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DISSERTATION SUBMITTED 2019

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ENGLISH SUMMARY

Diabetes mellitus represents a major and growing public health problem, which is partly due to the diabetes related burden of cardiovascular disease. Hence, a continued focus on cardiovascular disease prevention is needed and risk stratification in patients with diabetes is an important priority in the management of diabetes-related morbidity and mortality.

The aim of this thesis was to contribute to the identification of new potential prognostic factors for cardiovascular disease among patients with diabetes. Specifically, we aimed to investigate the prognostic influence of diabetes type and glycaemic status in patients with diabetes and atrial fibrillation, and to investigate the prognostic influence of albuminuria in a general cohort of type 2 diabetes patients.

The studies of this dissertation was based on data from Danish national registries. In study I, we found that the type of diabetes was generally not associated with a higher risk of ischemic stroke. However, in a subgroup of patients below 65 years, type 2 diabetes was associated with a higher risk of ischaemic stroke. In study II, we found that increasing levels of glycaemic status were associated with an increased risk of ischaemic stroke in patients with a shorter duration of diabetes but not in patients with a longer duration of diabetes. In study III, we found that micro- and macroalbuminuria were associated with a higher risk of incident ischaemic stroke and myocardial infarction in patients with type 2 diabetes.

The studies of this dissertation have contributed to the identification of a number of risk factors for ischaemic stroke and myocardial infarction both in a general cohort of patients with diabetes and in patients with diabetes and concomitant atrial fibrillation. Identifying high- and low-risk subgroups provides basis for evidence-based clinical risk stratification that may serve as a valuable clinical tool in aiding clinical counselling of patients and guiding treatment decisions.

DANSK RESUME

Diabetes er en hyppigt forekommende sygdom, der udgør et voksende folkesundheds problem, idet der medfølger en stor byrde af kardiovaskulær sygdom. Derfor er der fortsat et behov for fokus på forebyggelse af kardiovaskulær sygdom blandt patienter med diabetes. Anvendelse af risikostratificering af patienter med diabetes er et vigtigt redskab i håndteringen af diabetesrelateret morbiditet og mortalitet.

Det overordnede formål med denne afhandling var, at identificere potentielle prognostiske faktorer for udviklingen af kardiovaskulær sygdom blandt patienter med diabetes. De specifikke formål var at undersøge om risikoen for iskæmisk apopleksi er associeret med typen af diabetes og blodsukkerniveauet blandt patienter med diabetes og atrieflimren, samt at undersøge om risikoen for iskæmisk apopleksi og akut myokardieinfarkt er associeret med albuminudskillelsen i urinen iblandt patienter med diabetes.

Studierne i denne afhandling er baseret på data fra danske nationale registre. I studie I fandt vi, at typen af diabetes overordnet set ikke var associeret med risikoen for iskæmisk apopleksi. Dog var det blandt patienter under 65 år en betydeligt større risiko forbundet med at have type 2 diabetes sammenlignet med type 1 diabetes. I studie II fandt vi, at stigende blodsukkerniveauer var forbundet med en betydeligt højere risiko for at udvikle iskæmisk apopleksi blandt patienter med kortere varighed af diabetes, hvorimod der blandt patienter med længere varighed af diabetes ikke var nogen sammenhæng mellem blodsukkerniveauet og risikoen for iskæmisk apopleksi. I studie III fandt vi, at stigende niveauer af albuminuri var forbundet med en højere risiko for både iskæmisk apopleksi og akut myokardieinfarkt blandt patienter med diabetes.

Studierne i denne afhandling har bidraget til at identificere risikofaktorer for iskæmisk apopleksi og myokardieinfarkt blandt patienter med diabetes. Identificering af høj- og lavrisikogrupper blandt patienter med diabetes kan danne grundlag for evidensbaseret risikostratificering, som kan anvendes som et klinisk redskab til at rådgive patienter og til at træffe behandlingsmæssige beslutninger i den kliniske hverdag.

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Mia Vicki Fangel

LIST OF PAPERS

The thesis is based on the following papers:

1. Fangel MV, Nielsen PB, Larsen TB, Christensen B, Overvad TF, Lip GYH, Goldhaber SZ, Jensen MB. Type 1 versus type 2 diabetes and thromboembolic risk in patients with atrial fibrillation: A Danish nationwide cohort study. *Int J Cardiol*. 2018;268:137-142.

2. Fangel MV, Nielsen PB, Kristensen JK, Larsen TB, Overvad TF, Lip GYH, Jensen MB. Glycemic Status and Thromboembolic Risk in Patients with Atrial Fibrillation and Type 2 Diabetes: A Danish Cohort Study. *Circ Arrhytm Electrophysiol.* 2019;12:e007030.

3. Fangel MV, Nielsen PB, Kristensen JK, Larsen TB, Overvad TF, Lip GYH, Jensen MB. Albuminuria and Risk of Cardiovascular Events and Mortality in a General Population of Patients with Type 2 Diabetes without Cardiovascular Disease: A Danish Cohort Study. [submitted].

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CHAPTER 1. INTRODUCTION

Diabetes mellitus, commonly referred to as diabetes, is a group of metabolic disorders characterized by abnormally increased concentrations of glucose in the blood over a prolonged period of time. The overall prevalence of diabetes is reaching pandemic proportions, affecting more than 450 million people worldwide and 260,000 people in Denmark alone.[1,2] The global prevalence of diabetes in adults has been increasing over recent decades and is expected to continue to rise in upcoming years.[2–4]

Diabetes can be divided into several subtypes, whereof the vast majority of patients fall into two pathogenetic categories; type 1 and type 2 diabetes.[5] With type 2 diabetes accounting for about 80% of all diabetes cases and type 1 diabetes accounting for about 10%. Type 2 diabetes is a metabolic disorder characterized by impaired insulin secretion and insulin resistance. The pathophysiological changes in type 2 diabetes include dysfunction of the insulin-producing β-cells in the pancreas, inadequate sensitivity of the body cells to the action of the insulin produced, and chronic inflammation.[6] Type 2 diabetes is a multifactorial disease involving both genetic and lifestyle factors,[7] with adiposity being the single most important risk factor for the development of type 2 diabetes.[8] However, physical inactivity and genetic components affecting the development of obesity, insulin secretion, and insulin resistance also play important roles.[9] Type 2 diabetes usually develops during adulthood, but a rise in the incidence of Type 2 diabetes in children and adolescents has been observed.[10] Type 1 diabetes is an autoimmune chronic condition characterized by insulin deficiency due to destruction of insulin-producing β-cells in the pancreas.[11] The process usually progresses over months to years during which the subject is asymptomatic. Type 1 diabetes develops during childhood and adolescents and may be triggered by environmental agents in genetically susceptible individuals.[11]

People with diabetes have an increased risk of developing serious life-threatening health problems resulting in a reduced quality of life, increased mortality, and higher medical care costs.[12–15] Persistently high blood glucose levels cause generalized vascular damage that affect nearly all blood vessel types and sizes, which leads to an increased risk of both microvascular complications, such as retinopathy, nephropathy, and neuropathy and macrovascular complications including coronary artery disease, peripheral arterial disease, and ischaemic stroke.[16–19] Adults with diabetes have a two- to threefold increased risk of myocardial infarction and ischaemic stroke compared to adults without diabetes[20] and two-thirds of deaths in patients with diabetes are related to cardiovascular disease.[21] Furthermore, the severity of diabetes mellitus, determined by disease duration, worse glycaemic control, or requirement for insulin treatment, has been associated with cardiovascular disease development.[22] Not only, is diabetes associated with higher risk of cardiovascular disease, worse outcomes after myocardial infarction and ischaemic stroke have been observed in patients with diabetes compared to patients without diabetes.[23–29]

The underlying pathophysiological mechanisms explaining this observed excess risk are complex. Insulin resistance and hyperglycaemia plays a pivotal role in the accelerated atherosclerotic process in diabetes. Furthermore, chronic low-grade inflammation, primary haemostasis changes, increased levels of clotting factors, impaired fibrinolysis, and enhanced oxidative stress may be mediators of the prothrombotic state observed in diabetes.[30,31]

Even though patients with diabetes have a two- to threefold increased risk of cardiovascular morbidity and mortality compared to individuals without diabetes,[16–19] diabetes is no longer considered a coronary risk equivalent.[32] In fact, several studies suggest that a considerable part of patients are in a lower cardiovascular risk category.[33,34] Hence, international guidelines now recommend cardiovascular risk stratification for patients with diabetes in order to determine the intensity of prevention strategies.[35–37]

Prevention of cardiovascular disease in patients with type 2 diabetes can include antiplatelet treatment and management of abnormalities in blood glucose, lipids, and blood pressure. Generally these preventive strategies are divided into primary prevention and secondary prevention, applying to patients without cardiovascular disease and with cardiovascular disease, respectively. The essential difference in primary and secondary prevention is differences in treatment thresholds for lipid lowering and the recommendation regarding antiplatelet treatment. Intensified lipid lowering and antiplatelet treatment are generally recommended in secondary prevention. These recommendations are supported by several randomized controlled trials that have shown a net benefit of antiplatelet treatment and intensified lipid lowering in patients with type 2 diabetes and established cardiovascular disease.[38–46] More uncertainty about the intensity of treatment exists in treatment guidelines for primary prevention of cardiovascular disease in patients with diabetes as randomized controlled trials have not shown an overall net benefit of intensified lipid lowering and antiplatelet treatment in patients with diabetes but without cardiovascular disease.[35,36] Guidelines from both the European Society of cardiology (ESC) and the American Diabetes Association (ADA) state that assessing the risk of a first-time atherosclerotic cardiovascular event can aid the clinician in discriminating higher- from lower-risk patients and thereby make informed decisions about the intensity of preventive treatment.[35,47]

Identifying potential prognostic factors for the development of cardiovascular disease in patients with diabetes may optimize risk stratification. This may allow us to identify high-risk subgroups in the diabetes population who would benefit from intensive preventive treatment, while avoiding overtreatment in lower risk cases.

1.1. DIABETES AND ISCHAEMIC STROKE

The link between diabetes and stroke is complex, for instance, diabetes has been linked to ischaemic stroke but not to haemorrhagic stroke.[48] To add to the complexity, ischaemic stroke is an umbrella term covering several subtypes of ischaemic stroke, e.g. atherosclerotic and cardioembolic strokes. Whereas the link between diabetes and atherosclerotic strokes are well established, the role of diabetes as an independent risk factor for cardioembolic stroke is more conflicting.[49,50]

Cardioembolic stroke is often caused by atrial fibrillation and approximately 20% of all ischaemic strokes are estimated to be attributed to atrial fibrillation.[4] Atrial fibrillation is a supraventricular arrhythmia characterised by disorganized electrical activity in the atria causing an irregular and often rapid heart rhythm. Atrial fibrillation is the most common sustained arrhythmia affecting more than 33.5 million people worldwide.[51] The prevalence of atrial fibrillation more or less doubles with each decade of age, rising to almost 9% at 80-90 years.[52] It is expected that the prevalence will increase in upcoming years, primarily due to the increased aging of the population but also due to an increase in established risk factors for atrial fibrillation among others diabetes.[53,54] In fact, it has been estimated that people with diabetes have an approximately 40% greater risk of incident atrial fibrillation compared with people without diabetes.[55] Not only is diabetes associated with increased risk of developing atrial fibrillation, diabetes has also been associated with a higher risk of ischaemic stroke in patients who have already developed atrial fibrillation[56–58] Hence, diabetes is incorporated in the guideline-recommended thromboembolic risk stratification tool, the CHA_2DS_2-VASc score (congestive heart failure, hypertension, age $\geq$75, diabetes, ischaemic stroke/transient ischaemic attack, myocardial infarction/peripheral artery disease, age $\geq$65, and female sex).[59] According to guidelines, anticoagulation treatment should be considered for men with a CHA_2DS_2-VASc score of 1 and women with a score of 2, balancing the expected stroke reduction, bleeding risk, and patient preference. Consequently, all patients with diabetes mellitus are hereby potential candidates for life-long anticoagulant treatment. This simplistic approach to stroke risk stratification neglects the diversity of the diabetes population. By exploring whether it is possible to identify prediction markers for thromboembolic events in patients with diabetes and atrial fibrillation, we can clarify if it is possible to identify subgroups of patients with different thromboembolic risk, which could lead to more differentiated treatment recommendations.

CHAPTER 2. AIMS AND HYPOTHESES

The overall aim of this PhD project was to contribute to the identification of new potential prognostic factors for cardiovascular disease as well as potential therapeutic targets for cardiovascular risk reduction among patients with diabetes.

Study I
Aim: To examine whether type 1 and type 2 diabetes are associated with different risks of thromboembolism among patients with incident nonvalvular atrial fibrillation.

Hypothesis: Type 2 diabetes is associated with a higher risk of thromboembolism than type 1 diabetes in patients with incident nonvalvular atrial fibrillation.

Study II
Aim: To examine the effect of glycaemic status as reflected by haemoglobin A1c (HbA1c) on the risk of thromboembolism in patients with atrial fibrillation and type 2 diabetes; and to examine whether the effect of elevated HbA1c on the risk of thromboembolism was modified by the duration of diabetes.

Hypothesis: Increasing levels of HbA1c is associated with a higher risk of thromboembolism in patients with atrial fibrillation and type 2 diabetes and the association between HbA1c and thromboembolism is modified by diabetes duration.

Study III
Aim: To examine the association between micro- and macroalbuminuria and incident ischaemic stroke, myocardial infarction, and all-cause mortality in a nationwide cohort study of patients with type 2 diabetes without prevalent atherosclerotic cardiovascular disease.

Hypothesis: In patients with type 2 diabetes, micro- and macroalbuminuria are associated with a higher risk of incident ischaemic stroke, myocardial infarction, and all-cause mortality.

CHAPTER 3. METHODS

3.1. SETTING AND DESIGN

The studies in this dissertation were cohort studies based on data from Danish nationwide registries. Our studies were based on historical data, mainly collected for administrative purposes; however, the studies were designed and analysed prospectively, thereby ensuring that disease under study occurred after the exposure of interest.

3.2. DATA SOURCES

Denmark has a long history of collecting information on vital status, immigration/emigration, redeemed prescriptions, and disease incidence in Danish administrative and clinical registries.[60] By using a unique personal identification number (CPR-number), which is assigned to all residents in Denmark, it is possible to link data from the various registries to obtain individual level data.[61] The registries contain data reflecting clinical practice and covers the entire population. This creates the possibility for large sample sizes and a high representativeness of the results.[62] Accordingly, the Danish registries have been extensively used for epidemiological research.[62]

All studies in this dissertation are based on the Danish National Prescription Registry,[63] the Danish National Patient Registry,[64] and the Danish Civil Registration System.[61] Moreover, study II and III are also based on data from the Danish Adult Diabetes Registry.[65] The data was linked through Statistics Denmark. In the following section a concise description of the aforementioned registries is provided.

The Danish Civil Registration System
The Danish Civil Registration System was established in 1968. The registry holds information on date of birth, vital status, sex, and migration.[61] Contemporary information on migration and vital status allows for long-term follow-up in nationwide cohort studies with accurate censoring at emigration or death.[66]

The Danish National Patient Registry
The Danish National Patient Registry is a nationwide hospital based registry that provides longitudinal registration of comprehensive administrative and clinical data.[64] Since 1977, information on all patients discharged from Danish non-psychiatric wards has been registered. Moreover, information from psychiatric wards, outpatient contacts, and emergency departments has been registered since 1995 and from private hospitals since 2003.[62] For each patient contact the physician, discharging the patient, registers one primary diagnosis and optional secondary diagnoses. The diagnoses are

classified according to the International Classification of Diseases (ICD). Originally the 8th revision of ICD was used and since 1994 the 10th revision has been used.[62] The registry was used in all three studies to obtain information on the incidence of the study outcomes: ischaemic stroke, systemic embolism, and acute myocardial infarction. Moreover, information on the patients' comorbidity was partly obtained from the Danish National Patient Registry.

The Danish National Prescription Registry

The Register of Medicinal Product Statistics was established in 1994 and collects individual-level data on all prescriptions redeemed by Danish residents in Danish pharmacies. Since 2003, these data were made available for researchers in an independent sub-registry named the Danish National Prescription Registry.[63,67] The Registry holds information on all prescription drugs sold in Denmark since 1995. Nonetheless, information on over the counter drugs is only registered as aggregated data. For each redeemed prescription an electronic record is generated. The record includes the date of dispensing and the type and amount of the prescribed drug, coded according to the Anatomical Therapeutic Chemical (ATC) Classification System.[63] In all studies, the Danish National Prescription Registry was used to identify the use of medication at inclusion and throughout the study. Furthermore, the registry was used in combination with the Danish National Patient Registry to define specific comorbid conditions.

Danish Adult Diabetes Registry

The Danish Adult Diabetes Registry is a nationwide quality database that collects data from annual diabetes care check-ups in outpatient clinics and in general practice. Data has been collected from outpatient clinics since 2004 whereas data from general practice has been reported to the registry since 2006.[65] Among other things, information on date of diabetes mellitus diagnosis, HbA1c measurements, urinary albumin-to-creatinine ratio (UACR) or the urinary albumin excretion rate (UAE) measurements, smoking status, body mass index, blood pressure measurements, and lipid status is reported to the registry.[12] Approximately 90% of the data are captured directly from the electronic medical record systems, which minimizes the risk of data entry errors. The registry was used in study II and III to assess the patients' different levels of the exposures HbA1c and albuminuria. Furthermore, the registry was used to assess patient characteristics such as body mass index, blood pressure, and lipid status. The outpatient clinics have had an obligation to report to the registry since 2004. General practitioners have reported to the registry since 2006, however, it did not become mandatory before 2014. Therefore, the data coverage from outpatient clinics is generally high, whereas the data coverage from general practice is still limited.[65]

3.3. STUDY POPULATION

The study populations of study I-III comprised Danish patients with diabetes. In study I, the source population was patients with diabetes identified based a combination of ICD-10 diagnostic codes for diabetes in the Danish National Patient Registry and ATC-codes for claimed prescriptions of glucose-lowering drugs from the Danish National prescription registry. From the source population of diabetes patients, we included all inpatients and outpatients registered in the Danish National Patient Registry who were discharged with a hospital diagnosis of non-valvular atrial fibrillation from January 1, 2005 to December 31, 2015. The index date (baseline) was defined as the date of the incident atrial fibrillation diagnosis. The specific exclusion criteria are described in Table 1.

In study II, the source population comprised patients with type 2 diabetes identified through the Danish Adult Diabetes Registry. From the source population of type 2 diabetes patients, we included all inpatients and outpatients registered in the Danish National Patient Registry who were discharged with a hospital diagnosis of non-valvular atrial fibrillation from May 1, 2005 to December 31, 2015. The index date was defined as the date of the incident atrial fibrillation diagnosis. The specific exclusion criteria are described in Table 1.

In study III, the source population comprised patients with type 2 diabetes identified through the Danish Adult Diabetes Registry. From the source population of type 2 diabetes patients, we included all patients that were registered with two consecutive measurements of the UACR or UAE within 15 months during the inclusion period of May 1, 2005 to June 30, 2015. The index date was defined as the date of the latter albuminuria measurement. The specific exclusion criteria are described in Table 1.

Table 1. Overview of study populations for study I-III

Study	Period	Population	Exclusion Criteria
I	January 1, 2005 to December 31, 2015	Diabetes and incident non-valvular atrial fibrillation	• Valvular atrial fibrillation • Inconsistent information from CRS • Patients not habitually residing in Denmark* • Ischaemic stroke/systemic embolism on index date • Prior anticoagulation treatment†
II	May 1, 2005 to December 31, 2015	Type 2 diabetes and incident non-valvular atrial fibrillation	• Valvular atrial fibrillation • Inconsistent information from CRS • Patients not habitually residing in Denmark* • Ischaemic stroke/systemic embolism on index date • Anaemia or end stage kidney disease‡ • No available HbA1c measurements within the past two years before or four weeks after inclusion.
III	May 1, 2005 to June 30, 2015	Type 2 diabetes patients with two consecutive measurements of the UACR or the UAE within 15 months	• Prior diagnosis of ischaemic stroke, ischaemic heart disease, or peripheral arterial disease • Inconsistent information from CRS • Patients not habitually residing in Denmark*

* Immigration within one year before entrance in the study. †patients who were baseline users of anticoagulation treatment were excluded to assess ischaemic stroke risk in atrial fibrillation free from stroke prevention treatment. ‡Patients with anaemia or end stage kidney disease were excluded because HbA1c values can be misleadingly low in this patient group. CRS: The Danish Civil Registration System.

3.4. EXPOSURES

Type of diabetes

In study I, patients were identified as patients with type 1 diabetes or type 2 diabetes by using an algorithm developed by the Danish Health Data Authority (see Appendix A).[68] The algorithm is based on a combination ICD-10 diagnostic codes for diabetes and ATC codes for claimed prescriptions of glucose-lowering drugs.

Glycaemic status

In study II, glycaemic status was determined with HbA1c measurements from the Danish Adult Diabetes Registry. A patient's baseline HbA1c value was determined as the most recent HbA1c measurement within two years before inclusion or the first HbA1c measurement within four weeks after inclusion. HbA1c values were categorized according to the clinical cut points suggested by the National Institute for Health and Care Excellence guidelines (HbA1c $\leq$48 mmol/mol, HbA1c = 49-58 mmol/mol, and HbA1c >58 mmol/mol) and also investigated as a continuous variable.[69]

Albuminuria status
In study III, patients were categorised in the following categories: Normoalbuminuria (UAE <30 mg/day or UACR <30 mg/g), microalbuminuria (UAE = 30-299 mg/day or UACR of 30-299 mg/g), and macroalbuminuria (UAE ≥300 mg/day or UACR ≥300 mg/g).

3.5. OUTCOMES AND FOLLOW-UP

In all three studies, patients were followed in the Danish National Patient Registry for the occurrence of ischaemic stroke. Furthermore, systemic embolism was an outcome in study I and II and myocardial infarction and death was an outcome in study III. We only used primary diagnoses of the outcomes as the primary diagnosis is the main reason for the hospital contact. We did not use secondary diagnoses as they are a supplement to the primary diagnosis and therefore meant to express diseases related to the current hospital contact.[62] Therefore, secondary diagnoses are more likely to be an expression of a prior event of the outcome under study. Patients were followed from their individual index date to the occurrence of an outcome event, emigration, death, or end of follow-up (31th of December 2015), which ever came first. Furthermore, initiation of anticoagulation treatment was considered as a censoring event in study I, to focus on non-anticoagulated patients.

3.6. STATISTICS

In all studies, we assessed the incidence rates of the outcomes under study. The incidence rate of the outcome under study during a specific time interval was calculated as the number of events occurring during that time interval divided by the total observation time in that same interval.

For the purpose of assessing the association between the different exposures and outcomes of interest we used time-to-event analysis. Specifically, we used the Cox regression model. In Cox regression the hazard rate among exposed is compared with the hazard rate among non-exposed. The hazard rate it determined as the rate (probability per unit time) of experiencing a specific event at time *t* among all subjects who have not experienced any prior event up to time *t*. Hazard rates are calculated in a dynamic population, the 'at risk' population, as opposed to cumulative risk which is derived from fixed cohorts and relative to the baseline population. The hazard rate can be viewed as the average frequency or "speed" with which an event occurs and it is often referred to as the instantaneous risk of a given outcome.[70] In a Cox regression model no assumption is made about the underlying hazard function. This particular property results in the underlying assumption that the hazards in different exposure groups must be proportional throughout the study. We checked the proportional hazard assumption for all covariates in all studies with visual inspection of log-log plots. The underlying time axis in the Cox proportional hazard models was time since

inclusion into the study, and, as such, either time since incident atrial fibrillation (study I and II) or time since the latter albuminuria measurements (study III).

In study II and III, extreme outliers of body mass index and systolic blood pressure (susceptible to erroneous registration) were categorized as missing variables. Missing variables of body mass index, smoking status, systolic blood pressure, and HbA1c were handled with the 'missing indicator' method. When using the 'missing indicator' method to handle missing covariate data, missing observations are set to a fixed value, and an extra dummy variable is added to the multivariable model to indicate whether the value for that variable is missing.

In all analyses, we adjusted for selected risk factors or available confounders as appropriate. In study I, we sought to establish whether type of diabetes was associated with ischaemic stroke independently of the factors in the current European stroke risk stratification tool (the CHA_2DS_2-VASc score) in atrial fibrillation. Therefore, we included the CHA_2DS_2-VASc score components (congestive heart disease, hypertension, age, ischaemic stroke, vascular disease, and sex) and antiplatelet treatment in the Cox regression analysis. As we did not seek to investigate an aetiological relationship between type of diabetes and the risk of ischaemic stroke, we did not adjust for all factors that may confound the association between type of diabetes and the risk of thromboembolism.

In study II, we aimed to elucidate the aetiological relationship between glycaemic status and the risk of thromboembolism. Therefore, we adjusted for all available confounders that were selected a priori based on current literature, including congestive heart failure, hypertension, prior ischaemic stroke/transient ischaemic attack, vascular disease, sex, chronic kidney disease, smoking status, body mass index, age, diabetes duration, statin treatment, antiplatelet treatment, metformin treatment, and anticoagulation treatment.

In study III, we sought to examine the association between albuminuria and cardiovascular events/all-cause mortality, independent of other known risk factors for these outcomes. Therefore, we adjusted for other known cardiovascular risk factors. Adjustment was applied in different steps: model 1 included: Age and sex; model 2 included: Cardiovascular risk profile (in addition to the variables included in model 1 we adjusted for atrial fibrillation, congestive heart failure, diabetes duration, HbA1c, body mass index, smoking status, low-density lipoprotein cholesterol, systolic blood pressure); model 3 included: Cardiovascular risk profile and cardiovascular preventive treatment (in addition to the variables included in model 2 we adjusted for anticoagulation treatment, antiplatelet treatment, angiotensin-converting enzyme inhibitors/ angiotensin-receptor blockers).

Data were analysed with Stata Statistical Software: Release 14 (College Station, TX: StataCorp LP) and R version 3.3.3 (R Foundation for statistical Computing, Vienna, Austria). Results are reported with 95% confidence intervals (CIs).

Secondary analyses

In study I, we performed a secondary analysis where patients were stratified by baseline age into categories of: age <65 years, 65-74 years, and ≥75 years. The purpose of this analysis was to elucidate whether the association between type of diabetes and thromboembolism differed among different age groups.

In study II, we performed a secondary analysis where patients were stratified by baseline duration of diabetes into categories of: diabetes duration <10 years and ≥10 years. The purpose of this analysis was to examine whether the association between glycaemic status and thromboembolism differed among patients with different duration of diabetes. We chose the arbitrary cut point of <10 years and ≥10 years of diabetes duration based on evaluations of the clinical trials investigating the effect of intensive glycaemic control on the risk of cardiovascular disease in patients with diabetes. The evaluation of these trials have suggested that the primary effect of intensive treatment was within patients with a shorter duration of diabetes, which was also estimated to be a cut point of approximately 10 years.[71]

Sensitivity analyses

In study I, we classified patients as either type 1 or type 2 diabetes with the use of an algorithm developed by the Danish Health Data Authority,[68] however, after the initial classification around 24% of the patients with type 1 diabetes were baseline users of an oral glucose-lowering drug. Patients using oral glucose lowering drugs are most likely to be patients with type 2 diabetes, which may indicate that we misclassified some patients. To assess whether our analysis was affected by misclassification of the exposure groups, we relocated all baseline users of oral blood glucose-lowering drugs in the 'type 1 diabetes' group to the 'type 2 diabetes' group.

In study II, we restricted the study population to patients who had an HbA1c measurement within the past year before or 4 weeks after a first-time atrial fibrillation diagnosis. The analysis was intended to assess whether the use of up to two years old HbA1c measurements induced misclassification of the exposure. Furthermore, the baseline characteristics of patients who were excluded due to not having a HbA1c measurement within the past two years before or 4 weeks after index date was compared to the baseline characteristics of the included patients. We performed this analysis to assess whether our results could be generalized to a broad population of patients with type 2 diabetes and incident atrial fibrillation.

In study III, we repeated the approach in the main analysis for patients with a consistent albuminuria category in both albuminuria measurements. This analysis was intended to assess whether the uncertain exposure categorization induced misclassification bias in the main analysis.

3.7. APPROVAL AND ETHICS

The studies were approved by the Danish Data Protection Agency, file No. 2008-58-0028. In Denmark, no ethical approval is required for anonymous registry studies.

CHAPTER 4. TYPE OF DIABETES AND RISK OF ISCHAEMIC STROKE IN ATRIAL FIBRILLATION

As outlined in Chapter 1, patients with atrial fibrillation and comorbid diabetes have a higher risk of ischaemic stroke compared to those without diabetes. However, estimated incidence rates have varied considerably ranging between 3.6 and 8.6 per 100 person-years in different studies.[72] The observed risk difference might be related to the diversity of the diabetes population, underlining the need to improve stroke risk stratification in patients with diabetes and atrial fibrillation.

In study I, we aimed to examine whether type 1 and type 2 diabetes were associated with different risks of thromboembolism among patients with incident nonvalvular atrial fibrillation. In the following section, the results of study I will be presented and discussed in relation to other studies that have aimed to identify prognostic factors for ischaemic stroke in patients with atrial fibrillation and diabetes.

4.1. CURRENT RISK STRATIFICATION IN ATRIAL FIBRILLATION AND DIABETES

In patients with atrial fibrillation, the European Society of Cardiology guidelines currently recommend to assess the risk of ischaemic stroke with the CHA_2DS_2-VASc score.[59] The CHA_2DS_2-VASc score is a point based prediction model that allocates one point for each of the risk factors; congestive heart failure, hypertension, diabetes, vascular disease, age 65 to 74 years, and female sex, and two points for age ≥75 years and previous ischaemic stroke, systemic embolism, and/or transient ischaemic attack. The guideline-recommended function of the prediction model is to assist the clinician in deciding whether or not to initiate oral anticoagulation treatment in patients with atrial fibrillation. The underlying principle is that the benefit from the expected absolute reduction in ischaemic stroke risk from oral anticoagulation treatment must exceed the expected harm from the increased risk of bleeding associated with the treatment itself. Currently, there is strong evidence to support that the high-risk group of patients with a CHA_2DS_2-VASc score of 2 or more in men, and 3 or more in women will benefit from treatment with oral anticoagulants.[73–76] Benefits are less clear in patients with a CHA_2DS_2-VASc score of 1 for men, and 2 for women.[77] Currently, the European guidelines recommend that oral anticoagulation treatment should be considered for this group, weighing the expected stroke risk reduction, bleeding risk, and patient preference.[59] The lack of clear-cut recommendations for this group of patients arise from the substantial variation of observed ischaemic stroke risk in various studies of patients with atrial fibrillation and a CHA_2DS_2-VASc score of 1.

Observed rates per 100 person-years from currently available studies in patients with a CHA_2DS_2-VASc score of 1 have ranged from 0.1 to 6.6.[78] Similarly, rates per 100 person-years for patients with a CHA_2DS_2-VASc score of 0 have ranged from 0.04 to 2.4.[79] As these event rates emerge from various data sources, the large variation in events rates may in part be due the variations in the validity of the stroke diagnosis in administrative databases and in particular methodological differences in study design.[80,81] However, it is also conceivable that the score is poorly calibrated across different cohorts. Calibration is the degree to which the predicted risk of the score matches the observed risks across the range of risk scores. In that regard, overall calibration of the CHA_2DS_2-VASc score is of less clinical importance as the primary function of the CHA_2DS_2-VASc score is identify patients who are at a 'truly low risk' and will not have a net clinical benefit from anticoagulation treatment. However, as observed rates per 100 person years in patients with a CHA_2DS_2-VASc score of 0 or 1 have ranged both above and below the suggested 'tipping point' for anticoagulation treatment (0.9 for dabigatran and 1.7 for warfarin)[82] there seems to be room for improvement.

A potential source of the mediocre performance of the CHA_2DS_2-VASc score may be the simple dichotomising of the risk factors incorporated in the score. This is the case for patients with diabetes. Diabetes is simply dichotomized into presence or absence of diabetes. Nonetheless, the diabetes population is diverse as it includes patients with different types of diabetes, varying duration of disease, and varying levels of glycaemic control. Assuming that all patients with diabetes and atrial fibrillation have the same risk of ischaemic stroke is a simplistic approach. Thus, for the purpose of risk stratification for ischaemic stroke prevention among patients with diabetes, the CHA_2DS_2-VASc score may be viewed as a too simple tool. The simplicity may allow for ease of use, which has been proposed to improve adherence to guideline recommendations.[83] However, the lack of granularity of the CHA_2DS_2-VASc score may give rise to treatment decisions that are suboptimal for the individual patient. As anticoagulation treatment is potentially a life-long treatment with a consequential increased bleeding risk, precision in risk stratification trumps ease of use. Thus, further development of the tool to improve the predictive performance may facilitate better risk stratification in the specific subgroup of patients with both atrial fibrillation and diabetes and thereby optimize clinical decisions about antithrombotic treatment in every day clinical practice.

4.2. IMPROVEMENT OF STROKE RISK STRATIFICATION IN DIABETES AND ATRIAL FIBRILLATION

Depending on the population studied the risk of ischaemic stroke in patients with diabetes and atrial fibrillation has varied considerably.[72,84–89] This is, however, not surprising as the diabetes population includes patients with different metabolic disorders. The majority of diabetes patients can be divided into type 1 and type 2 diabetes, diseases with different distribution of cardiovascular risk factors that are not

included in the current risk stratification tool. Patients with type 1 and type 2 diabetes may differ with regard to their duration of disease, physical activity level, body mass index, and presence of dyslipidaemia. These factors have been associated with a higher ischaemic stroke risk in atrial fibrillation,[90–93] but whether that translates into differences in the ischaemic stroke risk among patients with atrial fibrillation and type 1 or type 2 diabetes is unknown. To explore whether the type of diabetes could serve as an easily available clinical risk prediction marker, we aimed to investigate whether subdividing patients into categories of type 1 and type 2 diabetes would provide additional prognostic information beyond the CHA_2DS_2-VASc score.

Results of study I

In study I, we identified 10,058 non-anticoagulated patients with a prior diagnosis of diabetes and an incident nonvalvular atrial fibrillation diagnosis. A total of 762 (7.6%) patients had a thromboembolic event during the three years of follow-up. Incidence rates of thromboembolism at one and three-year follow-up, both overall and stratified by age, are listed in Table 2. Incidence rates generally attenuated at three-years of follow-up, indicating that most events occurred early in the follow-up period after the atrial fibrillation diagnosis. Overall, we did not find a difference in the risk of thromboembolism when comparing patients with type 2 diabetes to patients with type 1 diabetes (Table 3). However, in an additional age stratified analysis we found that in patients aged <65 years, type 2 diabetes was associated with a higher risk of thromboembolism as compared with type 1 diabetes.

Table 2. Crude incidence rates per 100 person-years of thromboembolism at one- and three-year follow-up, overall and stratified by age

	1 year follow-up		3 year follow-up	
	Type 1 diabetes	**Type 2 diabetes**	**Type 1 diabetes**	**Type 2 diabetes**
Total population				
Number of patients	1,277	8,781	1,277	8,781
Events no.	73	543	86	676
Incidence rate	12.49	14.32	7.15	8.62
Age <65 years				
Number of patients	342	1,403	342	1,403
Events no.	12	54	13	74
Incidence rate	6.20	8.26	2.91	5.01
Age 65-74 years				
Number of patients	393	2,487	393	2,487
Events no.	24	157	31	186
Incidence rate	15.26	15.36	9.90	8.69
Age ≥75 years				
Number of patients	542	4,891	542	4,891
Events no.	37	332	42	416
Incidence rate	15.83	15.70	9.45	9.85

Table 3. Hazard ratios (95% CI) of thromboembolism at one- and three-year follow-up, overall, and stratified by age (reference: type 1 diabetes)

	1 year follow-up	3 year follow-up
Overall	1.10 (0.85-1.40)	1.15 (0.91-1.44)
Age <65 years	1.47 (0.77-2.79)	2.01(1.09-3.68)
Age 65-74 years	1.07 (0.69-1.65)	1.00 (0.68-1.47)
Age ≥75 years	1.02 (0.73-1.44)	1.09 (0.79-1.50)

The analyses were adjusted for the components of the CHA2DS2-VASc score (congestive heart failure, hypertension, age [continuous covariate], prior ischaemic stroke/transient ischaemic attack, myocardial infarction/peripheral artery disease, and female sex) and baseline use of antiplatelet treatment.

In a sensitivity analysis, we reassigned around 24% of the patients with type 1 diabetes to the group with type 2 diabetes because they had a prescription of an oral glucose-lowering drug. The results were similar to those of the main analysis, with an adjusted HR of thromboembolism of 1.09 (95% CI: 0.85-1.40) when comparing type 2 diabetes with type 1 diabetes.

Discussion

Overall, our results do not suggest that the type of diabetes was associated with different risks of ischaemic stroke when adjusting for the individual risk factors in the CHA_2DS_2-VASc score. However, we did find a higher risk of ischaemic stroke in patients with type 2 diabetes in the subset of patients aged less than 65 years. This is the subset of patients for whom the observed thromboembolism risk is usually the lowest and therefore most uncertainty about initiation of anticoagulation treatment exist in this particular group. In that respect, replication of our study findings is of interest to extend the clinical evidence specifically in this subgroup.

Although, no other studies have specifically focused on type of diabetes as a predictor of ischaemic stroke in atrial fibrillation, several studies have focused on identifying stroke risk predictors among patients with diabetes and atrial fibrillation. Some studies have specifically aimed for refining the CHA_2DS_2-VASc score, whereas other studies have focused on identifying diabetes-related risk factors that may be associated with a higher ischaemic stroke risk, independently of other known stroke risk factors. Currently, factors such as duration of diabetes, glycaemic status, and microvascular complications have been investigated (see Table 4 for an overview of the results of other studies).[94–98] For instance, Overvad et al. investigated whether duration of diabetes could refine the CHA_2DS_2-VASc score in a large Danish register based study.[94] They found a clear dose-response relationship between duration of diabetes and the risk of ischaemic stroke after adjusting for the individual factors in the CHA_2DS_2-VASc score. Similarly, Ashburner et al. found that a duration of diabetes over three years was associated with a higher rate of ischaemic stroke when adjusting for several risk factors for ischaemic stroke including the individual elements of the CHA_2DS_2-VASc score.[95] Ashburner et al. also investigated the association between

glycaemic status as reflected by HbA1c, but did not find a difference in the risk of ischaemic stroke in different categories of HbA1c after adjustment for several known risk factors for ischaemic stroke. This finding is in contrast to the findings of Saliba et al. who performed a large register based study in Israel where HbA1c in quartiles exhibited a dose-response relationship with the risk of ischaemic stroke and when added to CHA_2DS_2-VASc score helped to improve the predictive accuracy.[96] Their finding is supported by our results in study II, where we observed a dose-response relationship between HbA1c levels and the risk of ischaemic stroke. However, only among patients with shorter duration diabetes. This result may suggest that HbA1c would only be a meaningful stroke risk marker in the subgroup of patients with diabetes duration under 10 years.

Not only diabetes duration and glycaemic status display a potential for refining stroke risk prediction in atrial fibrillation and diabetes. Subdivision of patients into groups of insulin-treated and noninsulin-treated diabetes may also be a potential approach for breaking down the diabetes category in the CHA_2DS_2-VASc score. This approach was examined by Patti et al. and Mentias et al. who both found that insulin-treated diabetes was associated with a higher risk of ischaemic stroke compared with noninsulin-treated diabetes.[99] It serves mentioning that insulin treatment itself may not be causally associated with an increased risk of ischaemic stroke, but is more likely to be a proxy measure for severity of type 2 diabetes and the degree of insulin resistance. Another marker of severity of disease is the presence of retinopathy, nephropathy, and neuropathy in patients with atrial fibrillation and diabetes. Two studies have examined the association between the presence of these microvascular complications and the risk of ischaemic stroke.[97,98] After adjusting for several risk factors also beyond those embedded in the CHA_2DS_2-VASc score, the risk of ischaemic stroke was similar between patients with and without microvascular complications. Thus, the presence or absence of retinopathy, nephropathy, and neuropathy may be less suitable for refining stroke risk stratification in diabetes and atrial fibrillation.

The current literature clearly indicate that the risk of ischaemic stroke is not uniform in patients with diabetes and atrial fibrillation. It also illustrates the potential for including other factors in risk stratification of patients with diabetes and atrial fibrillation. Potential risk factors may include diabetes duration, glycaemic status, type of diabetes, and insulin versus non-insulin treated diabetes. These factors have the advantage of being readily available in everyday clinical practice. Moreover, these potential risk markers are reasonably evenly distributed in a diabetes population making them suitable for risk prediction. Identifying risk prediction markers that are only prevalent in a minority of the population would provide little clinical value.

Table 4. Overview of studies investigating prognostic factors for ischaemic stroke or systemic embolism in patients with diabetes and atrial fibrillation

	Definition of subdivision	Incidence rate*	Hazard Ratio (95% CI)
Saliba et al.[96]	Glycated haemoglobin†		
	No diabetes	2.59	1 (reference)
	Quartile 1 (<46 mmol/mol)	3.22	1.04 (0.83-1.30)
	Quartile 2 (46-52 mmol/mol)	3.51	1.14 (0.92-1.42)
	Quartile 3 (52-61 mmol/mol)	4.50	1.46 (1.19-1.79)
	Quartile 4 (>61 mmol/mol)	4.56	1.63 (1.33-2.00)
Fangel et al.[100]	Glycated haemoglobin & diabetes duration<10 years		
	HbA1c <48 mmol/mol	1.44	1 (reference)
	HbA1c = 49-58 mmol/mol	2.88	2.38 (1.54-3.70)
	HbA1c >58 mmol/mol	2.73	2.58 (1.55-4.28)
	Glycated haemoglobin & diabetes duration ≥10 years		
	HbA1c ≤48 mmol/mol	3.07	1 (reference)
	HbA1c = 49-58 mmol/mol	2.47	0.86 (0.55-1.35)
	HbA1c >58 mmol/mol	2.75	0.96 (0.62-1.48)
Ashburner et al.[95]	Glycated haemoglobin*		
	HbA1c <53.0 mmol/mol	2.6	1 (reference)
	HbA1c = 53.0-74.9 mmol/mol	2.9	1.21 (0.77-1.91)
	HbA1c ≥75.0 mmol/mol	2.9	1.04 (0.57-1.92)
	Diabetes duration		
	No diabetes	1.8	1 (reference)
	Diabetes duration <3 years	1.9	1.15 (0.84-1.58)
	Diabetes duration ≥3 years	3.2	1.63 (1.29-2.05)
Overvad et al.[94]	Diabetes duration		
	No diabetes mellitus	2.4	1 (reference)
	Diabetes duration 0-4 years	2.8	1.11 (1.03-1.20)
	Diabetes duration 5-9 years	3.7	1.32 (1.20-1.44)
	Diabetes duration 10-14 years	4.0	1.28 (1.13-1.45)
	Diabetes duration ≥15 years	4.5	1.48 (1.29-1.70)
Lip et al.[98]	Retinopathy		
	No retinopathy	4.16	1 (reference)
	Retinopathy	4.86	1.21 (0.80-1.84)
Chou et al.[97]	Microvascular complications		
	None	4.65	1 (reference)
	Retinopathy	5.07	1.11 (0.95-1.30)
	Nephropathy	4.77	1.03 (0.93-1.14)
	Neuropathy	5.20	1.15 (1.07-1.24)
Patti et al.[99]	Insulin- vs. noninsulin-requiring diabetes		
	Noninsulin-requiring diabetes	1.8	1 (reference)
	Insulin-requiring diabetes	5.2	2.96 (1.49-5.87)
Mentias et al.[101]	Insulin- vs. noninsulin-requiring diabetes		
	Noninsulin-requiring diabetes	2.3	1 (reference)
	Insulin-requiring diabetes	2.6	1.15 (1.09-1.20)

* Incidence Rates are per 100 person-years. †HbA1c units are converted from DCCT (Diabetes Control and Complication) units to IFFC (International Federation of Clinical Chemistry) units.[102] CI: Confidence interval.

However, several limitations concerning the available evidence serves mentioning. Firstly, the potential prediction markers have only been investigated in a few population-based studies. Several of these studies did not include incident atrial fibrillation,[95,96,99] which may underestimate true stroke risk as several studies have shown that the risk of ischaemic stroke is higher in the first year after initial onset of atrial fibrillation.[103–105]

Secondly, a critical limitation in current evidence is the handling of patients on anticoagulation treatment in the available studies. As it is universally accepted that anticoagulation treatment is beneficial for high-risk atrial fibrillation patients, completely non-anticoagulated cohorts of patients do not exist. Therefore, studies may either choose to focus on mixed cohorts of both anticoagulated and non-anticoagulated patients or strictly evaluate ischaemic stroke risk in non-anticoagulated cohorts.[81] In study I and some of the other studies, non-anticoagulated cohorts were used to asses ischaemic stroke risk,[95–97] whereas others looked at mixed cohort of both anticoagulated and non-anticoagulated patients.[94,98,99] On one hand, studies including only patients who are not using anticoagulants, may result in a selected subtype of atrial fibrillation patients including low-risk patients and patients in whom the clinician is reluctant to initiate oral anticoagulant treatment. On the other hand, in studies of mixed cohorts where a large proportion or the majority of the patients were receiving anticoagulation, the results may not be applicable to patients with diabetes and atrial fibrillation who are not anticoagulated. This is a particularly important concern, since anticoagulant treatment decision rely on observed risks of ischaemic stroke in untreated populations. In study I, we chose not to include patients already receiving anticoagulants and furthermore, we censored patients when they initiated anticoagulation treatment. This approach was chosen because the CHA_2DS_2-VASc score is recommended by the guidelines for stroke risk assessment prior to anticoagulant treatment decisions in patients with atrial fibrillation. However, this choice limits the external validity of the study results.

Thirdly, an inherent problem in current literature is the lack of focus on ischaemic stroke subtype.[106] Although the majority of ischaemic strokes (up to 80%) in atrial fibrillation are presumed to be of cardioembolic origin, ischaemic strokes of other origins also occur.[107,108] Neither clinical trials nor the cohort studies seeking to identify ‘truly low risk’ patients have accounted for potential differences in observed results based on stroke subtypes. As diabetes is a strong risk factor for developing atherosclerotic cardiovascular disease and several studies have shown that in non-atrial fibrillation populations diabetes is associated with non-cardioembolic strokes,[50,109,110] but not cardioembolic strokes,[49,50] it raises the question whether the observed increased risk of stroke in prior cohort studies including diabetes patients and in study I and II are in fact cardioembolic. As oral anticoagulants primarily prevent ischaemic strokes of cardioembolic origin, ignoring stroke subtype could theoretically lead to suboptimal treatment decision for patients with atrial fibrillation and a concomitant strong atherosclerotic risk profile.

Finally, several studies did not aim to specifically refine the CHA_2DS_2-VASc score, but merely to clarify whether an association between the risk factor under study and ischaemic stroke was present, independent of other known risk factors. Thus, whether these risk factors would refine stroke risk stratification with the CHA_2DS_2-VASc score is unknown. Moreover, the accuracy of a model is often only modestly improved when the added novel risk factor is positively correlated with the existing predictor variables.[111] Thus, it is unclear whether the above-mentioned risk factors would provide additional information when added simultaneously to the same risk score. In that regard, testing all factors together in a single study to elucidate which factors improve risk prediction the most is warranted.[106] Evaluation of the performance of an extended prognostic model by testing the calibration of an extended model is also necessary. In terms of evaluating the predictive ability of the CHA_2DS_2-VASc score, focus has primarily been on determining the discriminative performance of the CHA_2DS_2-VASc score that is, the ability to rank people correctly in order of their risk and separate those who will develop an event from those who will not.[79] The discriminative performance of the CHA_2DS_2-VASc score has been estimated to be modest with a pooled C-statistics of 0.64 in general populations and 0.71 in a hospital setting.[112] However, from a clinical point of view the discriminative performance is of less interest as it does not directly influence treatment decisions. We did not evaluate the discriminative ability of a refined model in study I. As no prior studies have explored whether an association between the type of diabetes and risk of thromboembolism exist, we chose to start out by elucidating whether there was an association and whether that association was of a clinically meaningful magnitude.[113] Moreover, even though a novel risk factor only provides little or no improvement in the discriminative performance of the model, it does not exclude that novel risk factor from improving the usefulness of the model.[114]

Another concern may be the application of an extended model. Adding factors to the CHA_2DS_2-VASc score would add complexity and thereby make everyday use in the clinical practice more complicated. This may raise the concern that the application of the score would decrease. However, digital tools derived from e.g. machine learning and implemented through digital administrative registries may accommodate even more advanced risk prediction models in the future without compromising their use in everyday clinical practice.[115]

As previously stated, it is currently recommended that patients with atrial fibrillation and a CHA_2DS_2-VASc score of 1 should be considered for anticoagulation treatment, balancing stroke risk, bleeding risk, and patient preference. This makes all patients with diabetes potential candidates for life long anticoagulation treatment. The observed incidence rates of ischaemic stroke associated with diabetes in the studies of patients with diabetes and atrial fibrillation with a CHA_2DS_2-VASc score of 1 have ranged well above the current recommendation of 1 to 2 per 100 person years.[84,116] In study I, we also observed incidence rates for all subgroups that ranged well above the treatment recommendations. Based on these observations, having 1 point due to

diabetes may in itself carry a magnitude of risk that indicates a net clinical benefit from oral anticoagulation treatment. Nonetheless, the presence of type 2 diabetes should perhaps favour initiation of anticoagulation compared with type 1 diabetes, specifically in cases of doubt regarding initiation of oral anticoagulation.

In conclusion, study I showed no overall association between the type of diabetes and the risk of thromboembolism among patients with atrial fibrillation. However, among the subgroup of patients aged below 65 years, type 2 diabetes was associated with a higher risk of thromboembolism when compared with type 1 diabetes. Patients aged below 65 years are usually patients with the lowest risk of thromboembolism. Hence, most uncertainty about the benefit of anticoagulation treatment is related to this group of patients and our findings indicate that type 2 diabetes is associated with a higher risk of thromboembolism than type 1 diabetes in this particular group. The combined results of study I and the other above-mentioned studies provides a perspective on how risk prediction might be improved regarding the subpopulation of diabetes patients with atrial fibrillation. The observed associations suggest that adding factors like insulin-treated diabetes, diabetes duration, and HbA1c level may provide better prediction of ischaemic stroke in patients with atrial fibrillation than the simple presence or absence of diabetes. However, there is a need for further validation of current findings, specifically whether adding these factors to the CHA_2DS_2-VASc score may facilitate identification of 'truly low-risk' patients in the diabetes subgroup, who may be better off without anticoagulation, as opposed to the current approach where all diabetes patients are potential candidates for life long oral anticoagulation. At present, there is not enough evidence to support a subdivision of diabetes patients according to diabetes related factors and diabetes should continue to be incorporated into CHA_2DS_2-VASc score as an entity.

CHAPTER 5. GLYCAEMIA AND RISK OF THROMBOEMBOLISM IN ATRIAL FIBRILLATION

As outlined in Chapter 4, diabetes has been associated with a higher risk of ischaemic stroke in patients with atrial fibrillation.[72,84–89] However, the underlying biological aspects that explain the link between diabetes and thromboembolic events are unclear. Chronic exposure to raised concentrations of glucose has been suggested to contribute to the development of ischaemic stroke and the association between elevated HbA1c and ischaemic stroke in patients with diabetes has been studied extensively showing a clear dose-response relationship.[117–120] Moreover, it has been suggested that the risk of ischaemic stroke is increased in patients with diabetes and hyperglycaemia, but not in those without hyperglycemia.[121] Hence, the presence of hyperglycaemia in diabetes may in part explain the increased risk of ischaemic stroke in patients with diabetes.

Whether hyperglycaemia contributes to the development of ischaemic stroke in patients with atrial fibrillation, where the origin of ischaemic strokes are predominantly cardioembolic, is unclear.[107,108] A few studies have examined the relationship between glycaemic status and the risk of ischaemic stroke with conflicting findings.[95,96] In study II, we addressed this discrepancy, by exploring the effect of glycaemic status on the risk of thromboembolism in patients with atrial fibrillation and type 2 diabetes. In the following section, the results of study II will be presented and discussed in perspective to other studies that have aimed to examine the association between HbA1c and the risk of ischaemic stroke in patients with atrial fibrillation and diabetes.

Results of study II

The study population included 5,386 patients with incident nonvalvular atrial fibrillation and type 2 diabetes. The incidence rates per 100 person-years increased in a dose-response manner across increasing levels of HbA1c (Table 5). Compared with patients with HbA1c <48 mmol/mol, we observed a higher risk of thromboembolism among patients with HbA1c = 49-58 mmol/mol and HbA1c >58 mmol/mol in the adjusted Cox regression analysis (Table 6). In analyses stratified by diabetes duration, a strong association between elevated levels of HbA1c and thromboembolism was found in patients with diabetes duration of <10 years. In contrast, we observed no difference in thromboembolic risk across different levels of HbA1c among patients with a diabetes duration of ≥10 years.

Table 5. Crude incidence rates per 100 person-years of thromboembolism at five-year follow-up, overall and stratified by diabetes duration

	HbA1c ≤48 mmol/mol	HbA1c 49-58 mmol/mol	HbA1c >58 mmol/mol
Total population			
Number of patients	2,120	1,657	1,609
Events no.	74	91	98
Incidence rate	1.92	2.66	2.74
Diabetes duration <10 years			
Number of patients	1,428	782	465
Events no.	39	47	29
Incidence rate	1.44	2.88	2.73
Diabetes duration ≥10 years			
Number of patients	692	875	1,144
Events no.	35	44	69
Incidence rate	3.07	2.47	2.75

Table 6. Hazard ratios (95% CI) of thromboembolism at five-year follow-up, overall and according to diabetes duration (reference: HbA1c ≤48)

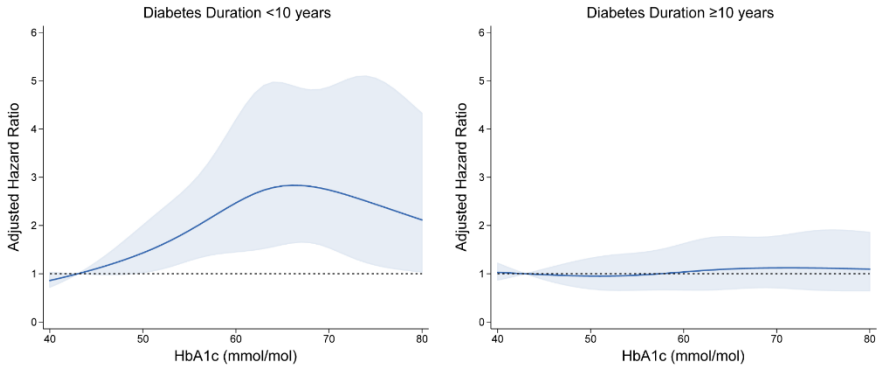
	HbA1c 49-58 mmol/mol	HbA1c >58 mmol/mol
Total population	1.49 (1.09-2.05)	1.59 (1.13-2.22)
Diabetes duration <10 years	2.38 (1.54-3.70)	2.58 (1.55-4.28)
Diabetes duration ≥10 years	0.86 (0.55-1.35)	0.96 (0.62-1.48)

The analysis was adjusted for congestive heart failure, hypertension, prior stroke/transient ischaemic attack, vascular disease, sex, chronic kidney disease, smoking status, body mass index, age, diabetes duration, statin treatment, antiplatelet treatment, metformin treatment, and anticoagulation treatment.

Sensitivity analysis

In a sensitivity analysis, we repeated the approach in the main analysis for the 3,857 patients who had an HbA1c measurement within the year before or four weeks after a first-time atrial fibrillation diagnosis. The results were similar to the main analysis with HR of 1.42 (95% CI: 0.98-2.06) and HR of 1.50 (95% CI: 1.02-2.22) for patients with HbA1c = 49-58 mmol/mol and HbA1c >58 mmol/mol, respectively. In addition, the baseline characteristics of patients excluded due to missing HbA1c values were assessed. On average this population was younger, had a shorter duration of diabetes, had a lower prevalence of comorbidity, and received less medication than the patients who were included in the study (data not shown).

Figure 1. Continuous analysis of HbA1c levels and adjusted hazard ratios of thromboembolism stratified by diabetes duration



HbA1c level was modelled with a restricted cubic spline. The applied reference value was 43 mmol/mol (calculated as the median HbA1c value of the reference group in the main analysis). The analysis was adjusted for congestive heart disease, hypertension, prior ischaemic stroke, vascular disease, sex, chronic kidney disease, smoking status, body mass index, age (modelled as a restricted cubic spline), statin treatment, antiplatelet treatment, metformin treatment, and anticoagulation treatment (as a time-varying covariate). Solid blue lines indicate the hazard function, and the blue shaded areas are 95% confidence intervals. Reproduced from Circ Arrhytm Electrophysiol. 2019;12:e007030, Copyright American Heart Association.

Discussion

In study II, we observed a dose-response pattern between increasing levels of HbA1c and the risk of thromboembolism in patients with a shorter duration of diabetes. In contrast, we observed no difference in the associated thromboembolic risk between the different levels of HbA1c among patients with a longer duration of diabetes. Recent cohort studies have sought to explore the role of glycaemic status on the risk of ischaemic stroke in patients with atrial fibrillation and the results have been conflicting. In a cohort of 37,358 patients with atrial fibrillation, Saliba et al. demonstrated that patients with diabetes and elevated HbA1c levels had a higher rate of ischaemic stroke when compared with patients without diabetes (Table 4).[96] Moreover, they observed a dose-response pattern with the rates of ischaemic stroke increasing across the increasing quartiles of HbA1c. Contrastingly, in a cohort of 1,993 patients with atrial fibrillation and comorbid diabetes mellitus, Ashburner et al. observed that both poor (HbA1c >75 mmol/mol) and moderate (HbA1c = 53-75 mmol/mol) glycaemic status were not significantly associated with a higher rate of ischaemic stroke compared with HbA1c <53 mmol/mol (Table 4).[95]

In the interpretation and comparison of their findings with our observations, there are some essential methodological differences to consider. Firstly, our results suggest that the association between HbA1c level and the risk of ischaemic stroke may be modified by diabetes duration. In our study, the extent of this effect modification was

substantial as we observed a clear association between HbA1c and ischaemic stroke among patients with a shorter duration of diabetes and no association between HbA1c and ischaemic stroke among patients with a longer duration of diabetes. This finding suggests that it may be more appropriate to study the association between HbA1c level and the risk of ischaemic stroke separately for patients with shorter and longer duration of diabetes. Saliba et al. and Ashburner et al. presented results reflecting the association between hyperglycaemia and the risk of ischaemic stroke for an overall cohort of diabetes. In light of the observed interplay between glycaemic level and duration of diabetes in study II, their results may not be representative for subgroups of patients with different diabetes durations.

Secondly, the reference groups varied in all three studies. In the study by Saliba et al., the reference group was patients without diabetes whereas Ashburner et al. used patients with diabetes and an HbA1c <53 mmol/mol as their reference group. In study II, we used a cut-point of HbA1c <48 mmol/mol as the reference group. Moreover, we observed that the risk of thromboembolism increased from levels of HbA1c lower than 53 mmol/mol among patients with shorter duration of diabetes. This may, in part, explain why Ashburner et al. did not find that glycaemic status was associated with ischaemic stroke.

Thirdly, confounder control differed between the studies. In study II and in the study by Ashburner et al., the analysis was adjusted for a broad range of potential confounders. Saliba et al. only adjusted for the CHA_2DS_2-VASc score components which makes a direct comparison between their study and study II unfeasible.

Fourthly, in our study we excluded patients with anaemia and chronic kidney disease as HbA1c measurements may be misleadingly low in these patients.[122–124] Neither Saliba et al. or Ashburner et al. accounted for the potential misclassification bias that may have arisen from including these patient groups. The diabetes population in the study of Ashburner et al. included approximately 17% with significant kidney dysfunction, and as such a potential exposure misclassification cannot be viewed as a negligible problem.[95]

Finally, neither Ashburner et al. or Saliba et al. included patients with incident atrial fibrillation, which may have led to an underestimation of the ischaemic stroke risk, as several studies show that the risk of ischaemic stroke is higher during the first year after an atrial fibrillation diagnosis.[103–105]

In study II, we aimed to explore whether a potential aetiological relationship exists between glycaemic level and the risk of ischaemic stroke. Essentially, interpretation of an aetiological study with an observational design is challenging. The observational study design requires much stronger assumptions for making inference about causation than what is required for a randomized study design. Importantly, the strongest assumption (no residual or unobserved confounding) cannot be tested in an

observational study design, and therefore causal inference from observational studies is still controversial. In 1965, Austin Bradford Hill proposed nine viewpoints that may be taken into consideration when wishing to make causal inference based on an observed association.[125] The observed association between HbA1c level and thromboembolism among patients with a shorter duration of diabetes fulfils several of the so called 'Bradford Hill Criteria':

(1) Strength: Hill proclaimed that the larger an association between exposure and disease, the more likely it is to be causal. However, it is likely that most effects studied in the real world are small and the strength of association does not necessarily contribute to the determination of causality. In study II, we found a strong association between HbA1c level and thromboembolism among patients with a shorter duration of diabetes. Even so, we cannot rule out residual confounding as an explanation for some or the entire observed risk difference. For instance, we did not adjust for factors like dietary patterns and physical activity. Moreover, a large proportion of patients have undiagnosed diabetes[126–129] and previous reports have suggested that onset of diabetes occurs 4-7 years before a clinical diagnosis of diabetes.[130] Hence, discrepancies between the diabetes duration based on clinical diagnoses and the actual physiological duration of disease may exist. If the patients with a shorter duration of diabetes and a high level of HbA1c are in fact patients who have had undiagnosed diabetes for a longer period, then the observed results could be confounded by diabetes duration, despite the fact that we adjusted for the time since a clinical diabetes diagnosis.

(2) Consistency: Hill stressed that a single observational study cannot be relied on to draw valid inference on cause and effect, as internal validity of an observational study is always questionable. The findings of other studies are not entirely consistent with the findings in study II. Several studies of patients with diabetes have shown a dose-response relationship between glycaemic level and the risk of ischaemic stroke.[117–120] Prior studies exploring the association between HbA1c and the risk of thromboembolism specifically in patients with atrial fibrillation have provided inconsistent results.[95,96] Thus, further research should focus on exploring the association between glycaemic level and risk of thromboembolism specifically in patients with atrial fibrillation. Moreover, the results of study II suggest that it may be reasonable to focus on potential differences of the importance of glycaemic status among patients with different durations of diabetes.

(3) Specificity: the criteria of specificity covers the view that associations are more likely to be causal if the exposure causes only one disease. This criterion is not considered relevant in modern epidemiology, as many exposures are known to contribute to the development of several different diseases and several diseases are caused by a combination of exposures. The association between diabetes and the risk of ischaemic stroke in atrial fibrillation is undoubtedly multifactorial and by no means explained by glycaemic control alone.

(4) Temporality: the temporality criterion covers the principle that the exposure must precede the onset of disease and is widely accepted that this criterion is essential to argue causal inference. In study II, an appropriate temporal relationship existed, as the HbA1c level was determined prior to the development of thromboembolism.

(5) Biological gradient: Hill argued that if a dose-response relationship is observed between an exposure and an effect, it is more likely to be causal. In study II, we observed a dose-response relationship between HbA1c level and the risk of thromboembolism among patients with a shorter duration of diabetes. However, we also observed that the thromboembolic risk attenuated at the highest levels of HbA1c in patients with diabetes duration <10 years. This could be an expression of a threshold effect of glycaemic level, where glycaemic levels above a certain threshold do not add additional thromboembolism risk. However, another possible explanation for this finding may be that these patients are more closely monitored by their physician due to their "uncontrolled" HbA1c level, leading to an overall better quality of oral anticoagulation treatment and other preventive strategies. We were unable to test this hypothesis, as we did not have access to information regarding quality of oral anticoagulation treatment or other preventive strategies.

(6) Plausibility and (7) Coherence: these criteria covers the perception that the cause-and-effect interrelationship should make sense with the knowledge available to the researcher. The results of study II could be biologically plausible as hyperglycaemia has been associated with several biological mechanisms that may promote thrombogenesis in patients with atrial fibrillation. Hyperglycaemia has been proposed to increase coagulability, fibrinolytic impairment, and impaired vascular function.[131,132] Similar mechanism such as endocardial damage, endothelial dysfunction, platelet hyperactivity, and increased coagulability may play a role in the thrombogenesis in atrial fibrillation. However, the biological plausibility for the absence of a higher rate of ischaemic stroke in patients with a poor glycaemic status among the patient group with a diabetes duration ≥10 years is less clear. One explanation may be that thrombotic abnormalities have become manifest among patients with a longer duration of diabetes thereby leading to a lesser role of the glycaemic status. Another explanation could be that tight glycaemic control increases the risk of hypoglycemia especially in patients with a longer duration of disease[133,134] and hypoglycemia has been associated with a higher risk of ischaemic stroke.[135]

(8) Experiment: Evidence from experimental manipulation where cessation of an exposure leads to a decline in disease risk has been proposed to strongly support causal inference. Several randomized clinical trials have investigated the effect of intensive glycaemic control on the risk of cardiovascular disease among patients with diabetes. The results have been conflicting and suggests a limited effect of tight glycaemic control in patients with diabetes.[136] However, post-hoc analyses of these trials suggests a potential beneficial effect of intensive treatment among patients with a shorter duration of diabetes.[71] Our results are in support of these findings from the

general diabetes cohorts, as we observed a lower risk of thromboembolism among patients with atrial fibrillation. However, it must be noted that the stroke mechanisms in atrial fibrillation are different from those in diabetes in general. Hence, the results of these randomized controlled trials may not be applicable to an atrial fibrillation population. Moreover, it must be considered that thromboembolism may be a result of multiple exposures and arise from a complex progression pathway. Multiple risk factors, including lifestyle and genetic predisposition are likely to contribute to the occurrence of thromboembolism. While the combination of these factors may culminate in disease, interventions to manipulate glycaemic levels alone may or may not reverse or slow the progression of thromboembolism. Thus, the observed associative exposure-response relationships in study II might not translate into a corresponding manipulative causal exposure-response relationships. In other words, we did not study a specific treatment approach against another and, therefore, randomized controlled trials evaluating the effect of specific glucose-lowering strategies are needed to provide conclusive evidence.[137] For example, the effect of lowering glucose with drugs may differ from lifestyle-related interventions which are likely to have other beneficial health side effects.

(9) Analogy: Bradford Hill proposed that when one causal agent is known the evidence needed to argue causality is lowered for a similar agent causing a similar disease. However, this is considered a very week criterion for causation. Moreover, the excessive knowledge accessible today would make it possible to identify an analogy for most situation.

Even though the observed association between HbA1c and thromboembolism among patients with shorter duration of diabetes fulfils several of the Bradford Hill criteria, these viewpoints were not intended to be hard-and-fast rules of evidence to accept cause and effect and Hill stated that “None of the nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis”. Thus, the results of study II are essentially to be considered as hypothesis-generating.

In conclusion, we demonstrated that poor glycaemic status was associated with a higher risk of thromboembolism in patients with atrial fibrillation and type 2 diabetes among patients with diabetes duration <10 years. Combining these results with prior findings, we conclude that the importance of glycaemic control in patients with diabetes and atrial fibrillation remain unclear. Hence, randomizes trials assessing whether strict glycaemic control would prevent thromboembolism specifically in patients with atrial fibrillation and type 2 diabetes are warranted.

CHAPTER 6. ALBUMINURIA AND CARDIOVASCULAR RISK IN TYPE 2 DIABETES

Risk stratification in patients with type 2 diabetes continues to be an important priority in the management of diabetes-related morbidity and mortality. Several risk factors have been proposed for cardiovascular risk stratification, including albuminuria.[35–37] Albuminuria is very common in patients with type 2 diabetes and around one quarter of patients with newly diagnosed diabetes develop microalbuminuria or nephropathy within ten years.[138] Early albuminuria research focused primarily on microalbuminuria a marker for a higher risk of developing diabetic kidney disease. However, subjects with microalbuminuria are not necessarily destined for worse renal function,[139,140] in fact only 25-30% of patients with microalbuminuria progress to chronic kidney disease.[138,141,142] Contrastingly, progression of microalbuminuria into macroalbuminuria is most often an indication of underlying chronic kidney disease.[138,141] Over the past two decades there has been an increased focus on microalbuminuria as a marker of cardiovascular disease and it is currently widely accepted that microalbuminuria is strongly associated with the risk of cardiovascular disease. The association between albuminuria levels and risk of cardiovascular disease has displayed a dose-response relationship[142–144] that may well begin at lower levels than the traditional thresholds for defining microalbuminuria.[141,145–147] Furthermore, progression of microalbuminuria has been shown to be associated with an increase in the risk of cardiovascular disease independent of the initial albuminuria level.[138,148,149] However, there has been less focus on micro- and macroalbuminuria as markers of incident cardiovascular disease in patients with type 2 diabetes.[150–152] It is generally recognized that patients with diabetes and established cardiovascular disease are high-risk patients that benefit from intensive treatment, hence, focus should be on identifying prognostic factors for cardiovascular disease among diabetes patients without established cardiovascular disease.[35–37] Distinguishing between risk stratification of an incident cardiovascular event and recurrent cardiovascular events is necessary as risk factors may differ in their prognostic value for patients with and without established cardiovascular disease. Hence, we aimed to examine the association between micro- and macroalbuminuria and incident ischaemic stroke, myocardial infarction, and all-cause mortality in a nationwide cohort study of patients with type 2 diabetes without prevalent atherosclerotic cardiovascular disease. In the following section, the results of study III will be presented and discussed in relation to other studies that have explored the association between albuminuria and the risk of cardiovascular disease and mortality.

Results of study III

The study population included 69,532 patients with type 2 diabetes. Incidence rates per 100 person-years increased in a dose-response manner across increasing levels of albuminuria for ischaemic stroke, myocardial infarction, and all-cause mortality (Table 7). Compared with patients with normoalbuminuria, we observed a higher risk of ischaemic stroke, myocardial infarction, and all-cause mortality among patients with microalbuminuria and macroalbuminuria (Table 8).

Table 7. Crude incidence rates per 100 person-years of ischaemic stroke, myocardial infarction, and all-cause mortality

Outcome	Normoalbuminuria (n = 45,695)	Microalbuminuria (n = 7,254)	Macroalbuminuria (n = 1,910)
Ischaemic stroke			
Events number	739	155	101
Incidence rate	0.39	0.59	0.90
Myocardial infarction			
Events number	587	129	93
Incidence rate	0.31	0.49	0.83
All-cause mortality			
Events number	2,650	690	395
Incidence rate	1.40	2.62	3.48

Table 8. Hazard ratios (95% CI) of ischaemic stroke, myocardial infarction, and all-cause mortality at 5 years follow-up (reference: normoalbuminuria)

Outcome/adjustment strategy	Microalbuminuria (n = 7,254)	Macroalbuminuria (n = 1,910)
Ischaemic stroke		
Age & Sex*	1.32 (1.11-1.57)	2.04 (1.66-2.52)
Risk Profile†	1.28 (1.07-1.52)	1.81 (1.46-2.23)
Risk & Medication Profile‡	1.28 (1.07-1.52)	1.81 (1.46-2.24)
Myocardial infarction		
Age & Sex*	1.40 (1.15-1.69)	2.33 (1.87-2.90)
Risk Profile†	1.34 (1.10-1.62)	1.99 (1.59-2.48)
Risk & Medication Profile‡	1.34 (1.11-1.63)	2.02 (1.61-2.53)
All-cause mortality		
Age & Sex*	1.52 (1.39-1.65)	2.03 (1.82-2.25)
Risk Profile†	1.48 (1.36-1.61)	1.83 (1.64-2.04)
Risk & Medication Profile‡	1.49 (1.37-1.62)	1.84 (1.65-2.05)

*Age & Sex model: adjusted for age (modelled as a restricted cubic spline) and sex. †Risk Profile model: Adjusted as the Age & Sex model and for systolic blood pressure, smoking status, body mass index, low-density lipoprotein cholesterol, haemoglobin A1c, diabetes duration (modelled as a restricted cubic spline), congestive heart failure, and atrial fibrillation. ‡Risk & Medication Profile model: Adjusted as the Risk Profile model and for antiplatelet treatment, angiotensin converting enzyme inhibitors/ angiotensin-receptor blockers, and anticoagulation treatment.

Sensitivity analysis

In a sensitivity analysis, the approach in the main analyses was repeated for the 54,859 patients where the measurements of albuminuria were within the same albuminuria category. The results were similar to those from the main analysis (Table 9).

Table 9. Hazard ratios (95% CI) of ischaemic stroke, myocardial infarction, and all-cause mortality at five-year follow-up for 54,859 patients with consistent albuminuria status (reference: normoalbuminuria)

Outcome/adjustment strategy	Microalbuminuria (n = 7,254)	Macroalbuminuria (n = 1,910)
Ischaemic stroke		
Age & Sex*	1.35 (1.11-1.63)	2.45 (1.91-3.16)
Risk Profile†	1.31 (1.09-1.59)	2.06 (1.59-2.67)
Risk & Medication Profile‡	1.32 (1.09-1.60)	2.07 (1.60-2.69)
Myocardial infarction		
Age & Sex*	1.44 (1.17-1.77)	2.96 (2.29-3.82)
Risk Profile†	1.39 (1.13-1.71)	2.39 (1.84-3.10)
Risk & Medication Profile‡	1.40 (1.13-1.72)	2.43 (1.87-3.17)
All-cause mortality		
Age & Sex*	1.56 (1.42-1.72)	2.70 (2.38-3.06)
Risk Profile†	1.52 (1.38-1.67)	2.39 (2.10-2.72)
Risk & Medication Profile‡	1.53 (1.39-1.68)	2.41 (2.11-2.74)

*Age & Sex model: adjusted for age (modelled as a restricted cubic spline) and sex. †Risk Profile model: Adjusted as the Age & Sex model and for systolic blood pressure, smoking status, body mass index, low-density lipoprotein cholesterol, haemoglobin A1c, diabetes duration (modelled as a restricted cubic spline), congestive heart failure, and atrial fibrillation. ‡Risk & Medication Profile model: Adjusted as the Risk Profile model and for antiplatelet treatment, angiotensin converting enzyme inhibitors/ angiotensin-receptor blockers, and anticoagulation treatment.

Discussion

The results of study III show that both micro- and macroalbuminuria are risk markers for incident ischaemic stroke, incident myocardial infarction, and all-cause mortality in patients with type 2 diabetes without established cardiovascular disease. This finding is in line with the findings of several longitudinal observational studies that have assessed the association between albuminuria level and the risk of atherosclerotic cardiovascular disease and all-cause mortality among patients with type 2 diabetes (Table 10). These studies have unanimously showed that patients with increased levels of albuminuria had a higher risk of atherosclerotic cardiovascular events. Study III build upon these prior findings by focusing specifically on patients with type 2 diabetes and no established cardiovascular disease. The studies by Fung et al. and Chen et al. also limited their study population to patients without cardiovascular disease. However, extrapolation of results from Asian populations to a Scandinavian population may not be applicable as ethnic differences in risk of cardiovascular disease exist, which may relate to differences in gene-environment interactions and genetic susceptibility.[153]

A key limitation in current literature is the categorisation of albuminuria level. Of the twelve studies in Table 10 that explored albuminuria and the risk of either cardiovascular disease or cardiovascular mortality, nine studies based their albuminuria categorisation on a single albuminuria measurement. As the day to day variation of urinary albuminuria excretion is substantial this approach may lead to exposure misclassification bias.[154,155] Although, Svensson et al. based their categorisation of albuminuria on the presence of micro- or macroalbuminuria in at least two out of three samples, the categorisation of the exposure in their study was assessed by the individual physician's and as such the researchers did not have access to the actual measurements of albuminuria.[156] This limits the validity of their exposure classification significantly. Chen et al. and Targher et al. assessed the UACR in two and three consecutive samples, respectively.[157] The approach by Targher et al. is in line with current recommendations for the classification of albuminuria which require that micro- or macroalbuminuria should be present in two of three specimens within a 3 to 6-month period.[158] In study III, we were unable to apply that specific definition of albuminuria as we were limited by the data obtained through the registries. The Danish Adult Diabetes Registry contains data from annual diabetes check-ups, these annual check-ups are sometimes delayed a few months. Thus, to retrieve two consecutive measurements of UACR or UAE, we broadened the criteria to up to 15 months between two consecutive tests.

Both albuminuria and low estimated glomerular filtration rate (eGFR) are indicators of kidney decline and the Kidney Disease: Improving Global Outcomes organization guidelines state that the categorization of chronic kidney disease should be based on both the eGFR and the level of albuminuria.[159] As chronic kidney disease is a known risk factor for cardiovascular disease,[160] exploring both albuminuria and eGFR as markers of a higher risk of cardiovascular disease and all-cause mortality is a reasonable approach. However, data completeness is very limited for measurements of eGFR in the Danish Adult Diabetes Registry, which prohibited us from exploring the association between eGFR and the risk of cardiovascular disease. Several of the aforementioned studies have, however, explored the role of eGFR in predicting cardiovascular disease and all-cause mortality. These studies have provided conflicting results regarding the association between eGFR and the risk of cardiovascular disease. Some studies found no associations between baseline eGFR and the risk of cardiovascular events when adjusting for known cardiovascular risk factors.[151,152,161] Whereas other studies showed that lower levels of eGFR were associated with a higher risk of experiencing a cardiovascular event.[142,144,156] The findings are more consistent for all-cause mortality where several studies have shown that decreasing eGFR was associated with a higher risk of all-cause mortality.[142,144,157,162] Furthermore, it has been suggested that combining albuminuria and eGFR leads to improved accuracy in predicting cardiovascular risk and all-cause mortality.[150] Nevertheless, several studies have shown that micro- and macro-albuminuria at baseline were associated with a higher risk of cardiovascular event, regardless of eGFR status.[156,161,163] Hence, current literature suggest that albuminuria

may be a better predictor of cardiovascular disease compared to eGFR. This finding may be explained by albuminuria not only reflecting kidney function, but also a generalized abnormality of the vascular function. Although the mechanisms linking microalbuminuria to cardiovascular disease are not fully understood, a common pathophysiologic process involving endothelial dysfunction, chronic low-grade inflammation, and increased transvascular leakiness of albumin is believed to play a role.[164] It has been suggested that endothelial dysfunction heightens the atherogenic state, thereby increasing the risk of atherosclerotic cardiovascular disease and microalbuminuria may reflect this endothelial dysfunction.[165–168] Others have suggested that the association is simply explained by common underlying risk factors between microalbuminuria and cardiovascular disease. Nonetheless, several of the studies listed in Table 10 have found albuminuria to be associated with cardiovascular disease and all-cause mortality independent of other know cardiovascular risk factors. It should be emphasized that the spectrum of cardiovascular risk factors remains to be discovered fully. Hence, a hitherto undiscovered risk factor for the development of cardiovascular disease that is related with microalbuminuria could theoretically explain the link. However, we adjusted for a broad variety of known risk factors and still found a strong association between albuminuria and cardiovascular risk, hence, it would seem unlikely that other, yet undiscovered, risk factors would explain the association fully.

Table 10. Risk estimates for clinical outcomes according to albuminuria status reported in longitudinal observational studies of patients with type 2 diabetes

Author, year	No. of patients	Albuminuria level	HR	95% CI
Outcome: Cardiovascular event, *n* = 8				
Bouchi et al., 2010[169]	1,002	UACR <30 mg/g	1	(Reference)
		UACR: 30-299 mg/g	2.33	(1.52-3.57)
		UACR ≥300 mg/g	3.70	(2.21-6.23)
Chen et al., 2012[151]	487*	UACR <30 mg/g	1	(Reference)
		UACR: 30-299.9 mg/g	1.61	(0.75-3.44)
Miettinen et al., 1996[170]	1,056	UACR <150 mg/l	1	(Reference)
		UACR: 150-299 mg/l	1.30	(1.00-2.07)
		UACR ≥300 mg/l	2.16	(1.51-3.09)
Fung et al., 2017[150]	67,334*	Normoalbuminuria	1 (reference)	
		UACR >2.5 mg/mmol	1.58 (male)	
		UACR >3.5 mg/mmol	1.48 (female)	
		UACR >25 mg/mmol	2.57 (male)	
		UACR >25 mg/mmol	2.40 (female)	
Monseu et al., 2015[144]	1,371	Albuminuria (log UACR)	1.33	(1.13-1.56)
Svensson et al., 2013[156]	66,065	UAE <20 µg/min	1	(Reference)
		UAE: 20-200 µg/min	1.16	(1.09-1.23)
		UAE >200 µg/min	1.36	(1.25-1.47)
Viana et al., 2012[171]	199	UACR <30 mg/g	1	(Reference)
		UACR ≥30 mg/g	2.89	(1.29-6.45)
Wada et al., 2013[163]	4,328	UACR <30 mg/g	1	(Reference)
		UACR: 30-299 mg/g	1.38	(1.14-1.67)
		UACR ≥300 mg/g	2.05	(1.61-2.58)
Outcome: Cardiovascular mortality, *n* = 5				
Bruno et al., 2007[172]	1,538	UAE <20 µg/min	1	(Reference)
		UAE: 20-200 µg/min	1.06	(0.80-1.40)
		UAE >200 µg/min	2.00	(1.48-2.71)
Cox et al., 2013[142]	1,220	1 SD increment in UACR	1.47	(1.24-1.74)
Monseu et al., 2015[144]	1,371	Albuminuria (log mg/mmol)	1.46	(1.20-1.77)
Targher et al., 2011[157]	2,823	UACR <30 mg/g	1	(Reference)
		UACR: 30-299 mg/g	1.56	(0.85-3.1)
		UACR ≥300 mg/g	3.40	(1.50-7.80)
Valmadrid et al., 2000[173]	840	UACR <30 mg/g	1	(Reference)
		UACR: 30-299.9 mg/l	1.84	(1.42-2.40)
		UACR ≥300 mg/l	2.61	(1.99-3.43)

Table 10 -continued **Outcome: All-cause mortality, *n* = 12**				
Berhane et al., 2011[174]	2,420	UACR <30 mg/g†	1	(Reference)
		UACR: 30-299 mg/g†	2.0	(1.3-3.0)
		UACR ≥300 mg/g†	2.5	(1.8-3.43)
Bruno et al., 2007[172]	1,538	UAE <20 µg/min	1	(Reference)
		UAE: 20-200 µg/min	1.30	(1.08-1.57)
		UAE >200 µg/min	1.91	(1.54-2.38)
Cox et al., 2013[142]	1,220	1 SD increment in UACR	1.41	(1.25-1.59)
Chen et al., 2012[151]	487	UACR <30 mg/g	1	(Reference)
		UACR: 30-299.9 mg/g	1.80	(0.71-4.56)
Fung et al. 2017[150]	67,334	Normoalbuminuria	1 (Reference)	
		UACR >2.5 mg/mmol	2.08 (Male)	
		UACR >3.5 mg/mmol	1.78 (Female)	
		UACR >25 mg/mmol	4.36 (Male)	
		UACR >25 mg/mmol	3.07 (Female)	
Miyake et al., 2018[175]	385	Logarithm of UAE	1.32	(1.02-1.07)
Monseu et al., 2015[144]	1,371	Albuminuria (log UACR)	1.38	(1.19-1.59)
Murussi et al., 2007[176]	173	UAE <5µg/min	1	(Reference)
		UAE >5µg/min	2.70	(1.20-6.10)
Tanaka et al., 2015[162]	3,231	1 SD increment in log UACR	1.31	(0.99-1.72)
Targher et al., 2011[157]	2,833	UACR <30 mg/g	1	(Reference)
		UACR: 30-299 mg/g	1.17	(0.73-1.90)
		UACR ≥300 mg/g	2.86	(1.60-5.00)
Viana et al., 2012[171]	199	UACR <30 mg/g	1	(reference)
		UACR ≥30 mg/g	5.07	(1.01-24.88)
Wada et al., 2014[163]	4,328	UACR <30 mg/g	1	(reference)
		UACR: 30-299 mg/g	1.37	(0.99-1.89)
		UACR ≥300 mg/g	3.60	(2.53-5.20)

*Population without overt cardiovascular disease at inclusion. †eGFR = 90-119 ml/min per. 1.73. UACR: urinary albumin-to-creatinine ratio, UAE: urinary albumin excretion rate, SD: standard deviation, CI: confidence interval.

The 2016 guidelines from the ESC on cardiovascular disease prevention management recommend high intensity statin treatment for patients with type 2 diabetes at very high risk of cardiovascular disease, defined as a 10-year cardiovascular risk of more than 10%.[47] The 2019 guidelines from ADA on cardiovascular disease and risk management state that it may be appropriate with intensive lipid lowering for individuals with a 10-year cardiovascular risk of ≥20%.[35] Similarly, several guidelines state that aspirin may be considered in the context of high atherosclerotic cardiovascular risk with low bleeding risk.[35,177] In study III, both micro- and macroalbuminuria were associated with increased risk of cardiovascular disease and using albuminuria status in combination with other well-known cardiovascular risk markers may provide the basis for identifying patients with diabetes and a 10-year risk of 10-20% in whom intensive vascular risk reduction is currently advocated for.[35,47] However, the effect of intensive interventions in people with type 2 diabetes and microalbuminuria has yet to be established. In the Steno-2 randomized trial, lower mortality and lower incidence of cardiovascular events were observed when comparing an intensified multifactorial treatment including aspirin treatment and

intensified glycaemic control, lipid lowering, and blood pressure control to conventional treatment in patients with type 2 diabetes and microalbuminuria.[178] This may suggest a beneficial effect of intensified treatment in patients with type 2 diabetes and microalbuminuria. Nonetheless, a recent meta-analysis including the Steno-2 randomized trial and two subgroups from other randomized trials did not provide conclusive evidence to support that intensive multifactorial intervention reduces the risk of stroke, myocardial infarction, cardiovascular mortality, or all-cause mortality.[179] Hence, it is unclear whether the presence of microalbuminuria in patients with type 2 diabetes should result in intensified treatment. Similarly, it is unclear whether reducing albuminuria translates into a reduction in long-term development of cardiovascular disease and mortality. A meta-analysis found that pharmacological blockade of angiotensin II receptors was effective in reducing the risk of new-onset albuminuria in patients with type 2 diabetes. However, the study was unable to show a significant reduction in mortality.[180] Nonetheless, in patients with diabetes and nephropathy reducing albuminuria appears to be protective for the development of cardiovascular disease.[181] Hence, based on the current literature it is not clear whether microalbuminuria is an actual therapeutic target in itself.

In conclusion, the results of study III clearly indicate that micro- and macroalbuminuria are robust markers of increased risk of cardiovascular events. Using albuminuria status in combination with other well-known cardiovascular risk markers may provide the basis for clinically useful cardiovascular risk assessment among patients with diabetes and no established cardiovascular disease.

CHAPTER 7. METHODOLOGICAL CONSIDERATIONS

The overall aim of an epidemiological study is to obtain estimates of the frequency of an outcome or the effect of a given exposure on the outcome that are both accurate and precise. Obtaining both accuracy and precision of an estimated result requires minimal error in the estimation process. Errors can emerge through study design, conduct, and analysis. Often errors are separated into systematic and random error and whereas random error leads to loss of precision, systematic error may lead to loss of accuracy of the results. Accuracy of results are often referred to as the validity, and epidemiological studies are evaluated both on internal and external validity. Whereas internal validity raises the question of whether the results of a given study is true for the population studied, or an artefact of the way the study was designed, external validity raises the question of whether the study results are likely to apply to an external target population. Thus, internal validity is a prerequisite for external validity.[182] Internal validity of a cohort study can be threatened by systematic errors, also known as bias. Traditionally, bias is divided into selection bias, information bias, and confounding. In the following paragraphs, issues concerning selection bias, information bias, confounding, external validity, and random error in study I-III, will be discussed.

7.1. SELECTION BIAS

Selection bias occurs when the association between the exposure and the disease differs between those who are in the study and those who are not.[183] Selection bias can be introduced by the processes that leads to the overall loss of study population throughout the study. Thus, it can stem from the process of selecting the initial study population or from differential loss to follow-up or censoring during the study.

Selection of the study population

In study I and as a partial inclusion criterion in study II, the study populations were identified based on a first-time hospital diagnosis of atrial fibrillation in the Danish National Patients Registry. A diagnosis of atrial fibrillation has been found to have a high validity in the Danish National Patient Registry and validation studies have estimated the positive predictive value to range between 92-99%.[184–186] Thus, it is reasonable to assume that the vast majority of patients included in study I and II do in fact have atrial fibrillation. However, the sensitivity of a diagnosis of the atrial fibrillation in the Danish National Patient Registry has not been assessed. Thus, we have no estimate of the proportion of patients with incident atrial fibrillation we have actually captured during the inclusion period. Nevertheless, Danish guidelines recommend referring patients with newly diagnosed atrial fibrillation to a specialist

evaluation, and as such, it is likely that we captured the vast majority of patents with incident atrial fibrillation during the inclusion period.

All studies in this dissertation focused on patients with diabetes. However, patients with diabetes were identified with different criteria in the three studies. In study I, we identified patients with type 1 or type 2 diabetes by using an algorithm that relied on hospital-based diagnoses and claimed prescription drugs. This identification method made us unable to identify patients with diabetes managed exclusively in general practice who were solely receiving non-pharmacological treatment. Non-pharmacologically managed patients with type 2 diabetes are likely to be those with the lowest prevalence of cardiovascular risk factors. We did not adjust for all potential risk factors for ischaemic stroke, as the study aimed to explore the type of diabetes as an easily obtainable proxy for the differences in the distribution of those very same cardiovascular risk factors. Therefore, we may have induced selection bias as we may have compared high-risk type 2 diabetes patients with type 1 diabetes patients.

In Study II and III, patients with type 2 diabetes were identified through the Danish Adult Diabetes Registry. The data completeness from outpatient clinics is high in the Danish Adult Diabetes Registry, whereas the data completeness from primary care is considerably lower.[65] As the majority of patients with type 2 diabetes are treated in primary health care we did not capture the entire population of patients with type 2 diabetes in Denmark. Patients followed in secondary care are likely to have a higher burden of comorbidities and therefore our population are likely to be a high-risk population compared with the general diabetes population. Furthermore, in study II, we excluded patients who did not have an available HbA1c measurements within two years before the index date. This led to an exclusion of a large number of patients. These patients were generally younger and had a lower burden of comorbidity. In study III, we only included patients with two measurements of microalbuminuria within 15 months before the index date. Therefore, we did not capture all patients with diabetes during the study period. Hence, this population may be a high-risk population with more comorbidities than the general diabetes population which may have resulted in rates of ischaemic stroke that are higher than those of the general Danish diabetes population.

Nonetheless, selection bias is only induced when the association between the given exposures and the outcome differs between those who are included and those who are not. It is not likely that the association between glycaemic status/microalbuminuria and ischaemic stroke differs between those who were included and those who were not. Thus, the above-mentioned limitations in the selection of the study population are not expected to induce actual selection bias in study II and III and thereby threaten the internal validity of the study. Instead these limitations in the selection of the study populations can raise the question of the generalizability of the results to a target population.

Censoring

Loss to follow-up and losses due to competing risk can be handled with censoring in survival analysis such as the Cox regression analysis. Survival analysis relies on the independent censoring assumption which is that the censoring must be unrelated to the risk of the outcome within each exposure group. In other words, those who are censored should not be individuals with systematically higher or lower risk of the outcome compared to the individuals who remains at risk.

In study I-III, patients were followed in The Danish National Patient Registry for the outcomes under study and aside from a few patients emigrating during the study period this led to almost complete follow-up. A potential problematic cause of censoring was performed in study I where patients were censored when initiating anticoagulation treatment. In order to comply with the independent censoring assumption, patients initiating anticoagulation treatment needs to be a random subset of the population, this may not be the case. Therefore, censoring may be informative with respect to the outcome. As initiation of anticoagulation treatment was more frequent among patients with type 2 diabetes, this may have led to an underestimation of risk specifically among patients with type 2 diabetes as compared with type 1 diabetes.

In all studies, death was considered a competing risk for the other outcomes. Due to the register-based nature of the datasets, deaths can be categorised into two main categories; i) deaths that are due to undiagnosed cardiovascular events that otherwise would have been defined as an outcome, had they been diagnosed. These patients were censored but should really have been registered as having the outcome under study, and ii) deaths unrelated to the cardiovascular events of interest, which are the only deaths that are considered to be actual competing events. We were unable to make this distinction, since causes of death are poorly recorded in Danish registries; only 4% of people dying have an autopsy performed.[187] In a high mortality population like the diabetes population, some patients die from other causes before experiencing the outcome of interest. If the exposure is associated with a higher risk of death than the outcome of interest, it may lead to an overestimation of the association between the exposure and the outcome. In study III, the risk of all-cause mortality was higher among those with micro- and macroalbuminuria thereby potentially violating the independent censoring assumption. Moreover, it is likely that the exposures in study I and II, in a similar fashion, would increase the risk of death and thereby violate the independent censoring assumption. Nonetheless, one could argue that estimating associations between exposures and outcomes in a high mortality population where patients do not die belongs to a hypothetical world. By analysing associations with a cause specific Cox regression model, as we did in our studies, inference for disease rates are made in the presence of the competing risk of dying.[70]

7.2. INFORMATION BIAS

When information used in a study is erroneous, for example, due to incorrect measuring or classification of study variables (exposures, outcomes, and confounders/risk factors), it can lead to information bias.[15] When information bias leads to subjects being placed in an incorrect exposure or outcome category, it is denoted as misclassification bias. Misclassification can be differential or nondifferential. Nondifferential misclassification is a misclassification unrelated to other study variables and it leads to more predictable bias and will often affect the estimates towards the null. Differential misclassification occurs when the misclassified variable is related to other variables in the study and it leads to more unpredictable bias. Study outcomes were identified through the Danish National Patient Registry and as such is depended on the accuracy of the diagnoses in this registry. Incomplete or inaccurate registration can lead to information bias.

Exposure misclassification

In study I, patients with type 1 diabetes were compared with patients with type 2 diabetes in respect to the risk of thromboembolism. To classify patients as having either type 1 or type 2 diabetes, we used a combination of ICD-10 codes and information on claimed prescriptions of glucose-lowering drugs. This classification of patients can have induced misclassification bias – e.g., a patient with type 2 diabetes receiving monotherapy with insulin who is not registered with an ICD-10 code of type 2 diabetes or has an erroneous ICD-10 code of type 1 diabetes would be misclassified as a patient with type 1 diabetes. Furthermore, after the initial classification of patients, there was approximately 24% of patients classified as having type 1 diabetes that were receiving oral glucose lowering drugs.[105] This was likely a misclassification of patients with type 2 diabetes as type 1 diabetes patients. We addressed this potential error in a sensitivity analysis where these patients were relocated to the type 2 diabetes group. The analysis resulted in similar estimates as in the main analysis.

In study II, the exposure was glycaemic status as reflected by the measured HbA1c level. The HbA1c measurements were captured directly from the electronic medical record systems, which minimized the risk of data entry errors and thereby minimized misclassification bias. Patients were classified according to their baseline level of HbA1c defined as the most recent HbA1c measurement within the past two years before or the first four weeks after inclusion. Using up to two years old HbA1c measurements may have led to misclassification as patients' HbA1c measurements can change during this time period. In a sensitivity analysis we assessed whether using a more recent HbA1c measurement, obtained within one year before or the first four weeks after inclusion, would affect the results. This analysis resulted in similar estimates as the main analysis and thereby it supports the validity of our main findings. However, the potential effects of glycaemia on the risk of developing ischaemic stroke is presumably due to a long-term exposure to heightened levels of glucose, and we did not account for variations in glycaemic control over longer periods prior to

inclusion - nor did we account for the potential changes in HbA1c during follow-up. As it is unrealistic to assume that HbA1c values remain stable throughout this period of time, making inferences regarding the effect of glycaemic control on thromboembolic risk based on one static HbA1c value is questionable. Thus, we only examined the association between the risk of ischaemic stroke and glycaemic *status,* but not glycaemic *control.*

In study III, the exposure was albuminuria level. As the biological variability in urinary albumin excretion is substantial, we used two consecutive measurements of albuminuria to minimize misclassification bias. Moreover, we observed that some patients did not have a consistent categorization in their two measurements, therefore we performed a sensitivity analyses restricted to patients with a consistent categorization of albuminuria in both measurements. The sensitivity analysis resulted in similar results as the main analysis. To categorize albuminuria, measurements of either UACR or UAE were used. Both these measurements have been shown to have a high sensitivity and specificity for the detection of albuminuria. Moreover, exposure misclassification due to imprecise measurements is unlikely to be systematic and would therefore tend to draw associations towards the null.

Outcome misclassification

The results in all three studies consisted of a measure of event rates and HR's of the outcomes. Potential misclassification of the outcomes affects these measures differently. Whereas the HR's are primarily susceptible to bias if the misclassification is differential; event rates based on administrative data can be altered substantially depending on the extent and validity of the diagnoses used to define the outcome.

Often, an outcome can be defined by several diagnoses in the registries that more or less precisely captures patients with the actual outcome under study. Generally, a broad definition of an outcome leads to loss of specificity and will tend to overestimate event rates. Oppositely, a narrow definition leads to loss of sensitivity and will tend to underestimate the event rates. In all studies, ischaemic stroke was an outcome. This outcome was defined as a combination of ischaemic stroke and unspecified stroke. Using a diagnosis of unspecified stroke to define the outcome may have misclassified some patients as having had an ischaemic stroke when in fact they had that actually had a haemorrhagic stroke. Thus, including unspecified stroke in the definition of the outcome in all studies has likely led to an overestimation of the event rates. Nonetheless, most strokes coded as unspecific have been reported to be of ischaemic origin and as such we viewed it to be reasonable to include unspecified stroke in the outcome definition.[188] Furthermore, refraining from including these patients would most likely led to an underestimation of the event rates.

Another concern that may affect the observed event rates, is whether the outcome is defined by primary diagnoses only or by a combination of primary and secondary diagnoses. Secondary diagnoses are more likely to be an expression of an old event

(carry-over diagnosis) and will therefore tend to overestimate the event rates, whereas including only primary diagnoses may underestimate the event rates. As we only used primary diagnoses in our studies, we may have underestimated the event rates.

The validity of the individual diagnostic codes will also affect how precisely the outcomes are estimated. If the diagnostic code of an outcome has a low positive predictive value that will tend to overestimate the event rates, whereas a low sensitivity will tend to underestimate the event rates. In our studies, ischaemic stroke and acute myocardial infarction were some of the outcomes under study and the validity of a primary diagnoses of those diseases in the Danish National Patient Registry have been shown to be high, with positive predictive values of 81% and 97%, respectively.[189,190]

As previously mentioned, some patients may have died due to an unrecognized ischaemic stroke or myocardial infarction. By not being able to capture these patients we may have underestimated the 'true' risk of ischaemic stroke and myocardial infarction. If death due to ischaemic stroke and myocardial infarction had been included in the outcomes it would have resulted in more precise event rate estimates.

As described, there are various sources of potential errors when estimating the rates of ischaemic stroke and myocardial infarction through administrative registries. It is not possible to determine the sum of all these errors. We must merely conclude that the event rates have been estimated with errors and must be interpreted with caution. Nonetheless, the above-mentioned sources of information bias are not expected to be related to the exposures in study I-III, and as such a potential misclassification of the outcomes are expected to drive the HR estimates towards the null. Therefore misclassification bias is not expected to be the explanation for the observed differences in the rates of the outcomes.

Comorbidity misclassification

The comorbidity status of the individual patients was obtained through the registries. Thus, the precision with which we captured the actual comorbidity status of these patients depends on the validity of diagnoses and prescriptions in the registries. As the validity of these diagnoses vary,[189] some degree of misclassification of the comorbidity status of the study population occurred in all three studies. For variables obtained through the Danish Adult Diabetes Registry, we identified a varying degree of missing data. We handled missing variables with the 'missing indicator' method in study II and III. However, this method may have induced some degree of bias as data in the registries probably are not missing completely at random.[191]

Medication misclassification

The baseline medication status of the study cohorts was ascertained through the Danish National Prescription Registry which holds information regarding claimed prescriptions. Therefore, the information in the registries are only reflecting the

medications that have been claimed, which may not equal the medication that have been consumed. If there are differences in the compliance to the medication in the different exposure groups it may have led to differential misclassification bias. Another concern is the quality of treatment with anticoagulation treatment, specifically with vitamin k antagonist treatment. As we did not have access to information regarding international normalized ratio (INR) values, we do not know the time in therapeutic range for the patients receiving vitamin k antagonists. This may have led to residual confounding in study II.

7.3. CONFOUNDING

Confounding is defined as a confusion of effects. It occurs when the primary exposure of interest is mixed up with some other factor that causes the outcome under study. A confounder is a factor that is associated with the exposure of interest and a cause of the outcome under study. A confounder cannot be an intermediary step that lie on the causal pathway between the exposure and the outcome. Confounding as a concept is by definition only an issue in studies of aetiology where the objective is to determine the causal effect of an exposure on the outcome. Controlling for confounding is essential in aetiological studies and it can be performed with statistical modelling. Nonetheless, confounder control strategies are usually imperfect in cohort studies as a confounder may be measured imperfectly, modelled incorrectly, or be unavailable in the applied data. Study II was an aetiological study and therefore we adjusted for a wide range of potential confounders. However, as discussed in Chapter 5, the observational study design makes us unable to rule out residual confounding as part of the observed results.

7.4. RANDOM ERROR

So far potential sources of systematic errors have been described. Another source of error is random error. Random error is unexplained variation in the data. Generally, reducing random error and thereby increasing the precision of an estimate can be achieved by increasing the study size. In all three studies, the sample sizes were relatively high with an accompanying high number of outcomes, allowing for precise effect estimates with reasonable narrow CIs.

7.5. EXTERNAL VALIDITY

External validity, also denoted generalisability, refers to the extent to which an internally valid result in a study sample can be extrapolated to the target population. To make meaningful inference about the generalizability of the results of a study to a target population, the target population must be well-defined. Thus, whether the aim is to generalize the results to the population from which the sample population was derived or to an entirely different population will affect the extent to which the results are generalizable. Our studies comprised a reasonable large sample of the individual

populations that we view as our target populations in Denmark. However, as described previously, the selection into the study may have resulted in a high-risk study population in study II and III. This may limit the generalizability of the results. Furthermore, when generalizing our results to other populations it must be taken into account that the Danish population is ethnically non-diverse. Therefore, the results may not apply in more ethnically diverse populations.

CHAPTER 8. CONCLUSIONS AND PERSPECTIVES

The prevalence of diabetes has been and continues to be on the rise and the future burden of diabetes-related cardiovascular disease is likely to increase. Hence, a continued focus on cardiovascular disease prevention is paramount. The studies of this dissertation contributes to the identification of a number of risk factors for ischaemic stroke and myocardial infarction both in a general cohort of patients with diabetes and in patients with diabetes and concomitant atrial fibrillation. Identifying high- and low-risk subgroups in the diabetes population may provide the basis for evidence-based clinical risk stratification that may serve as a valuable clinical tool in aiding clinical counselling of patients and guiding treatment decisions.

We explored whether the type of diabetes and glycaemic status were associated with a higher risk of thromboembolism in patients with diabetes and atrial fibrillation. We found that overall, the type of diabetes was not associated with a higher risk of thromboembolism, while type 2 diabetes was associated with a substantially higher risk of ischaemic stroke in the subgroup of patients aged below 65 years. Furthermore, the results suggested that increasing levels of glycaemic status are associated with a higher risk of thromboembolism in patients with a shorter duration diabetes, but not in patients with a longer duration of diabetes. In study I and II, the association between the exposures and the outcomes were modified by age and diabetes duration, respectively. These findings underline the complexity of risk stratification in diabetes and atrial fibrillation. Our findings may indicate that type 2 diabetes is associated with a higher risk of thromboembolism among patients under 65 years and that poor glycaemic status is associated with a higher risk of thromboembolism among patients with a shorter duration of diabetes. However, studies investigating these specific exposures are limited and the role of both type of diabetes and glycaemic status in patients with diabetes and atrial fibrillation remains unclear. Hence, future studies are necessary to confirm our findings. Study I and II provides a perspective on how risk stratification in atrial fibrillation and diabetes could potentially be improved, however, there is currently no basis to support a subdivision of diabetes patients according to diabetes-related factors. Hence, diabetes should continue to be incorporated into the CHA_2DS_2-VASc score as an entity. Furthermore, both prior studies and the findings of our studies indicate that patients with atrial fibrillation and concomitant diabetes carry a high risk of ischaemic stroke and are therefore likely to benefit from anticoagulation treatment.

We also explored whether micro- and macroalbuminuria are associated with incident ischaemic stroke, myocardial infarction, and all-cause mortality among patients with type 2 diabetes without overt cardiovascular disease. We found that micro- and macroalbuminuria are robust markers of a higher risk of cardiovascular events. Hence,

the presence of micro- or macroalbuminuria should warrant careful evaluation of cardiovascular risk among patients with diabetes and considerations of suitable preventive strategies, due to the observed higher risk of cardiovascular events. Future studies may focus on including albuminuria status in combination with other well-known cardiovascular risk markers to develop cardiovascular risk stratification tools to identify high-risk diabetes patients that may benefit from intensified preventive strategies.

The observational nature of the studies in this dissertation makes us unable to conclude whether the identified high-risk groups of this dissertation may directly benefit from intensified treatment. Ultimately, randomized controlled trials investigating the effect of intensified treatment among high-risk groups are needed to provide conclusive evidence. However, currently such trials are either not available or have provided inconsistent results and until additional evidence from randomized controlled trials emerge, cohort studies detecting differences in risk profiles in subpopulations of diabetes patients should continue to inform and aid clinicians in their decision making.

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APPENDICES

Appendix A: Algorithm for identification of type 1 and type 2 diabetes

Appendix B: Paper I

Appendix C: Paper II

Appendix D: Paper III

APPENDIX A. ALGORITHM FOR IDENTIFICATION OF TYPE 1 AND TYPE 2 DIABETES

Algorithm developed by the Danish Health Data Authority for identifying type 1 and type 2 diabetes patients based on Danish register data from the Danish National Patient Registry and the Danish National Prescription Registry.

Algorithm for identification of Type 2 diabetes
Inclusion:

- Persons with a minimum of one prescription claim of glucose lowering drugs (ATC code: A10B) with exclusion of insulin or insulin analogues (ATC code: A10A) in the Danish National Prescription Registry.
- Persons with a relevant primary or secondary diagnosis (ICD-10 code: E11) as the last of specific diagnoses (ICD-10 code: E10 and E11) in the Danish National Patient Registry.

Exclusion:

- Persons who are solely registered the Danish National Prescription Registry with one claimed prescription of insulin or insulin analogues (ATC code: A10A) or other glucose lowering drugs (ATC code: A10B).
- Persons who are solely registered in the Danish National Patient Registry with one specific ICD-code for type 2 diabetes (ICD-10 code: E11).
- Women who have solely claimed a prescription of Metformin (ATC code: A10BA02) and have a claimed prescription of Clomiphene (ATC code: G03GB02) or an Antiandrogen combined with Oestrogen (ATC code: G03HB) or have a diagnosis of polycystic ovarian syndrome (ICD-10 code: E282) in the Danish National Patient Registry.
- Persons who are not registered in the Danish National Prescription Registry with a claimed prescription of insulin or insulin analogues (ATC code: A10A) or other

glucose lowering drugs (ATC code: A10B) or who does not have a relevant diagnosis in the Danish National Patient Registry 10 years from their index date.

Algorithm for identification of Type 1 diabetes
Inclusion:

- Persons with a minimum of one claimed prescription of insulin or insulin analogues (ATC code: A10A) in the Danish National Prescription Registry.
- Persons with relevant primary or secondary diagnosis (ICD-10 code: E10) as the last diagnose of specific diagnoses (ICD-10 code: E10 and E11) in the Danish National Patient Registry.

Exclusion:

- Persons who are solely registered in the Danish National Prescription Registry with one prescription of insulin or insulin analogues.
- Persons who are solely registered in the Danish National Patient Registry without a claimed prescription of insulin or insulin analogues (ATC code: A10A).
- Persons classified as type 2 diabetes patients according to the algorithm for identification of type 2 diabetes.
- Women with a diagnosis of gestational diabetes (ICD-10 code: 024.4) in the Danish National Patient Registry, who solely have a claimed a prescription of glucose lowering drugs in the Danish National Prescription Registry within 280 days before first contact or 280 days after last contact with gestational diabetes.
- Persons who are not registered in the Danish National Prescription Registry with a claimed prescription of insulin or insulin analogues (ATC code: A10A) 10 years from their index date.

APPENDIX B. PAPER I

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APPENDIX C. PAPER II

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