants in the odanacatib group having a higher risk of stroke and a numerically higher risk of new or recurrent atrial fibrillation (table 4). With the increased number of patients with events included from LOFT Extension (744 cardiovascular death, myocardial infarction, or stroke; 363 new or recurrent episodes of atrial fibrillation or flutter and 324 stroke), the HR for cardiovascular death, myocardial infarction, or stroke with odanacatib

versus placebo was significant (HR 1.17, 95% CI 1.02–1.36; p=0.029; incidence 5.0% [401/8043] *vs* 4.3% [343/8028]; table 4, figure 3), which was primarily due to an increased risk of stroke (HR 1.37, 1.10–1.71; p=0.0051; table 4, figure 3). We found no evidence of effect modification on the risk of stroke on the basis of age, previous stroke or transient ischaemic attack, diabetes, hypertension, or previous myocardial infarction in LOFT or LOFT Extension. In the on-treatment analysis, qualitatively consistent results occurred across all cardiovascular endpoints. To analyse total mortality, including cause of death, we used all available data on both continuing participants and those who had discontinued. In LOFT, there were numerically more deaths in the odanacatib group than in the placebo group (5.0% [401/8043] *vs* 4.4% [356/8028]; HR 1.13, 95% CI 0.98–1.30; p=0.10). Over the 5-year study period that included LOFT Extension, the numeric between-group difference in total mortality was less (odanacatib *vs* placebo: 8.5% [682/8043] *vs* 8.1% [651/8028]; HR 1.05, 95% CI 0.95–1.17; p=0.34). All reported deaths in LOFT and LOFT Extension were adjudicated as to cause by TIMI. In both LOFT and LOFT Extension, the largest between-group difference among adjudicated causes of death over the 5-year study period was in death due to malignancy, but assessment of the individual cases revealed no consistent pattern as to the reported type or types of malignancy.

6.4 Conclusions

In postmenopausal women with osteoporosis, treatment with odanacatib significantly reduced the risk of osteoporotic fractures, including fractures at the spine and hip. The relative reductions in fracture risk were similar to those in previous studies with other drugs for osteoporosis that inhibit bone resorption.[168,169,171] Over 5 years, spine and hip bone mineral density increased in the odanacatib group versus placebo. Protection from non-vertebral fractures appeared to increase with longer duration of therapy, consistent with the progressive changes in bone mineral density and the effects of odanacatib on strength of the cortical component of long bones in pre-clinical studies.[151,152,156,157]

The increase in subtrochanteric and femoral shaft fractures with odanacatib was unexpected and is not currently understood. All confirmed atypical femoral shaft fractures met American Society for Bone and Mineral Research criteria.[166,167] There was also an unexpected increase in the risk of stroke with odanacatib in both LOFT and LOFT Extension, with most events ischaemic in aetiology. This effect was consistent across pre-specified subgroups including those with history of cerebrovascular disease. However, odanacatib did not increase the risk of myocardial infarction and therefore there is no clear evidence it is prothrombotic or proatherogenic. We also noted a non-significant trend (p=0. 074) toward more episodes of new or recurrent atrial fibrillation or flutter with odanacatib, but this did not account for the increase in strokes given that few participants reported with a stroke were documented to have also had an atrial arrhythmia. However, we cannot exclude potential subclinical episodes of atrial fibrillation or flutter that were not detected or captured in the database.

The mechanistic underpinnings to explain these cardiovascular effects are unknown. Before our study, pre-clinical evidence suggested that odanacatib might have a cardioprotective role. Cathepsin K has highly potent elastase and collagenase activity and has therefore been hypothesised to contribute to atherosclerotic plaque instability by diminishing the structural integrity of the vascular wall. Increased expression of cathepsin K has been identified in macrophages and smooth muscle cells at sites of vascular matrix remodelling in human atheromas.[153,172,173] Deficiency of cathepsin K in apolipoprotein-E knockout mice was shown to reduce plaque progression and induce fibrosis, but promote macrophage foam cell formation by increasing scavenger receptor mediated uptake of modified LDL, leading to increased cellular storage of cholesterol esters.[151] Cathepsin K has been hypothesised to have a detrimental role in other cardiovascular disease states including heart failure and aortic and cerebral aneurysms,[174,175] further supporting the hypothesis that a cathepsin K inhibitor would be associated with cardiovascular benefit. However, since growing evidence suggests structural remodelling of the extracellular matrix might increase the risk of developing atrial

arrhythmia,[176,177] it is plausible that cathepsin K inhibition could drive the development of supraventricular arrhythmias by altering local cathepsin K elastase and collagenase activity in the atrium. Further assessment of vasculotropic effects of osteoporosis drugs and of cardiovascular risk might be warranted for future trials of established and novel therapies for osteoporosis.[178] To put the fracture efficacy and cardiovascular safety results in context, the results of LOFT suggest that for every 1000 women treated with odanacatib for 3 years, odanacatib might be expected to prevent approximately 40 vertebral and eight hip fractures, but could also lead to an increase of four strokes. Furthermore, there were numerically more deaths in the odanacatib group than in the placebo group; however, this difference was not significant. Based on the overall balance between benefit and risk, the study's sponsor announced that they would no longer pursue development of odanacatib for treatment of osteoporosis. Nonetheless, the current findings have important implications for the development of other novel drugs to treat osteoporosis.

Our study had several limitations. Approximately 50% of participants who entered LOFT did not enrol in LOFT Extension. Many of these were ineligible because they were not taking the study drug at the end of LOFT due to excessive bone loss, which occurred more often in the placebo group than the odanacatib group. Because excessive bone loss is a recognised risk factor for morbidity and mortality in older people,[179,180] this result might have led to informative censoring in LOFT Extension. Despite this limitation, analyses that include data from LOFT Extension are presented for completeness because of the substantial number of additional clinical events reported and because these results reflect the long-term profile of treatment with odanacatib. For example, 518 primary endpoint cardiovascular events of cardiovascular death, myocardial infarction, or stroke occurred during LOFT and an additional 226 occurred during LOFT Extension, and the total number of deaths reported was 757 in LOFT and an additional 576 occurred during the extension study. However, despite some baseline characteristics being similar in LOFT and LOFT Extension, between-group

comparison of adverse events based on those reported during LOFT Extension should be interpreted with caution because of the risk of bias in the setting of incomplete patient participation in LOFT Extension. Also, because LOFT was not a dedicated cardiovascular outcomes trial, the detail about a participant's cardiovascular history was less than would be expected for a trial designed to explicitly test this outcome. Another limitation was that the final cardiovascular event adjudication process was initiated during the LOFT Extension and therefore there might not have been enough detailed information surrounding earlier events in some instances (it was not always possible to obtain all documents requested for adjudication). Although no further clinical studies with odanacatib are planned, future biomarker and genetic analyses might determine whether genotypic or phenotypic markers can identify participants for whom the balance between efficacy and safety of odanacatib was more favourable. Such analyses might also yield valuable insight into the pathobiology of the observed increased risk of stroke.

In conclusion, in a trial of more than 16 000 postmenopausal women with osteoporosis followed for up to 5 years, treatment with the cathepsin K inhibitor odanacatib was associated with progressive increases in bone mineral density and reductions in the incidences of vertebral, hip, and non-vertebral fractures. However, treatment also increased the risk of stroke. Further investigation is warranted to understand the pathobiology of this finding, as it might provide insights into overlapping disease pathways in bone and cardiovascular biology that would guide future development of both osteoporosis and cardiovascular disease therapies. However, further development of odanacatib as a potential treatment for patients with osteoporosis was stopped based on the overall balance between benefit and risk.

6.5 Tables

Table 1. Baseline characteristics of the intention-to-treat population

	LOFT		LOFT Extension*	
	Odanacatib (n=8043)	Placebo (n=8028)	Odanacatib (n=4297)	Placebo (n=3960)
Age (years)	72·8 (5·4)	72·9 (5·3)	72·6 (5·2)	72·5 (5·0)
BMI (kg/m²)	25·4 (4·5)	25·5 (4·6)	25·6 (4·5)	25·7 (4·5)
Region†				
Americas	2848 (35·4%)	2827 (35·2%)	1817 (42·3%)	1676 (42·3%)
Asia-Pacific	1378 (17·1%)	1360 (16·9%)	384 (8·9%)	362 (9·1%)
Europe	3817 (47·5%)	3841 (47·8%)	2096 (48·8%)	1922 (48·5%)
BMD T score				
Lumbar spine	−2·68 (1·24)	−2·65 (1·23)	−2·64 (1·21)	−2·58 (1·18)
Total hip	−2·35 (0·70)	−2·35 (0·71)	−2·30 (0·67)	−2·27 (0·70)
Femoral neck	−2·66 (0·52)	−2·66 (0·52)	−2·62 (0·50)	−2·60 (0·50)
Previous vertebral fracture				
Yes	3733 (46·4%)	3737 (46·5%)	2012 (46·8%)	1837 (46·4%)
No	4310 (53·6%)	4291 (53·5%)	2285 (53·2%)	2123 (53·6%)
Serum 25–hydroxyvitamin D (ng/mL)	n=1392; 29·48 (11·49)	n=1416; 30·33 (11·52)	n=702; 31·12 (10·50)	n=641; 31·16 (11·70)
Biochemical markers				
Urine N-telopeptides of type I collagen:creatinine ratio (nmol BCE/mmol)	n=844; 47·31 (41·20)	n=858; 47·38 (33·21)	n=489; 44·44 (25·70)	n=426; 43·32 (25·00)
Serum N-terminal propeptides of type 1 collagen (ng/mL)	n=852; 58·30 (25·95)	n=868; 58·94 (31·38)	n=491; 58·21 (24·74)	n=423; 56·24 (24·46)
Cardiovascular risk factors				
Current or previous smoker	1962 (24·4%)	1919 (23·9%)	NA	NA
Hypertension	4426 (55·0%)	4554 (56·7%)	NA	NA
Dyslipidaemia	3281 (40·8%)	3267 (40·7%)	NA	NA
Diabetes	938 (11·7%)	921 (11·5%)	NA	NA
Known coronary artery disease	946 (11·8%)	999 (12·4%)	NA	NA
History of heart failure	202 (2·5%)	2017 (2·6%)	NA	NA
History of stroke or TIA	281 (3·5%)	298 (3·7%)	NA	NA
Family history of cardiovascular disease	2687 (33·4%)	2716 (33·8%)	NA	NA
eGFR <60 mL/min per 1·73m² (CKD-EPI)	1798 (22·4%)	1870 (23·4%)	NA	NA
LDL-cholesterol (mg/dL)	122 (98–148)	123 (99–148)	NA	NA
Baseline medication use			NA	NA
Antiplatelet	1688 (21·0%)	1721 (21·4%)	NA	NA
Statin	1595 (19·8%)	1527 (19·0%)	NA	NA
Anticoagulant	106 (1·3%)	109 (1·4%)	NA	NA

BMD=bone mineral density. BCE=bone collagen equivalents. NA=not available. TIA=transient ischaemic attack. eGFR=estimated glomerular filtration rate. CKD-EPI=Chronic Kidney Disease Epidemiology Collaboration. *Baseline characteristics are data from the time of randomisation in LOFT. †All study sites in India (19 centres) and Peru (10 centres) did not participate in LOFT Extension because the protocol did not receive regulatory approval there. The single study site in Lebanon also did not participate in LOFT Extension because of resourcing issues. An additional 104 study sites (in 24 countries) chose not to participate in LOFT Extension.

Table 2. Effect of odanacatib on the risk of fractures

	LOFT			LOFT plus LOFT Extension		
	Odanacatib (n=8043)	Placebo (n=8028)	Hazard ratio (95% CI)	Odanacatib (n=8043)	Placebo (n=8028)	Hazard ratio (95% CI)
Primary endpoints*						
Radiographic vertebral fracture†	251 (3·7%)‡	542 (7·8%)‡	0·46 (0·40–0·53)§	341 (4·9%)‖	675 (9·6%)‖	0·48 (0·42–0·55)§
Hip fracture¶	65 (0·8%)	125 (1·6%)	0·53 (0·39–0·71)§	86 (1·1%)	162 (2·0%)	0·52 (0·40–0·67)§
Non-vertebral fracture¶	412 (5·1%)	541 (6·7%)	0·77 (0·68–0·87)§	512 (6·4%)	675 (8·4%)	0·74 (0·66–0·83)§
Secondary endpoints						
Clinical vertebral fracture¶	37 (0·5%)	133 (1·7%)	0·28 (0·19–0·40)§	55 (0·7%)	162 (2·0%)	0·33 (0·24–0·45)§
Any clinical fracture¶	445 (5·5%)	662 (8·2%)	0·67 (0·60–0·76)§	561 (7·0%)	812 (10·1%)	0·67 (0·60–0·75)§

Data are n (%) except where stated otherwise. Traumatic fractures were excluded from the analysis (74 and 83 fractures for odanacatib and placebo, respectively, in LOFT; and 100 and 105 fractures for odanacatib and placebo, respectively, in LOFT plus LOFT Extension). *If the p values for all three primary endpoints were lower than their corresponding boundaries via the α-spending function ($p \leq 0.008$ at the first interim analysis), the data monitoring committee could recommend early stopping of the trial for efficacy. †Interval censored analysis: generalised linear model for binary data with cloglog link and terms for time interval, treatment, stratum, and geographic region. ‡In LOFT, there were 6770 participants in the odanacatib group and 6910 in the placebo group with both baseline and at least one post-baseline evaluable radiograph values for determination of radiographic vertebral fractures. §$p<0.0001$. ¶Cox proportional hazards model with terms for treatment, stratum, and geographic region. ‖In LOFT plus LOFT Extension, there were 6909 participants in the odanacatib group and 7011 in the placebo group with evaluable radiographs available at baseline and at least one other timepoint for determination of radiographic vertebral fractures.

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Table 3. Summary of adverse events

	LOFT			LOFT plus LOFT Extension		
	Odanacatib (n=8043)	Placebo (n=8028)	Estimated difference in rates per 100 patient-years (odanacatib minus placebo)	Odanacatib (n=8043)	Placebo (n=8028)	Estimated difference in rates per 100 patient-years (odanacatib minus placebo)
All	6893 (85·7%)	6887 (85·8%)	1·52 (–1·80 to 4·84)	7101 (88·3%)	7084 (88·2%)	0·88 (–2·30 to 4·07)
Serious	1907 (23·7%)	1962 (24·4%)	–0·19 (–0·79 to 0·41)	2440 (30·3%)	2444 (30·4%)	–0·22 (–0·77 to 0·32)
Leading to discontinuation of blinded study drug	662 (8·2%)	611 (7·6%)	0·27 (–0·04 to 0·58)	777 (9·7%)	730 (9·1%)	0·11 (–0·16 to 0·37)
Adjudication confirmed						
Femoral shaft fracture*	22 (0·3%)	13 (0·2%)	0·04 (–0·01 to 0·09)	26 (0·3%)	7 (0·1 %)	0·06 (0·03 to 0·11)
Atypical femoral shaft fracture†	5 (0·1%)	0	0·02 (0·01 to 0·05)	10 (0·1%)	0	0·03 (0·02 to 0·06)
Delayed fracture union‡	10 (2·1%)	16 (2·6%)	–0·16 (–0·78 to 0·50)	18 (3·1%)	18 (2·4%)	0·15 (–0·29 to 0·64)
Osteonecrosis of the jaw	0	0	0 (-0.01 to 0.01)	0	0	0 (-0.02 to 0.02)
Morphea-like skin lesion	12§ (0·1%)	3 (<0·1%)	0·04 (0·01 to 0·08)	13§ (0·2%)	3 (<0·1%)	0·03 (0·01 to 0·07)
Systemic sclerosis	2 (<0·1%)	1 (<0·1%)	0·00 (–0·02 to 0·03)	2 (<0·1%)	1 (<0·1%)	0·00 (–0·01 to 0·02)
Serious respiratory infection	101 (1·3%)	123 (1·5%)	–0·09 (–0·22 to 0·04)	129 (1·6%)	147 (1·8%)	–0·07 (–0·18 to 0·04)

Data are n (%) or estimated difference (95% CI). *Shaft indicates subtrochanteric/femoral shaft. †All femoral fractures with atypical features met both American Society for Bone and Mineral Research 2010 and 2013 Task Force criteria. ‡473 patients in the odanacatib group and 611 in the placebo group in LOFT; and 588 patients in the odanacatib group and 761 in the placebo group in LOFT plus LOFT Extension. §A 13th case of morphea with odanacatib occurred during LOFT but was reported after the initial database lock so was not included in the analysis of LOFT.

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Table 4. Pre-specified cardiovascular endpoints of interest

	LOFT					LOFT plus LOFT Extension				
	Odanacatib (n=8043)	Placebo (n=8028)	Hazard ratio (95% CI)	p value	Risk difference (95% CI)	Odanacatib (n=8043)	Placebo (n=8028)	Hazard ratio (95% CI)	p value	Risk difference (95% CI)
Primary cardiovascular endpoints										
Cardiovascular death, myocardial infarction, or stroke	273 (3·4%)	245 (3·1%)	1·12 (0·95–1·34)	0·18	0·3 (–0·20 to 0·89)	401 (5·0%)	343 (4·3%)	1·17 (1·02–1·36)	0·029	0·7 (0·06 to 1·37)
New onset (or presumed new onset) atrial fibrillation or atrial flutter	112 (1·4%)	96 (1·2%)	1·18 (0·90–1·55)	0·24	0·2 (–0·15 to 0·55)	164 (2·0%)	141 (1·8%)	1·17 (0·93–1·46)	0·18	0·2 (–0·14 to 0·71)
Secondary cardiovascular endpoints										
Four-point MACE	293 (3·6%)	264 (3·3%)	1·12 (0·95 to 1·32)	0·181	0·3 (–0·21 to 0·92)	422 (5·2%)	371 (4·6%)	1·14 (0·99–1·31)	0·062	0·6 (–0·04 to 1·30)
Cardiovascular death	115 (1·4%)	99 (1·2%)	1·16 (0·89 to 1·52)	0·28	0·2 (–0·16 to 0·56)	185 (2·3%)	164 (2·0%)	1·13 (0·92–1·40)	0·25	0·3 (–0·19 to 0·71)
Myocardial infarction	60 (0·7%)	74 (0·9%)	0·82 (0·58 to 1·15)	0·26	–0·2 (–0·46 to 0·11)	84 (1·0%)	90 (1·1%)	0·94 (0·70–1·26)	0·67	–0·1 (–0·40 to 0·25)
Stroke*	136 (1·7%)	104 (1·3%)	1·32 (1·02 to 1·70)	0·034	0·4 (0·02 to 0·78)	187 (2·3%)	137 (1·7%)	1·37 (1·10–1·71)	0·0051	0·6 (0·18 to 1·06)
Ischaemic stroke	107 (1·3%)	81 (1·0%)	1·33 (1·00 to 1·78)	0·052	0·3 (–0·01 to 0·66)	143 (1·8%)	102 (1·3%)	1·41 (1·09–1·81)	0·0084	0·5 (0·13 to 0·89)
Haemorrhagic stroke	14 (0·2%)	9 (0·1%)	1·56 (0·68 to 3·61)	0·30	0·1 (–0·06 to 0·19)	20 (0·2%)	16 (0·2%)	1·25 (0·65–2·41)	0·51	0·0 (–0·10 to 0·21)
Undetermined stroke	17 (0·2%)	15 (0·2%)	1·14 (0·57 to 2·29)	0·70	0·0 (–0·12 to 0·17)	27 (0·3%)	20 (0·2%)	1·35 (0·76–2·41)	0·31	0·1 (–0·08 to 0·26)
Admission to hospital for unstable angina	23 (0·3%)	22 (0·3%)	1·06 (0·59 to 1·89)	0·86	0·0 (–0·16 to 0·18)	25 (0·3%)	32 (0·4%)	0·79 (0·47–1·33)	0·37	–0·1 (–0·28 to 0·099)
New onset (or presumed new onset) atrial fibrillation (ECG confirmed only†)	60 (0·7%)	55 (0·7%)	1·10 (0·76 to 1·59)	0·61	0·0 (–0·20 to 0·33)	88 (1·1%)	72 (0·9%)	1·23 (0·90–1·68)	0·20	0·2 (–0·11 to 0·51)
New or recurrent episode of atrial fibrillation or atrial flutter	141 (1·8%)	114 (1·4%)	1·25 (0·98 to 1·60)	0·074	0·4 (–0·05 to 0·72)	199 (2·5%)	164 (2·0%)	1·22 (0·99–1·50)	0·059	0·5 (–0·03 to 0·89)
All-cause death	401 (5·0%)	356 (4·4%)	1·13 (0·98 to 1·30)	0·10	0·6 (–0·10 to 1·21)	682 (8·5%)	651 (8·1%)	1·05 (0·95–1·17)	0·34	0·4 (–0·48 to 1·22)

Data are n (%) unless otherwise stated. Four-point MACE included cardiovascular death, myocardial infarction, stroke, or admission to hospital for unstable angina. MACE=major adverse cardiovascular events. ECG=electrocardiogram. *Each individual row in the table reflects the complete number of first events for that particular event type. Some participants had more than one stroke type (eg, ischaemic and haemorrhagic) and, therefore, the sum might exceed the total number of strokes. †ECG or similar electrical tracing was available and provided to the clinical events committee to confirm an episode of atrial fibrillation or atrial flutter.

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6.6 Figures

Figure 1. Trial profile

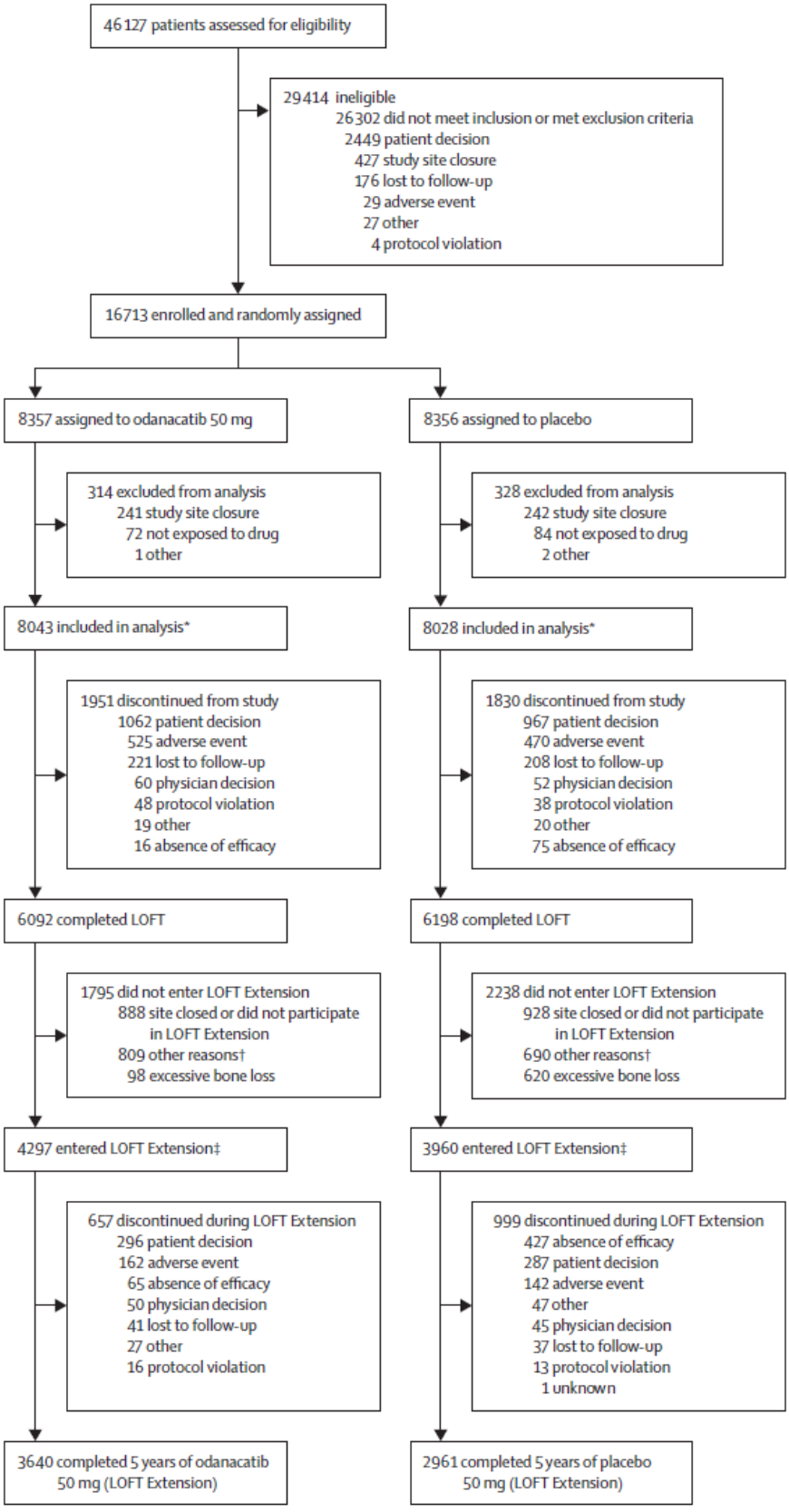
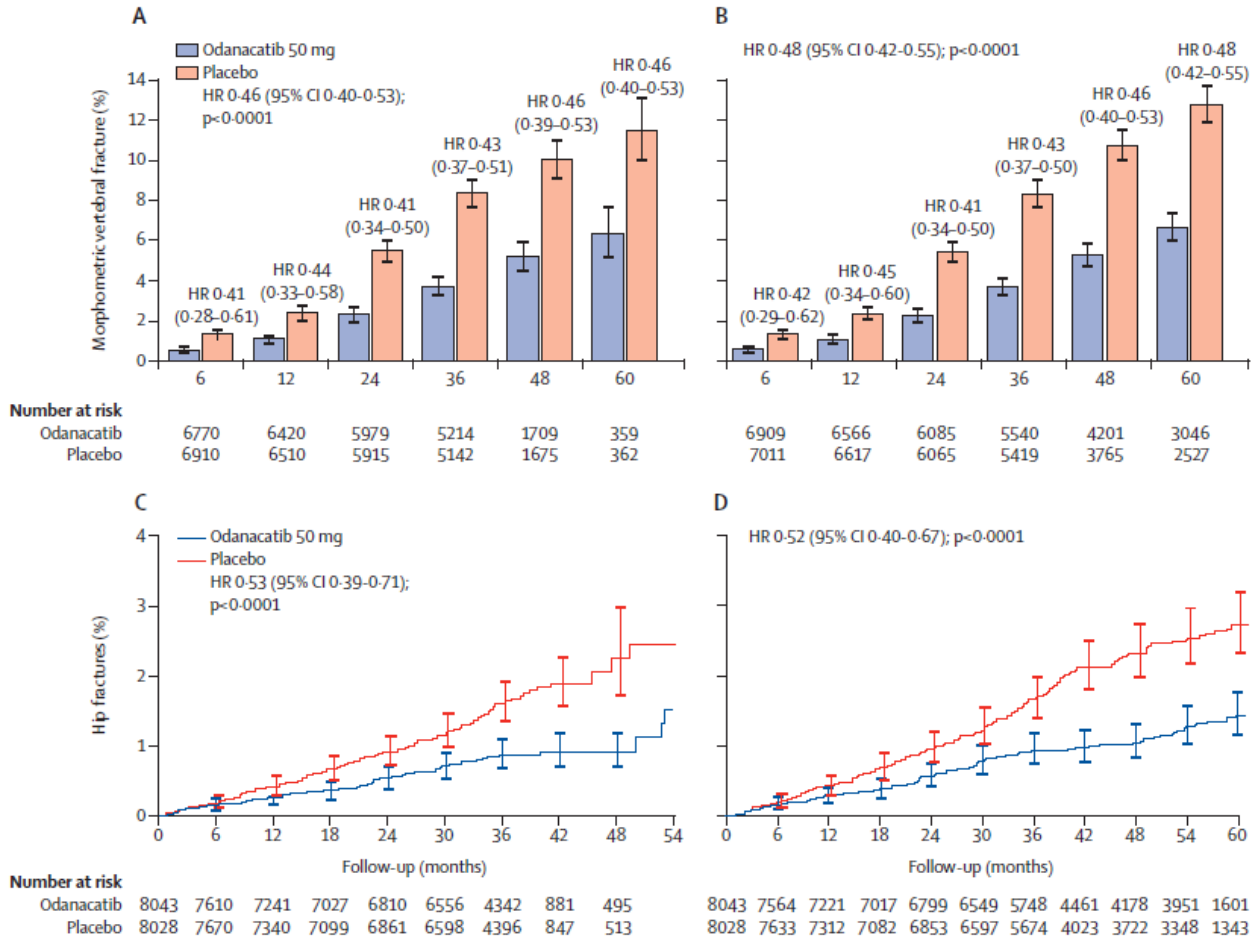
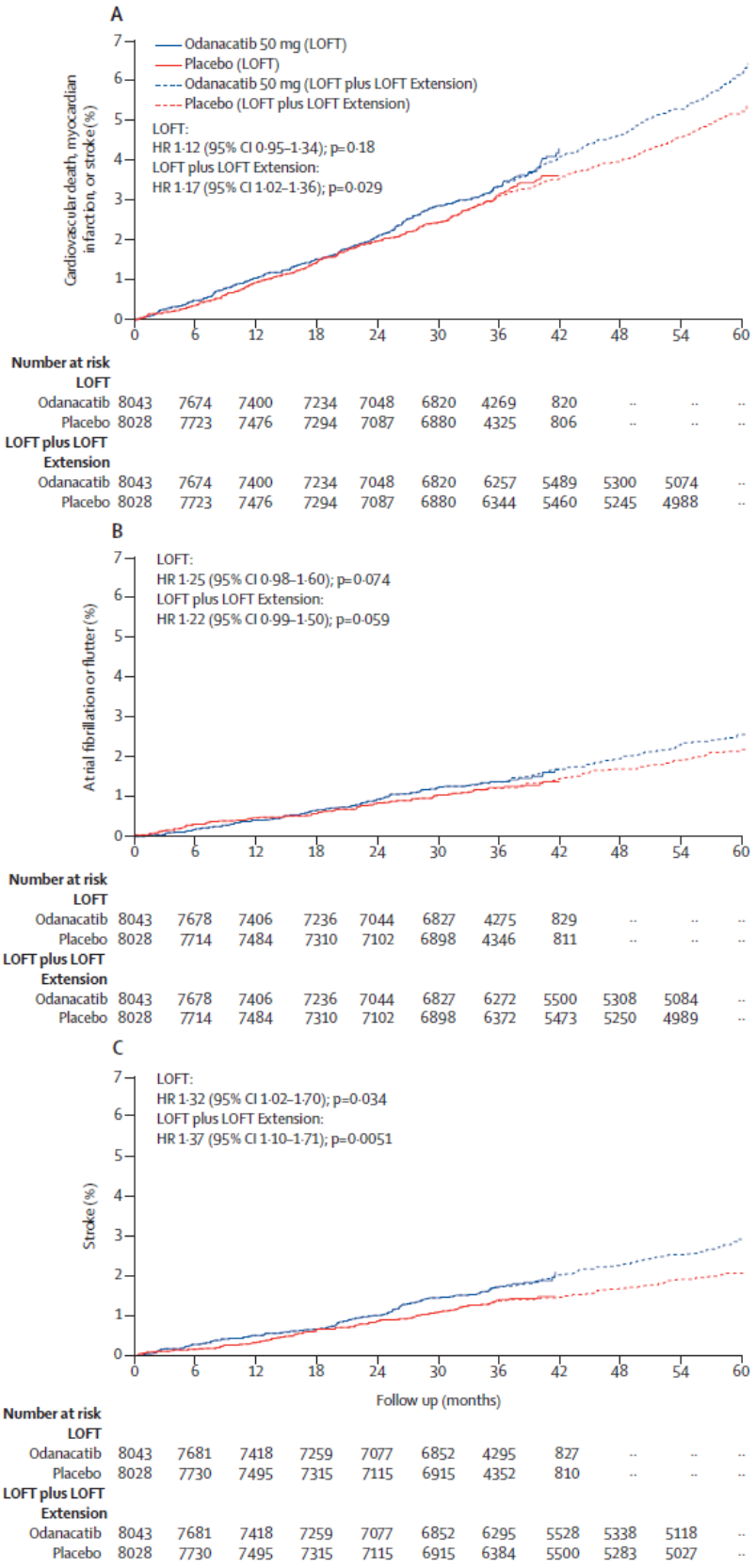


Figure 2. Vertebral and hip fractures



Cumulative incidence and life-table estimates of time to incident new or worsening radiographic vertebral fracture shown in (A) for LOFT and (B) for LOFT plus LOFT Extension. Cumulative incidence curves for incident hip fracture shown in (C) for LOFT and (D) for LOFT plus LOFT Extension. HR=hazard ratio.

Figure 3. Cardiovascular events



Cumulative incidence during LOFT (solid lines) and LOFT plus LOFT Extension (dashed lines) for (A) cardiovascular death, myocardial infarction, or stroke; (B) new or recurrent atrial fibrillation or atrial flutter; and (C) fatal or non-fatal stroke.

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List of full publications during the three-year Ph.D. course

1. Patti G, Pengo V, Marcucci R, Cirillo P, Renda G, Santilli F, Calabrò P, De Caterina AR, Cavallari I, Ricottini E, Parato VM, Zoppellaro G, Di Gioia G, Sedati P, Cicchitti V, Davì G, Golia E, Pariggiano I, Simeone P, Abbate R, Prisco D, Zimarino M, Sofi F, Andreotti F, De Caterina R; Working Group of Thrombosis of the Italian Society of Cardiology. The left atrial appendage: from embryology to prevention of thromboembolism. Eur Heart J. 2017:38(12):877-87.

2. Cavallari I, Bonaca MP. Antiplatelet Therapy for Secondary Prevention After Acute Myocardial Infarction. Interv Cardiol Clin. 2017;6:119-129.

3. Patti G, Di Gioia G, Cavallari I, Nenna A. Safety and efficacy of nonvitamin K antagonist oral anticoagulants versus warfarin in diabetic patients with atrial fibrillation: A study-level meta-analysis of phase III randomized trials. Diabetes Metab Res Rev. 2017; 33 (3).

4. Patti G, Lucerna M, Cavallari I, Ricottini E, Renda G, Pecen L, Romeo F, Le Heuzey JY, Zamorano JL, Kirchhof P, De Caterina R. Insulin-Requiring Versus Noninsulin-Requiring Diabetes and Thromboembolic Risk in Patients With Atrial Fibrillation: PREFER in AF. J Am Coll Cardiol. 2017;69(4):409-419.

5. Patti G, Lucerna M, Pecen L, Siller-Matula JM, Cavallari I, Kirchhof P, De Caterina R. Thromboembolic Risk, Bleeding Outcomes and Effect of Different Antithrombotic Strategies in Very Elderly Patients With Atrial Fibrillation: A Sub-Analysis From the PREFER in AF (PREvention oF Thromboembolic Events-European Registry in Atrial Fibrillation). J Am Heart Assoc. 2017;6(7).

6. Patti G, Cavallari I, Antonucci E, Calabrò P, Cirillo P, Gresele P, Palareti G, Pengo V, Pignatelli P, Ricottini E, Marcucci R. Prevalence and Predictors of Dual Antiplatelet Therapy Prolongation Beyond One Year in Patients with Acute Coronary Syndrome. Plos One 2017;12(10):e0186961.

7. Cavallari I, Morrow DA, Creager MA, Olin J, Bhatt DL, Steg PG, Storey RF, Cohen M, Scirica BS, Piazza G, Goodrich EL, Braunwald E, Sabatine MS, Bonaca MP. Frequency, Predictors, and Impact of Combined Antiplatelet Therapy on Venous Thromboembolism in Patients With Symptomatic Atherosclerosis. Circulation. 2018 Feb 13;137(7):684-692.

8. Cavallari I, Patti G. Meta-Analysis Comparing the Safety and Efficacy of Dual Versus Triple Antithrombotic Therapy in Patients With Atrial Fibrillation Undergoing Percutaneous Coronary Intervention. Am J Cardiol. 2018 Mar 15;121(6):718-724.

9. Cavallari I, Ruff CT, Nordio F, Deenadayalu N, Shi M, Lanz H, Rutman H, Mercuri MF, Antman EM, Braunwald E, Giugliano RP. Clinical events after interruption of anticoagulation in patients with atrial fibrillation: An analysis from the ENGAGE AF-TIMI 48 trial. Int J Cardiol. 2018 Apr 15;257:102-107.

10. Mangiacapra F, Colaiori I, Ricottini E, Creta A, Di Gioia G, Cavallari I, Bressi E, Capuano M, Barbato E, Di Sciascio G. Impact of platelet reactivity on 5-year clinical outcomes following percutaneous coronary intervention: a Landmark analysis. J Thromb Thrombolysis. 2018 May;45(4):496-503.

11. Cavallari I, Cannon CP, Braunwald E, Goodrich EL, Im K, Lukas MA, O'Donoghue ML. Metabolic syndrome and the risk of adverse cardiovascular events after an acute coronary syndrome. Eur J Prev Cardiol. 2018 May;25(8):830-838.

12. Cavallari I, Patti G. Clinical effects with inhibition of multiple coagulative pathways in patients admitted for acute coronary syndrome. Intern Emerg Med. 2018 Mar 21. doi: 10.1007/s11739-018-1834-x. [Epub ahead of print]

13. Ricottini E, Mangiacapra F, Nusca A, Melfi R, Cavallari I, Miglionico M, Gallo P, Pozzilli P, Di Sciascio G. Relation of Platelet Indexes to Platelet Reactivity and Periprocedural Myocardial Infarction in Patients Who Underwent Percutaneous Coronary Angioplasty. Am J Cardiol. 2018 May 1;121(9):1027-1031.

14. Maddaloni E, Cavallari I, Napoli N, Conte C. Vitamin D and Diabetes Mellitus. Front Horm Res. 2018;50:161-176.

15. Patti G, Cavallari I, Hanon O, De Caterina R. The safety and efficacy of non-vitamin K antagonist oral anticoagulants in atrial fibrillation in the elderly. Int J Cardiol. 2018 Aug 15;265:118-124.

16. Patti G, Ricottini E, Nenna A, Cavallari I, Antonucci E, Calabrò P, Cirillo P, Gresele P, Palareti G, Pengo V, Pignatelli P, Bisignani A, Marcucci R. Impact of Chronic Renal Failure on Ischemic and Bleeding Events at 1 Year in Patients With Acute Coronary Syndrome

(from the Multicenter START ANTIPLATELET Registry). Am J Cardiol. 2018 Sep 15;122(6):936-943.

17. Patti G, Cavallari I, Andreotti F, Calabrò P, Cirillo P, Denas G, Galli M, Golia E, Maddaloni E, Marcucci R, Parato VM, Pengo V, Prisco D, Ricottini E, Renda G, Santilli F, Simeone P, De Caterina R; Working Group on Thrombosis of the Italian Society of Cardiology. Prevention of atherothrombotic events in patients with diabetes mellitus: from antithrombotic therapies to new-generation glucose-lowering drugs. Nat Rev Cardiol. 2019 Feb;16(2):113-130.

18. Cavallari I, Patti G. Efficacy and safety of oral anticoagulation in elderly patients with atrial fibrillation. Anatol J Cardiol. 2018 Jan;19(1):67-71.

19. Cavallari I, Patti G. Author`s Reply. Anatol J Cardiol. 2018 Apr;19(4):292.

20. Patti G, Cavallari I. [Antithrombotic approaches in patients undergoing left atrial appendage occlusion: current evidence and future perspectives]. G Ital Cardiol (Rome). 2019 Mar;20(3):23-27.

21. Bressi E, Mangiacapra F, Ricottini E, Cavallari I, Colaiori I, Di Gioia G, Creta A, Capuano M, Viscusi MM, Di Sciascio G. Impact of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio on 5-Year Clinical Outcomes of Patients with Stable Coronary Artery Disease Undergoing Elective Percutaneous Coronary Intervention. J Cardiovasc Transl Res. 2018 Dec;11(6):517-523.

22. Cavallari I, Delli Veneri A, Maddaloni E, Melfi R, Patti G, Napoli N, Pozzilli P, Di Sciascio G. Comparison of Lipid-Lowering Medications and Risk for Cardiovascular Disease in Diabetes. Curr Diab Rep. 2018 Oct 29;18(12):138.

23. Cavallari I, Maddaloni E. Cardiovascular effects of SGLT-2 inhibitors: What we have learned from cardiovascular outcome trials and what we still need to understand. Diabetes Metab Res Rev. 2019 May;35(4):e3124.

24. Calabrò P, Moscarella E, Gragnano F, Cesaro A, Pafundi PC, Patti G, Cavallari I, Antonucci E, Cirillo P, Pignatelli P, Palareti G, Sasso FC, Pengo V, Gresele P, Marcucci R; START-ANTIPLATELET collaborators, Conte M, Fimiani F, Di Serafino L, Del Pinto M, Denas G, Pastori D, Eleonora C, Fierro T. Effect of Body Mass Index on Ischemic and Bleeding

Events in Patients Presenting With Acute Coronary Syndromes (from the START-ANTIPLATELET Registry). Am J Cardiol. 2019;124(11):1662-1668.

25. McClung MR, O'Donoghue ML, Papapoulos SE, Bone H, Langdahl B, Saag KG, Reid IR, Kiel DP, Cavallari I, Bonaca MP, Wiviott SD, de Villiers T, Ling X, Lippuner K, Nakamura T, Reginster JY, Rodriguez-Portales JA, Roux C, Zanchetta J, Zerbini CAF, Park JG, Im K, Cange A, Grip LT, Heyden N, DaSilva C, Cohn D, Massaad R, Scott BB, Verbruggen N, Gurner D, Miller DL, Blair ML, Polis AB, Stoch SA, Santora A, Lombardi A, Leung AT, Kaufman KD, Sabatine MS; LOFT Investigators. Odanacatib for the treatment of postmenopausal osteoporosis: results of the LOFT multicentre, randomised, double-blind, placebo-controlled trial and LOFT Extension study. Lancet Diabetes Endocrinol. 2019 Dec;7(12):899-911.

26. Calabrò P, Gragnano F, Cesaro A, Pariggiano I, Patti G, Cavallari I, Parato VM, Renda G, De Caterina R; a nome del Gruppo di Studio sulla Trombosi della Società Italiana di Cardiologia. [Atrial and left atrial appendage thrombosis in patients with atrial fibrillation: from pathophysiology to treatment]. G Ital Cardiol (Rome). 2019 Jun;20(6):374-383.

27. Mangiacapra F, Bressi E, Colaiori I, Ricottini E, Cavallari I, Capuano M, Viscusi MM, Spoto S, Barbato E, Di Sciascio G. Interaction Between Diabetes Mellitus and Platelet Reactivity in Determining Long-Term Outcomes Following Percutaneous Coronary Intervention. J Cardiovasc Transl Res. 2019 Nov 15. doi:10.1007/s12265-019-09931-z. [Epub ahead of print]

28. Cavallari I, Verolino G, Romano S, Patti G. Efficacy and Safety of Non-Vitamin K Oral Anticoagulants in Patients With Atrial Fibrillation and Cancer: a Study-Level Meta-Analysis. Thromb Haemost 2020 Feb;120(2):314-321.

29. Cavallari I, Patti G. Primum non nocere does not justify clinical inertia for stroke prevention in elderly patients with atrial fibrillation in the era of direct oral anticoagulants. Eur J Intern Med. 2020 Jan;71:11-12.

Book chapters

1. Patti G, Cavallari I, Kaski JC. Atrial Fibrillation and Percutaneous Coronary Intervention. Case-based Recommendations on Oral Anticoagulation, Antiplatelet Therapy and Stenting. Editors: Gregory YH Lip and Andrea Rubboli. Chapter 10. Urgent surgery early after

coronary stenting in a patient with atrial fibrillation on triple therapy of NOAC (a factor Xa-inhibitor), aspirin and clopidogrel.

2. Cavallari I, Bonaca MP. Antiplatelet and Anticoagulation Therapy In PCI, An Issue of Interventional Cardiology Clinics, 1st Edition. Elsevier. Editor-in-Chief: Matthew J. Price, Editors: Matthew J. Price and Dominick J. Angiolillo. Chapter 10. Antiplatelet Therapy for Secondary Prevention After Acute Myocardial Infarction.

References

1 Colwell JA, Lopes-Virella M, Halushka P V. Pathogenesis of atherosclerosis in diabetes mellitus. *Diabetes Care*; **4**: 121–33.

2 Ferreiro JL, Angiolillo DJ. Diabetes and antiplatelet therapy in acute coronary syndrome. *Circulation* 2011; **123**: 798–813.

3 Blache D, Bourdon E, Salloignon P, *et al.* Glycated albumin with loss of fatty acid binding capacity contributes to enhanced arachidonate oxygenation and platelet hyperactivity: relevance in patients with type 2 diabetes. *Diabetes* 2015; **64**: 960–72.

4 Sagel J, Colwell JA, Crook L, Laimins M. Increased platelet aggregation in early diabetus mellitus. *Ann Intern Med* 1975; **82**: 733–8.

5 Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost* 2004; **2**: 1282–91.

6 Eibl N, Krugluger W, Streit G, Schrattbauer K, Hopmeier P, Schernthaner G. Improved metabolic control decreases platelet activation markers in patients with type-2 diabetes. *Eur J Clin Invest* 2004; **34**: 205–9.

7 Vazzana N, Ranalli P, Cuccurullo C, Davì G. Diabetes mellitus and thrombosis. *Thromb Res* 2012; **129**: 371–7.

8 Yudkin JS. Abnormalities of coagulation and fibrinolysis in insulin resistance. Evidence for a common antecedent? *Diabetes Care* 1999; **22 Suppl 3**: C25-30.

9 Alzahrani SH, Ajjan RA. Coagulation and fibrinolysis in diabetes. *Diabetes Vasc Dis Res* 2010; **7**: 260–73.

10 Andreotti F, Coluzzi G, Pafundi T, *et al.* Anemia contributes to cardiovascular disease through reductions in nitric oxide. *J Appl Physiol* 2017; **122**: 414–7.

11 Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, *et al.* Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet (London, England)* 2009; **373**: 1849–60.

12 Collaborative overview of randomised trials of antiplatelet therapy--I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. *BMJ* 1994; **308**: 81–106.

13 CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet (London, England)* 1996; **348**: 1329–39.

14 Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W, Topol EJ. Amplified benefit of

clopidogrel versus aspirin in patients with diabetes mellitus. *Am J Cardiol* 2002; **90**: 625–8.

15 Bhatt DL, Flather MD, Hacke W, *et al.* Patients with prior myocardial infarction, stroke, or symptomatic peripheral arterial disease in the CHARISMA trial. *J Am Coll Cardiol* 2007; **49**: 1982–8.

16 Dasgupta A, Steinhubl SR, Bhatt DL, *et al.* Clinical outcomes of patients with diabetic nephropathy randomized to clopidogrel plus aspirin versus aspirin alone (a post hoc analysis of the clopidogrel for high atherothrombotic risk and ischemic stabilization, management, and avoidance [CHARISMA] tri. *Am J Cardiol* 2009; **103**: 1359–63.

17 Steinhubl SR, Berger PB, Mann JT, *et al.* Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA* 2002; **288**: 2411–20.

18 Patti G, Colonna G, Pasceri V, Pepe LL, Montinaro A, Di Sciascio G. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the ARMYDA-2 (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty). *Circulation* 2005; **111**: 2099–106.

19 Taubert D, Kastrati A, Harlfinger S, *et al.* Pharmacokinetics of clopidogrel after administration of a high loading dose. *Thromb Haemost* 2004; **92**: 311–6.

20 Gargiulo G, Windecker S, da Costa BR, *et al.* Short term versus long term dual antiplatelet therapy after implantation of drug eluting stent in patients with or without diabetes: systematic review and meta-analysis of individual participant data from randomised trials. *BMJ* 2016; **355**: i5483.

21 Connolly SJ, Eikelboom JW, Bosch J, *et al.* Rivaroxaban with or without aspirin in patients with stable coronary artery disease: an international, randomised, double-blind, placebo-controlled trial. *Lancet (London, England)* 2017; published online Nov 10. DOI:10.1016/S0140-6736(17)32458-3.

22 Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 2002; **105**: 1650–5.

23 CURRENT-OASIS 7 Investigators, Mehta SR, Bassand J-P, *et al.* Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. *N Engl J Med* 2010; **363**: 930–42.

24 Yusuf S, Zhao F, Mehta SR, *et al.* Effects of Clopidogrel in Addition to Aspirin in Patients with Acute Coronary Syndromes without ST-Segment Elevation. *N Engl J Med* 2001; **345**: 494–502.

25 Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, *et al.* Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; **54**: 2430–5.

26 Mangiacapra F, Patti G, Peace A, *et al.* Comparison of platelet reactivity and periprocedural outcomes in patients with versus without diabetes mellitus and treated with clopidogrel and percutaneous coronary intervention. *Am J Cardiol* 2010; **106**: 619–23.

27 Patti G, Proscia C, Di Sciascio G. Antiplatelet therapy in patients with diabetes mellitus and acute coronary syndrome. *Circ J* 2014; **78**: 33–41.

28 Ueno M, Ferreiro JL, Tomasello SD, *et al.* Functional profile of the platelet $P2Y_{12}$ receptor signalling pathway in patients with type 2 diabetes mellitus and coronary artery disease. *Thromb Haemost* 2011; **105**: 730–2.

29 Angiolillo DJ, Jakubowski JA, Ferreiro JL, *et al.* Impaired responsiveness to the platelet P2Y12 receptor antagonist clopidogrel in patients with type 2 diabetes and coronary artery disease. *J Am Coll Cardiol* 2014; **64**: 1005–14.

30 Angiolillo DJ, Badimon JJ, Saucedo JF, *et al.* A pharmacodynamic comparison of prasugrel vs. high-dose clopidogrel in patients with type 2 diabetes mellitus and coronary artery disease: results of the Optimizing anti-Platelet Therapy In diabetes MellitUS (OPTIMUS)-3 Trial. *Eur Heart J* 2011; **32**: 838–46.

31 Laine M, Frère C, Toesca R, *et al.* Ticagrelor versus prasugrel in diabetic patients with an acute coronary syndrome. A pharmacodynamic randomised study. *Thromb Haemost* 2014; **111**: 273–8.

32 Wiviott SD, Braunwald E, Angiolillo DJ, *et al.* Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial I. *Circulation* 2008; **118**: 1626–36.

33 Roe MT, Armstrong PW, Fox KAAA, *et al.* Prasugrel versus Clopidogrel for Acute Coronary Syndromes without Revascularization. *N Engl J Med* 2012; **367**: 1297–309.

34 Dalby AJ, Gottlieb S, Cyr DD, *et al.* Dual antiplatelet therapy in patients with diabetes and acute coronary syndromes managed without revascularization. *Am Heart J* 2017; **188**: 156–66.

35 James S, Angiolillo DJ, Cornel JH, *et al.* Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATelet inhibition and patient Outcomes (PLATO) trial. *Eur Heart J* 2010; **31**: 3006–16.

36 Franchi F, Rollini F, Aggarwal N, *et al.* Pharmacodynamic Comparison of Prasugrel Versus

Ticagrelor in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease: The OPTIMUS (Optimizing Antiplatelet Therapy in Diabetes Mellitus)-4 Study. *Circulation* 2016; **134**: 780–92.

37 Schulz S, Angiolillo DJ, Antoniucci D, *et al.* Randomized comparison of ticagrelor versus prasugrel in patients with acute coronary syndrome and planned invasive strategy--design and rationale of the iNtracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment (ISAR-REA. *J Cardiovasc Transl Res* 2014; **7**: 91–100.

38 Mauri L, Kereiakes DJ, Yeh RW, *et al.* Twelve or 30 months of dual antiplatelet therapy after drug-eluting stents. *N Engl J Med* 2014; **371**: 2155–66.

39 Meredith IT, Tanguay J-F, Kereiakes DJ, *et al.* Diabetes Mellitus and Prevention of Late Myocardial Infarction After Coronary Stenting in the Randomized Dual Antiplatelet Therapy Study. *Circulation* 2016; **133**: 1772–82.

40 Bhatt DL, Bonaca MP, Bansilal S, *et al.* Reduction in Ischemic Events With Ticagrelor in Diabetic Patients With Prior Myocardial Infarction in PEGASUS-TIMI 54. *J Am Coll Cardiol* 2016; **67**: 2732–40.

41 Huxley RR, Filion KB, Konety S, Alonso A. Meta-analysis of cohort and case-control studies of type 2 diabetes mellitus and risk of atrial fibrillation. *Am J Cardiol* 2011; **108**: 56–62.

42 Fatemi O, Yuriditsky E, Tsioufis C, *et al.* Impact of intensive glycemic control on the incidence of atrial fibrillation and associated cardiovascular outcomes in patients with type 2 diabetes mellitus (from the Action to Control Cardiovascular Risk in Diabetes Study). *Am J Cardiol* 2014; **114**: 1217–22.

43 Kirchhof P, Benussi S, Kotecha D, *et al.* 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J* 2016; **37**: 2893–962.

44 Stroke Risk in Atrial Fibrillation Working Group. Independent predictors of stroke in patients with atrial fibrillation: a systematic review. *Neurology* 2007; **69**: 546–54.

45 Ashburner JM, Go AS, Chang Y, *et al.* Effect of Diabetes and Glycemic Control on Ischemic Stroke Risk in AF Patients: ATRIA Study. *J Am Coll Cardiol* 2016; **67**: 239–47.

46 Patti G, Lucerna M, Cavallari I, *et al.* Insulin-Requiring Versus Noninsulin-Requiring Diabetes and Thromboembolic Risk in Patients With Atrial Fibrillation: PREFER in AF. *J Am Coll Cardiol* 2017; **69**. DOI:10.1016/j.jacc.2016.10.069.

47 Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. *Ann Intern Med* 2007; **146**: 857–67.

48 Ruff CT, Giugliano RP, Braunwald E, *et al.* Comparison of the efficacy and safety of new

oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. *Lancet (London, England)* 2014; **383**: 955–62.

49 Itzhaki Ben Zadok O, Eisen A. Use of non-vitamin K oral anticoagulants in people with atrial fibrillation and diabetes mellitus. *Diabet Med* 2018; **35**: 548–56.

50 Patti G, Di Gioia G, Cavallari I, Nenna A. Safety and efficacy of nonvitamin K antagonist oral anticoagulants versus warfarin in diabetic patients with atrial fibrillation: A study-level meta-analysis of phase III randomized trials. *Diabetes Metab Res Rev* 2017; **33**. DOI:10.1002/dmrr.2876.

51 Federation ID. No Title. In: IDF Diabetes Atlas, 8th edn. Brussels, Belgium, 2017.

52 Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. *JAMA* 1999; **281**: 1291–7.

53 Emerging Risk Factors Collaboration, Di Angelantonio E, Kaptoge S, *et al.* Association of Cardiometabolic Multimorbidity With Mortality. *JAMA* 2015; **314**: 52–60.

54 Cavender MA, Steg PG, Smith SC, *et al.* Impact of Diabetes Mellitus on Hospitalization for Heart Failure, Cardiovascular Events, and Death: Outcomes at 4 Years From the Reduction of Atherothrombosis for Continued Health (REACH) Registry. *Circulation* 2015; **132**: 923–31.

55 Rao Kondapally Seshasai S, Kaptoge S, Thompson A, *et al.* Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* 2011; **364**: 829–41.

56 Rawshani A. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2018; **379**: 633–44.

57 Gaede, P, Oellgaard J, Carstensen B, Rossing P, Lund-Andersen H PH, O. P. Years of life gained by multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: 21 years follow-up on the Steno-2 randomised trial. *Diabetologia* 2016; **59**: 2298–307.

58 Das SR, Everett BM, Birtcher KK, Brown JM, Cefalu WT, Januzzi JL Jr, Kalyani RR, Kosiborod M, Magwire ML, Morris PB SL. 2018 ACC Expert Consensus Decision Pathway on Novel Therapies for Cardiovascular Risk Reduction in Patients With Type 2 Diabetes and Atherosclerotic Cardiovascular Disease: A Report of the American College of Cardiology Task Force on Expert Consensus Deci. *J Am Coll Cardiol* 2018; **72**: 3200–23.

59 Scirica BM, Bhatt DL, Braunwald E, *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317–26.

60 Scirica BM, Bhatt DL, Braunwald E, *et al.* The design and rationale of the saxagliptin assessment of vascular outcomes recorded in patients with diabetes mellitus-thrombolysis in

myocardial infarction (SAVOR-TIMI) 53 study. *Am Heart J* 2011; **162**: 818-825.e6.

61 Administration F and D. No Title. Stand. Defin. end point events Cardiovasc. trials. 2010. http://www.cdisc.org/stuff/contentmgr/files/0/ 2356ae38ac190ab8ca4ae0b222392b37/ misc/cdisc_november_16__2010.pdf).

62 Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. *Circulation* 2016; **133**: 601–9.

63 Fine JP GR. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *J Am Stat Assoc* 1999; **94**: 496–509.

64 Austin PC FJ. Practical recommendations for reporting Fine-Gray model analyses for competing risk data. *Stat Med* 2017; **36**: 4391–400.

65 D'Agostino RB, Grundy S, Sullivan LM, Wilson P, CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. *JAMA* 2001; **286**: 180–7.

66 Stevens RJ, Kothari V, Adler AI, Stratton IM, United Kingdom Prospective Diabetes Study (UKPDS) Group. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond)* 2001; **101**: 671–9.

67 Yeboah J, Erbel R, Delaney JC, *et al.* Development of a new diabetes risk prediction tool for incident coronary heart disease events: the Multi-Ethnic Study of Atherosclerosis and the Heinz Nixdorf Recall Study. *Atherosclerosis* 2014; **236**: 411–7.

68 Wells BJ, Jain A, Arrigain S, Yu C, Rosenkrans WA, Kattan MW. Predicting 6-year mortality risk in patients with type 2 diabetes. *Diabetes Care* 2008; **31**: 2301–6.

69 Hong Kong Diabetes Registry, Yang X, So WY, *et al.* Development and validation of an all-cause mortality risk score in type 2 diabetes. *Arch Intern Med* 2008; **168**: 451–7.

70 McEwen LN, Karter AJ, Waitzfelder BE, *et al.* Predictors of mortality over 8 years in type 2 diabetic patients: Translating Research Into Action for Diabetes (TRIAD). *Diabetes Care* 2012; **35**: 1301–9.

71 De Cosmo S, Copetti M, Lamacchia O, *et al.* Development and validation of a predicting model of all-cause mortality in patients with type 2 diabetes. *Diabetes Care* 2013; **36**: 2830–5.

72 Raffield LM, Hsu F-C, Cox AJ, Carr JJ, Freedman BI, Bowden DW. Predictors of all-cause and cardiovascular disease mortality in type 2 diabetes: Diabetes Heart Study. *Diabetol Metab Syndr* 2015; **7**: 58.

73 Tancredi M, Rosengren A, Svensson A-M, *et al.* Excess Mortality among Persons with Type 2 Diabetes. *N Engl J Med* 2015; **373**: 1720–32.

74 Bergmark BA, Bhatt DL, Braunwald E, *et al.* Risk Assessment in Patients With Diabetes With the TIMI Risk Score for Atherothrombotic Disease. *Diabetes Care* 2018; **41**: 577–85.

75 Jouven X, Desnos M, Guerot C, Ducimetière P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation* 1999; **99**: 1978–83.

76 Balkau B, Jouven X, Ducimetière P, Eschwège E. Diabetes as a risk factor for sudden death. *Lancet (London, England)* 1999; **354**: 1968–9.

77 Albert CM, Chae CU, Grodstein F, *et al.* Prospective study of sudden cardiac death among women in the United States. *Circulation* 2003; **107**: 2096–101.

78 Jouven X, Lemaître RN, Rea TD, Sotoodehnia N, Empana J-P, Siscovick DS. Diabetes, glucose level, and risk of sudden cardiac death. *Eur Heart J* 2005; **26**: 2142–7.

79 Fox CS, Evans JC, Larson MG, Kannel WB, Levy D. Temporal trends in coronary heart disease mortality and sudden cardiac death from 1950 to 1999: the Framingham Heart Study. *Circulation* 2004; **110**: 522–7.

80 Zheng ZJ, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. *Circulation* 2001; **104**: 2158–63.

81 Sharma A, Green JB, Dunning A, *et al.* Causes of Death in a Contemporary Cohort of Patients With Type 2 Diabetes and Atherosclerotic Cardiovascular Disease: Insights From the TECOS Trial. *Diabetes Care* 2017; **40**: 1763–70.

82 Scirica BM, Bhatt DL, Braunwald E, *et al.* Prognostic Implications of Biomarker Assessments in Patients With Type 2 Diabetes at High Cardiovascular Risk: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Cardiol* 2016; **1**: 989–98.

83 Everett BM, Brooks MM, Vlachos HEA, *et al.* Troponin and Cardiac Events in Stable Ischemic Heart Disease and Diabetes. *N Engl J Med* 2015; **373**: 610–20.

84 Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB. Clustering of metabolic factors and coronary heart disease. *Arch Intern Med* 1999; **159**: 1104–9.

85 Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the metabolic syndrome in the United States, 2003-2012. *JAMA* 2015; **313**: 1973–4.

86 Mottillo S, Filion KB, Genest J, *et al.* The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **56**: 1113–32.

87 Kahn R, Buse J, Ferrannini E, Stern M, American Diabetes Association, European Association for the Study of Diabetes. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2005; **28**: 2289–304.

88 Norhammar A, Mellbin L, Cosentino F. Diabetes: Prevalence, prognosis and management of

a potent cardiovascular risk factor. *Eur J Prev Cardiol* 2017; **24**: 52–60.

89 Maddaloni E, Pozzilli P. SMART diabetes: the way to go (Safe and Multifactorial Approach to reduce the Risk for Therapy in diabetes). *Endocrine* 2014; **46**: 3–5.

90 O'Donoghue ML, Braunwald E, White HD, *et al.* Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. *JAMA* 2014; **312**: 1006–15.

91 O'Donoghue ML, Braunwald E, White HD, *et al.* Study design and rationale for the Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial in patients after an acute coronary syndrome. *Am Heart J* 2011; **162**: 613-619.e1.

92 The IDF consensus worldwide definition of the metabolic syndrome 2005. https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome.

93 Grundy SM, Cleeman JI, Daniels SR, *et al.* Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; **112**: 2735–52.

94 Netzer NC, Stoohs RA, Netzer CM, Clark K, Strohl KP. Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. *Ann Intern Med* 1999; **131**: 485–91.

95 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and T of HBC in A (Adult TPI. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143–421.

96 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595–607.

97 DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991; **14**: 173–94.

98 Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994; **267**: E187-202.

99 Haffner SM, Katz MS, Dunn JF. Increased upper body and overall adiposity is associated with decreased sex hormone binding globulin in postmenopausal women. *Int J Obes* 1991; **15**: 471–8.

100 Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. *Am J Med* 2006; **119**: 812–9.

101 Gami AS, Witt BJ, Howard DE, *et al.* Metabolic syndrome and risk of incident

cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007; **49**: 403–14.

102 Lee Y, Lim Y-H, Shin J-H, Park J, Shin J, Kim K-S. The Impact of Metabolic Syndrome on Clinical Outcomes After Everolimus-Eluting Stent Implantation. *Am J Cardiol* 2015; **116**: 717–24.

103 Won K-B, Kim B-K, Chang H-J, *et al.* Metabolic syndrome does not impact long-term survival in patients with acute myocardial infarction after successful percutaneous coronary intervention with drug-eluting stents. *Catheter Cardiovasc Interv* 2014; **83**: 713–20.

104 Mehta RH, Westerhout CM, Zheng Y, *et al.* Association of metabolic syndrome and its individual components with outcomes among patients with high-risk non-ST-segment elevation acute coronary syndromes. *Am Heart J* 2014; **168**: 182-8.e1.

105 Dohi T, Miyauchi K, Kasai T, *et al.* Impact of metabolic syndrome on 10-year clinical outcomes among patients with acute coronary syndrome. *Circ J* 2009; **73**: 1454–8.

106 Boulon C, Lafitte M, Richeboeuf V, *et al.* Prevalence of metabolic syndrome after acute coronary syndrome and its prognostic significance. *Am J Cardiol* 2006; **98**: 1429–34.

107 Isomaa B, Almgren P, Tuomi T, *et al.* Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001; **24**: 683–9.

108 Lakka H-M, Laaksonen DE, Lakka TA, *et al.* The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002; **288**: 2709–16.

109 Ford ES. The metabolic syndrome and mortality from cardiovascular disease and all-causes: findings from the National Health and Nutrition Examination Survey II Mortality Study. *Atherosclerosis* 2004; **173**: 309–14.

110 Malik S, Wong ND, Franklin SS, *et al.* Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation* 2004; **110**: 1245–50.

111 Butler J, Rodondi N, Zhu Y, *et al.* Metabolic syndrome and the risk of cardiovascular disease in older adults. *J Am Coll Cardiol* 2006; **47**: 1595–602.

112 Monami M, Marchionni N, Masotti G, Mannucci E. IDF and ATP-III definitions of metabolic syndrome in the prediction of all-cause mortality in type 2 diabetic patients. *Diabetes Obes Metab* 2007; **9**: 350–3.

113 Marroquin OC, Kip KE, Kelley DE, *et al.* Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women: a report from the Women's Ischemia Syndrome Evaluation. *Circulation* 2004; **109**: 714–21.

114 Levantesi G, Macchia A, Marfisi R, *et al.* Metabolic syndrome and risk of cardiovascular

events after myocardial infarction. *J Am Coll Cardiol* 2005; **46**: 277–83.

115 Feinberg MS, Schwartz R, Tanne D, *et al.* Impact of the metabolic syndrome on the clinical outcomes of non-clinically diagnosed diabetic patients with acute coronary syndrome. *Am J Cardiol* 2007; **99**: 667–72.

116 Stern MP, Williams K, Hunt KJ. Impact of diabetes/metabolic syndrome in patients with established cardiovascular disease. *Atheroscler Suppl* 2005; **6**: 3–6.

117 Driver SL, Martin SS, Gluckman TJ, Clary JM, Blumenthal RS, Stone NJ. Fasting or Nonfasting Lipid Measurements: It Depends on the Question. *J Am Coll Cardiol* 2016; **67**: 1227–34.

118 Bruthans J, Mayer O, De Bacquer D, *et al.* Educational level and risk profile and risk control in patients with coronary heart disease. *Eur J Prev Cardiol* 2016; **23**: 881–90.

119 Kotseva K, Wood D, De Bacquer D, *et al.* EUROASPIRE IV: A European Society of Cardiology survey on the lifestyle, risk factor and therapeutic management of coronary patients from 24 European countries. *Eur J Prev Cardiol* 2016; **23**: 636–48.

120 Schiele F, Ecarnot F, Chopard R. Coronary artery disease: Risk stratification and patient selection for more aggressive secondary prevention. *Eur J Prev Cardiol* 2017; **24**: 88–100.

121 Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of clinical classification schemes for predicting stroke: results from the National Registry of Atrial Fibrillation. *JAMA* 2001; **285**: 2864–70.

122 European Heart Rhythm Association, European Association for Cardio-Thoracic Surgery, Camm AJ, *et al.* Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *Eur Heart J* 2010; **31**: 2369–429.

123 Camm AJ, Lip GYH, De Caterina R, *et al.* 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. *Eur Heart J* 2012; **33**: 2719–47.

124 Targher G, Chonchol M, Zoppini G, Franchini M. Hemostatic disorders in type 1 diabetes mellitus. *Semin Thromb Hemost* 2011; **37**: 58–65.

125 Kirchhof P, Ammentorp B, Darius H, *et al.* Management of atrial fibrillation in seven European countries after the publication of the 2010 ESC Guidelines on atrial fibrillation: Primary results of the PREvention of thromboemolic events-European Registry in Atrial Fibrillation (PREFER in AF). *Europace* 2014; **16**: 6–14.

126 Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2011; **34**: S62–9.

127 Giugliano RP, Ruff CT, Braunwald E, *et al.* Edoxaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2013; **369**: 2093–104.

128 Sacco RL, Kasner SE, Broderick JP, *et al.* An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2013; **44**: 2064–89.

129 Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ* 1980; **58**: 113–30.

130 Berisso MZ, Fabbri G, Gonzini L, *et al.* Antiarrhythmic strategies in patients with atrial fibrillation managed by cardiologists and internists: Antithrombotic Agents in Atrial Fibrillation (ATA-AF) survey. *J Cardiovasc Med (Hagerstown)* 2014; **15**: 626–35.

131 Lip GYH, Varughese GI. Diabetes mellitus and atrial fibrillation: perspectives on epidemiological and pathophysiological links. *Int J Cardiol* 2005; **105**: 319–21.

132 Komajda M, Tavazzi L, Francq BG, *et al.* Efficacy and safety of ivabradine in patients with chronic systolic heart failure and diabetes: an analysis from the SHIFT trial. *Eur J Heart Fail* 2015; **17**: 1294–301.

133 Seidl K, Hauer B, Schwick NG, Zellner D, Zahn R, Senges J. Risk of thromboembolic events in patients with atrial flutter. *Am J Cardiol* 1998; **82**: 580–3.

134 Patients with nonvalvular atrial fibrillation at low risk of stroke during treatment with aspirin: Stroke Prevention in Atrial Fibrillation III Study. The SPAF III Writing Committee for the Stroke Prevention in Atrial Fibrillation Investigators. *JAMA*; **279**: 1273–7.

135 Petersen P, Kastrup J, Helweg-Larsen S, Boysen G, Godtfredsen J. Risk factors for thromboembolic complications in chronic atrial fibrillation. The Copenhagen AFASAK study. *Arch Intern Med* 1990; **150**: 819–21.

136 Aronow WS, Ahn C, Kronzon I, Gutstein H. Risk factors for new thromboembolic stroke in patients > or = 62 years of age with chronic atrial fibrillation. *Am J Cardiol* 1998; **82**: 119–21.

137 Stöllberger C, Chnupa P, Abzieher C, *et al.* Mortality and rate of stroke or embolism in atrial fibrillation during long-term follow-up in the embolism in left atrial thrombi (ELAT) study. *Clin Cardiol* 2004; **27**: 40–6.

138 el Khawand C, Jamart J, Donckier J, *et al.* Hemostasis variables in type I diabetic patients without demonstrable vascular complications. *Diabetes Care* 1993; **16**: 1137–45.

139 Fasching P, Veitl M, Rohac M, *et al.* Elevated concentrations of circulating adhesion molecules and their association with microvascular complications in insulin-dependent

diabetes mellitus. *J Clin Endocrinol Metab* 1996; **81**: 4313–7.

140 Elhadd TA, Kennedy G, Hill A, *et al.* Abnormal markers of endothelial cell activation and oxidative stress in children, adolescents and young adults with type 1 diabetes with no clinical vascular disease. *Diabetes Metab Res Rev*; **15**: 405–11.

141 Rathcke CN, Persson F, Tarnow L, Rossing P, Vestergaard H. YKL-40, a marker of inflammation and endothelial dysfunction, is elevated in patients with type 1 diabetes and increases with levels of albuminuria. *Diabetes Care* 2009; **32**: 323–8.

142 Carmassi F, Morale M, Puccetti R, *et al.* Coagulation and fibrinolytic system impairment in insulin dependent diabetes mellitus. *Thromb Res* 1992; **67**: 643–54.

143 Oggianu L, Lancellotti S, Pitocco D, *et al.* The oxidative modification of von Willebrand factor is associated with thrombotic angiopathies in diabetes mellitus. *PLoS One* 2013; **8**: e55396.

144 Nemmar A, Subramaniyan D, Yasin J, Ali BH. Impact of experimental type 1 diabetes mellitus on systemic and coagulation vulnerability in mice acutely exposed to diesel exhaust particles. *Part Fibre Toxicol* 2013; **10**: 14.

145 Haitas B, Barnes AJ, Cederholm-Williams SA, Moore J, Shogry ME, Turner RC. Abnormal endothelial release of fibrinolytic activity and fibronectin in diabetic microangiopathy. *Diabetologia* 1984; **27**: 493–6.

146 Hess K, Alzahrani SH, Mathai M, *et al.* A novel mechanism for hypofibrinolysis in diabetes: the role of complement C3. *Diabetologia* 2012; **55**: 1103–13.

147 Madonna R, De Caterina R. Cellular and molecular mechanisms of vascular injury in diabetes--part I: pathways of vascular disease in diabetes. *Vascul Pharmacol*; **54**: 68–74.

148 Madonna R, De Caterina R. Cellular and molecular mechanisms of vascular injury in diabetes--part II: cellular mechanisms and therapeutic targets. *Vascul Pharmacol*; **54**: 75–9.

149 Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res* 2007; **22**: 465–75.

150 Duong LT. Therapeutic inhibition of cathepsin K-reducing bone resorption while maintaining bone formation. *Bonekey Rep* 2012; **1**: 67.

151 Duong LT, Pickarski M, Cusick T, *et al.* Effects of long term treatment with high doses of odanacatib on bone mass, bone strength, and remodeling/modeling in newly ovariectomized monkeys. *Bone* 2016; **88**: 113–24.

152 Duong LT, Crawford R, Scott K, *et al.* Odanacatib, effects of 16-month treatment and discontinuation of therapy on bone mass, turnover and strength in the ovariectomized rabbit

model of osteopenia. *Bone* 2016; **93**: 86–96.

153 Lutgens E, Lutgens SPM, Faber BCG, *et al.* Disruption of the cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation. *Circulation* 2006; **113**: 98–107.

154 Lutgens SPM, Kisters N, Lutgens E, *et al.* Gene profiling of cathepsin K deficiency in atherogenesis: profibrotic but lipogenic. *J Pathol* 2006; **210**: 334–43.

155 Bone HG, McClung MR, Roux C, *et al.* Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. *J Bone Miner Res* 2010; **25**: 937–47.

156 Brixen K, Chapurlat R, Cheung AM, *et al.* Bone density, turnover, and estimated strength in postmenopausal women treated with odanacatib: a randomized trial. *J Clin Endocrinol Metab* 2013; **98**: 571–80.

157 Cheung AM, Majumdar S, Brixen K, *et al.* Effects of odanacatib on the radius and tibia of postmenopausal women: improvements in bone geometry, microarchitecture, and estimated bone strength. *J Bone Miner Res* 2014; **29**: 1786–94.

158 Eisman JA, Bone HG, Hosking DJ, *et al.* Odanacatib in the treatment of postmenopausal women with low bone mineral density: three-year continued therapy and resolution of effect. *J Bone Miner Res* 2011; **26**: 242–51.

159 Langdahl B, Binkley N, Bone H, *et al.* Odanacatib in the treatment of postmenopausal women with low bone mineral density: five years of continued therapy in a phase 2 study. *J Bone Miner Res* 2012; **27**: 2251–8.

160 Rizzoli R, Benhamou C-L, Halse J, *et al.* Continuous treatment with odanacatib for up to 8 years in postmenopausal women with low bone mineral density: a phase 2 study. *Osteoporos Int* 2016; **27**: 2099–107.

161 Bone HG, Dempster DW, Eisman JA, *et al.* Odanacatib for the treatment of postmenopausal osteoporosis: development history and design and participant characteristics of LOFT, the Long-Term Odanacatib Fracture Trial. *Osteoporos Int* 2015; **26**: 699–712.

162 Peroni A, Zini A, Braga V, Colato C, Adami S, Girolomoni G. Drug-induced morphea: report of a case induced by balicatib and review of the literature. *J Am Acad Dermatol* 2008; **59**: 125–9.

163 Rünger TM, Adami S, Benhamou C-L, *et al.* Morphea-like skin reactions in patients treated with the cathepsin K inhibitor balicatib. *J Am Acad Dermatol* 2012; **66**: e89-96.

164 Drake MT, Clarke BL, Oursler MJ, Khosla S. Cathepsin K Inhibitors for Osteoporosis: Biology, Potential Clinical Utility, and Lessons Learned. *Endocr Rev* 2017; **38**: 325–50.

165 Papapoulos S, Chapurlat R, Libanati C, *et al.* Five years of denosumab exposure in women with postmenopausal osteoporosis: results from the first two years of the FREEDOM extension. *J Bone Miner Res* 2012; **27**: 694–701.

166 Shane E, Burr D, Ebeling PR, *et al.* Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res* 2010; **25**: 2267–94.

167 Shane E, Burr D, Abrahamsen B, *et al.* Atypical subtrochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res* 2014; **29**: 1–23.

168 Black DM, Delmas PD, Eastell R, *et al.* Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N Engl J Med* 2007; **356**: 1809–22.

169 Black DM, Cummings SR, Karpf DB, *et al.* Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. *Lancet (London, England)* 1996; **348**: 1535–41.

170 Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med*; **4**: 213–26.

171 Cummings SR, San Martin J, McClung MR, *et al.* Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009; **361**: 756–65.

172 Faber BC, Cleutjens KB, Niessen RL, *et al.* Identification of genes potentially involved in rupture of human atherosclerotic plaques. *Circ Res* 2001; **89**: 547–54.

173 Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest* 1998; **102**: 576–83.

174 Aoki T, Kataoka H, Ishibashi R, Nozaki K, Hashimoto N. Cathepsin B, K, and S are expressed in cerebral aneurysms and promote the progression of cerebral aneurysms. *Stroke* 2008; **39**: 2603–10.

175 Cheng XW, Shi G-P, Kuzuya M, Sasaki T, Okumura K, Murohara T. Role for cysteine protease cathepsins in heart disease: focus on biology and mechanisms with clinical implication. *Circulation* 2012; **125**: 1551–62.

176 Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol* 2014; **63**: 2335–45.

177 Nattel S. Molecular and Cellular Mechanisms of Atrial Fibrosis in Atrial Fibrillation. *JACC Clin Electrophysiol* 2017; **3**: 425–35.

178 Towler DA. Commonalities Between Vasculature and Bone: An Osseocentric View of Arteriosclerosis. *Circulation* 2017; **135**: 320–2.

179 Kado DM, Browner WS, Blackwell T, Gore R, Cummings SR. Rate of bone loss is associated with mortality in older women: a prospective study. *J Bone Miner Res* 2000; **15**: 1974–80.

180 Nguyen ND, Center JR, Eisman JA, Nguyen T V. Bone loss, weight loss, and weight fluctuation predict mortality risk in elderly men and women. *J Bone Miner Res* 2007; **22**: 1147–54.