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# **DRUG SAFETY AND EFFECTIVENESS IN RELATION TO KIDNEY FUNCTION: A PHARMACOEPIDEMIOLOGICAL APPROACH**

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# Drug safety and effectiveness in relation to kidney function: a pharmacoepidemiological approach

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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*To my family - you made all this possible*

*“In God we trust, all others must bring data”*

W.E. Deming



## ABSTRACT

Kidney function plays an important role in drug safety and effectiveness. As many medications are excreted by the kidneys, patients with reduced kidney function are at a higher risk of supra-therapeutic or toxic drug levels. At the same time, drug-induced nephrotoxicity is common due to the high filtration capacity and metabolic activity of the kidneys. Patients with chronic kidney disease (CKD) are at high risk for adverse drug event and drug overdosing. Therefore, randomized controlled trials (RCTs) have generally excluded patients with CKD or included only a small proportion that precludes strong conclusions about the safety and effectiveness in this segment of the population. Pharmacoepidemiological studies performed in real-world settings can help provide complementary evidence and expand findings of RCTs to the general population. However, existing observational studies are often limited in sample size, length of follow-up and inappropriate management of confounding and biases.

The presented work aims to expand existing knowledge on drug safety and effectiveness of common cardiovascular and antidiabetic medications used in routine practice and to investigate differences in drug risk-benefit across levels of kidney function.

**Study I** describes the frequency of hyperkalemia in a cohort of new users of mineralocorticoid receptor antagonist (MRA) identified from the Stockholm CREA<sup>t</sup>inine Measurement (SCREAM) project during 2007-2010. During the 1-year follow-up after treatment initiation, 18% of the patients experienced hyperkalemia in the overall cohort and 26% among patients with heart failure history. After hyperkalemia, 47% of patients discontinued the therapy and only 10% reduced the dose. CKD was common (28%) and it was a major risk factor for both hyperkalemia and MRA discontinuation.

**Study II** examines safety and effectiveness associated with continuing vs stopping MRA treatment after an episode of hyperkalemia in routine care. A cohort of new users of MRA surviving an incident hyperkalemia during 2007-2018 was identified from the SCREAM project. Target trial emulation methods were applied to assess the association between treatment strategies (stopping vs continuing MRA within 6 months after hyperkalemia) and subsequent outcomes. Compared to the “continue MRA” strategy, patients who stopped MRA were at higher risk of cardiovascular events and mortality but lower risk of recurrent hyperkalemia. These associations were consistent across eGFR strata.

**Study III** investigates the cardiovascular effectiveness associated to Glucagon-Like Peptide-1 Receptor Agonists (GLP-1 RAs) use, compared to a standard diabetic care, after an acute myocardial infarction (MI). A cohort of patients with diabetes surviving an acute MI during 2010-2017 were selected from the Swedish Web-system for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies (SWEDEHEART) registry. Results from the multivariable Cox regression showed a 28% relative risk reduction associated with GLP-1 RAs use compared with standard care. There was no suggestion of effect modification across stages of CKD.

**Study IV** compares the risk of cardiorenal outcomes among patients with non-valvular atrial fibrillation (AF) initiating direct oral anticoagulants (DOAC) or vitamin K antagonists (VKA) treatment. Using data from the SCREAM project, we identified a cohort of patients who started oral anticoagulants (OAC) between 2011 and 2018. Propensity-score weighted Cox regression was used to estimate the treatment-outcomes associations adjusting for 50 measured confounders. Results showed a lower risk of CKD progression, acute kidney injury (AKI) and major bleeding associated with DOAC use compared to VKA treatment. No statistical difference was observed between treatment groups for the composite outcome of stroke/systemic embolism and mortality. The observed associations were mostly similar across levels of baseline kidney function.

In conclusion, this work emphasizes the importance of pharmacoepidemiology in expanding trial evidence on the safety and effectiveness of medications in real-world settings. Moreover, this thesis also highlights the important role of kidney function in assessing the risk–benefit of medications.



## LIST OF SCIENTIFIC PAPERS

- I. **Trevisan M**, de Deco P, Xu H, Evans M, Lindholm B, Bellocco R, Barany P, Jernberg T, Lund LH, Carrero JJ. Incidence, predictors and clinical management of hyperkalaemia in new users of mineralocorticoid receptor antagonists. *European Journal of Heart Failure* 2018; 20(8): 1217-26.
- II. **Trevisan M**, Fu EL, Xu Y, Savarese G, Dekker FW, Lund LH, Clase CM, Sjölander A, Carrero JJ. Stopping mineralocorticoid receptor antagonists after hyperkalaemia: trial emulation in data from routine care. *European Journal of Heart Failure* 2021; 23(10): 1698-707
- III. **Trevisan M**, Fu EL, Szummer K, Norhammar A, Lundman P, Wanner C, Sjölander A, Jernberg T, Carrero JJ. Glucagon-like peptide-1 receptor agonists and the risk of cardiovascular events in diabetes patients surviving an acute myocardial infarction. *European heart journal Cardiovascular pharmacotherapy* 2021; 7(2): 104-11
- IV. **Trevisan M**, Hjemdahl P, Clase CM, de Jong Y, Evans M, Bellocco R, Fu EL, Juan Jesus Carrero. Cardiorenal outcomes associated with oral anticoagulant use in patients with atrial fibrillation. *Manuscript submitted*

These articles are referred to by their roman numerals throughout, and are presented in full at the end of this thesis.

## RELATED PAPERS

(In appendix)

- **Trevisan M**, Fu EL, Xu Y, Jager K, Zoccali C, Dekker FW, Carrero JJ. Pharmacoepidemiology for nephrologists (part 1): concept, applications and considerations for study design. *Clinical kidney journal* 2020; 14(5): 1307-16.

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- Fu EL, van Diepen M, Xu Y, **Trevisan M**, Dekker FW, Zoccali C, Jager K, Carrero JJ. Pharmacoepidemiology for nephrologists (part 2): potential biases and how to overcome them. *Clinical kidney journal* 2020; 14(5): 1317-26.
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## LIST OF ABBREVIATIONS

ACEi	Angiotensin-Converting-Enzyme inhibitor
ACS	Acute Coronary Syndrome
AER	Albumin Excretion Rate
AF	Atrial Fibrillation
AKI	Acute Kidney Injury
ARB	Angiotensin Receptor Blocker
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CI	Confidence Interval
CKD	Chronic Kidney Disease
DKD	Diabetic Kidney Disease
DOAC	Direct Oral Anticoagulant
eGFR	estimated Glomerular Filtration Rate
ESKD	End-Stage Kidney Disease
GFR	Glomerular Filtration Rate
GLP-1 RA	Glucagon-Like Peptide-1 Receptor Agonist
HF	Heart Failure
HR	Hazard Ratio
ICD-10	International Classification of Diseases Version 10
IPTW	Inverse Probability of Treatment Weighting
IQR	Interquartile range
K <sup>+</sup>	Potassium
KRT	Kidney Replacement Therapy
LISA	Longitudinell Integrationsdatabas för Sjukförsäkrings- och Arbetsmarknadsstudier
MACE	Major Adverse Cardiovascular Events
MI	Myocardial Infarction
MRA	Mineralocorticoid Receptor Antagonist
OAC	Oral Anticoagulants
PS	Propensity Score

RAASi	Renin-Angiotensin-Aldosterone System inhibitor
RAS	Renin-Angiotensin System
RCT	Randomized Controlled Trial
SCREAM	Stockholm CREAtinine Measurement project
SLL	Stockholms Läns Sjukvårdsområde
STEMI	ST-segment elevation MI
SWEDEHEART	Swedish Web-system for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies registry
U.S.	United States
VAL	Vårdanalysdatabasen
VKA	Vitamin K Antagonist
WHO	World Health Organization



# 1 BACKGROUND

## 1.1 Kidney function and chronic kidney disease (CKD)

The kidneys play an important role in maintaining the balance of the body's fluids and filtering the blood from waste products and drugs. The Glomerular filtration rate (GFR) is one of the measures that characterize the excretory function of the kidneys and represents the amount of fluid that is filtered through the nephrons in a unit of time. Chronic kidney disease (CKD) is diagnosed in the presence of persistent abnormalities in the structure or function of the kidneys. In particular, it is commonly defined as  $\text{GFR} < 60 \text{ ml/min/1.73m}^2$  that persists for more than 3 months<sup>1</sup>. Current equations that estimate GFR (eGFR, estimated GFR) use laboratory information on serum creatinine levels along with demographic information on age, sex and race<sup>2</sup>. According to GFR levels, five stages of CKD have been defined: G1 (Normal or high),  $\text{GFR} \geq 90 \text{ ml/min/1.73m}^2$ ; G2 (Mildly decreased),  $\text{GFR} 60\text{-}90 \text{ ml/min/1.73m}^2$ ; G3 (mildly to severe decreased),  $\text{GFR} 30\text{-}60 \text{ ml/min/1.73m}^2$ ; G4 (severely decreased),  $\text{GFR} 15\text{-}30 \text{ ml/min/1.73m}^2$ ; G5 (end-stage kidney disease (ESKD)),  $\text{GFR} < 15 \text{ ml/min/1.73m}^2$  or undergoing kidney replacement therapy (KRT: chronic dialysis or transplantation).

Another marker of kidney damage is albuminuria<sup>1</sup>, which represents the abnormal loss of the protein albumin in the urine that is symptomatic of an increase glomerular permeability. Albuminuria is one of the earliest signs of glomerular damage and is not always accompanied by a decrease in GFR. Albuminuria can be measured through the albumin excretion rate (AER) and categorized as: A1 (normal or mildly increased),  $\text{AER} < 30 \text{ mg/g}$ ; A2 (moderately increased),  $\text{AER} 30\text{-}300 \text{ mg/g}$ ; (A3 severely increased),  $\text{AER} > 300 \text{ mg/g}$ . Current guidelines propose to define the severity of CKD based on the combination of GFR and albuminuria categories (**Figure 1**). Patients that score high in both categories are at higher risk of CKD progression. This definition has proponents and opponents: Glassock *et al.*<sup>3</sup> suggest that CKD definitions should be based on age-specific references to overcome the problem of false positive, in particular when there are no signs of kidney damage (e.g. proteinuria).

CKD is usually irreversible, but there are treatments that aim at slowing the progression of the kidney failure. When patients reach ESKD, preparations start for commencement of chronic dialysis or be placed in the kidney transplant list. These practices, although life-saving therapies, also increase the risk of adverse outcomes (e.g. death, infections and cardiovascular events<sup>4,5</sup>). The management of CKD is very costly for healthcare, being estimated to account for 3% of the total healthcare budget of developed countries, which is, to a large extent, attributed to the costs of dialysis<sup>6-8</sup> and development of subsequent cardiovascular events<sup>9</sup>.

**Figure 1. Chronic kidney disease classification based on glomerular filtration rate and albuminuria**

				Persistent albuminuria categories		
				Description and range		
				A1	A2	A3
				Normal to mildly increase	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
GFR categories (ml/min/1.73m <sup>2</sup> ) Description and range	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Data from the KDIGO CKD Work Group clinical practice guidelines<sup>1</sup>.

Abbreviations: GFR; glomerular filtration rate

Colors indicate the prognosis by GFR and albuminuria category: Green, low risk; Yellow, moderately increased risk; Orange, high risk; Red, very high risk.

## 1.2 CKD incidence and prevalence

CKD has been recognized as a public health priority only in recent decades<sup>10, 11</sup>, especially after the development of simple creatinine-based equations to estimate GFR<sup>12</sup>. Evaluating CKD prevalence and incidence is a fundamental step towards better strategies for CKD prevention and management at the population level. It has been estimated that CKD afflicts 10–15% of the adult general population in developed countries<sup>13-15</sup> but it has a wider range of variation when we include also developing countries<sup>15</sup>.

CKD represents an important comorbidity in terms of mortality and quality of life. Results from the 2016 Global Burden of disease report showed that CKD has climbed the ranking among the causes of death in the decade 1990-2016, with 2.1% of deaths attributable to this disease<sup>16</sup>. Accordingly to the projection of the World Health Organization (WHO), the death rate associated with CKD is expected to increase by 2060, ranking as the 7th top among all-cause of death worldwide (currently 12th)<sup>16</sup>. To some extent, the growth of CKD is attributed to the increase in life expectancy (i.e., ageing).

There is still poor awareness of CKD by both patients and physicians<sup>17</sup>, which poses some challenges in the identification of these patients in population-based data, as diagnostic codes are seldom used. Using data from the Stockholm CREAtinine Measurement (SCREAM) project, Gasparini *et al.*<sup>14</sup> have shown that only 12% of persons with CKD in Stockholm identified on the basis of eGFR, carried a CKD diagnosis or were seen by a nephrologist. Relying on measures of creatinine or albuminuria may allow better identification of patients

with CKD and better ascertainment of kidney measures as study outcomes or mediators. However, there are challenges in translating laboratory measurements from healthcare databases into clinical diagnoses of CKD. For instance, the presence of eGFR measurements is determined by testing indication and disease, and patients with two consecutive measurements of  $\text{GFR} < 60 \text{ ml/min/1.73 m}^2$  are probably sicker than those without these tests, as it requires them coming to healthcare repeatedly within a short period of time. Further, the longer the period between the measurements is, the higher are the chances of loss at follow-up with consequent lower CKD prevalence<sup>18</sup>. For example, a patient can experience a competing event (e.g. death) before he/she gets the chance of a second confirmatory test. Another challenge is presented by the low rate of albuminuria (or proteinuria) testing, directed mainly at persons at risk<sup>13, 14</sup>. Infrequent testing might also impact negatively on a correct estimation of the CKD prevalence or staging of CKD<sup>19</sup>.

### 1.3 Kidney function and cardiovascular diseases

In the early 19th century, Dr. Richard Bright was the first to suggest an association between impairment of the kidneys and cardiovascular abnormalities<sup>20</sup>. He observed that, in the majority of the cases of heart size increase (hypertrophy), there were co-existing signs of advanced kidney damage. Since then, many epidemiological studies have investigated and confirmed this association, showing a strong link between CKD and cardiovascular outcomes.

Two large meta-analyses on population cohorts described the strong association between kidney function and risk of cardiovascular mortality<sup>21, 22</sup>. Matsushita *et al.*<sup>21</sup> combined information from 21 cohorts for a total of approximately 1.2 million individuals. The pooled results showed that there is a significant increase in the risk of cardiovascular mortality associated with decline in kidney function. Compared to eGFR  $95 \text{ ml/min/1.73 m}^2$ , the hazard ratios (HR) for cardiovascular mortality at eGFR 65, 45 and  $15 \text{ ml/min/1.73 m}^2$  were 1.40 (95% Confidence Interval (CI): 1.25-1.57), 1.99 (95% CI: 1.73-2.28) and 2.66 (95% CI: 2.04-3.46), respectively. Similar findings were also observed in terms of albuminuria, with a risk that was two-fold higher in the microalbuminuria category (30-300 mg/g) compared to normal albuminuria.

Impaired kidney function has also been associated with the incidence of a variety of specific cardiovascular diseases. The risk of heart failure (HF) is approximately two times higher in patients with eGFR  $< 60 \text{ ml/min/1.73 m}^2$  and it is even higher in more severe CKD stages<sup>23-25</sup>. These results are similar when comparing severe and normal albuminuria<sup>24</sup>. Decline in eGFR and increasing albuminuria seem to be also associated with higher risk of stroke<sup>26, 27</sup>, atrial fibrillation (AF)<sup>28, 29</sup> and coronary heart disease<sup>30</sup>. The association between kidney impairment and risk of cardiovascular disease has been shown to be irrespective of other cardiovascular risk factors such as age<sup>31</sup>, sex<sup>32</sup>, diabetes<sup>33</sup> and hypertension<sup>34</sup>. At the same time, reduced kidney function plays an important role in increasing the likelihood of having cardiovascular outcomes in patients with pre-existing cardiovascular risk factors<sup>33, 34</sup>.

Part of the association between impaired kidney function and cardiovascular risk is possibly explained, as mentioned above, by shared risk factors (e.g. diabetes and hypertension). However, these comorbidities are not sufficient to explain such strong relationship. Other complications such as dyslipidemia<sup>35</sup>, inflammation<sup>36</sup>, anemia<sup>37</sup>, left-ventricular hypertrophy<sup>38</sup>, and atherosclerosis<sup>39</sup> have all been associated with CKD progression and increased risk of cardiovascular events (especially in patients with ESKD and undergoing dialysis). Finally, reduced kidney function can also affect pharmacokinetics<sup>40</sup>, which can reduce the effectiveness and safety of medications.

#### **1.4 CKD and adverse drug effects**

Prescribed drugs epitomize healthcare. In 2006, approximately 82% of individuals in the United States (U.S.) population used at least one prescribed drug, over-the-counter medication or dietary supplement, and 29% reported using five or more prescribed drugs<sup>41</sup>. In Sweden, where drugs are less commonly prescribed, still 2.8 million men (59%) and 3.6 million women (76%) received at least one drug prescription in 2010<sup>42</sup>. Inappropriate drug utilization (e.g. unnecessary prescription, incorrect dosing or insufficient monitoring of drugs) is common in clinical practice and can lead to adverse events while incurring in increased cost for healthcare<sup>43</sup>. Each year in the U.S., adverse drug events result in approximately 2 million hospitalizations<sup>44</sup>, cause 3.5 million office visits and 1 million emergency department visits<sup>45</sup>,<sup>46</sup>, and add \$3.5 billion to healthcare costs<sup>47</sup>.

Individuals with CKD are at particular high risk of adverse drug events<sup>48, 49</sup>, which can be explained by a variety of reasons. First, because impaired kidney function affects the pharmacokinetics<sup>40</sup>. Many medications are excreted by the kidneys, and lower GFR results in lower kidney excretion and a greater potential for supra-therapeutic or toxic drug levels. Clearance of highly protein-bound medications may be affected by the health of the kidneys' proximal tubule, the usual site of active secretion<sup>50</sup>. Kidney disease itself can alter hepatic and intestinal metabolism of drugs, exaggerating or attenuating drug efficacy<sup>51</sup>.

Second, pharmacokinetic data is not always available for old drugs, and is often obtained from small sample sizes and patients with limited comorbidities, which limits our understanding of drug safety for persons with CKD and often leads to contradictory recommendations for medication use and dosing<sup>52</sup>. Third, because the majority of CKD patients have concomitant comorbidities that may require multiple medications for optimal management<sup>53, 54</sup>, polypharmacy increases their risk of adverse drug reactions<sup>54-59</sup>. Finally, and in view of these adverse drug reaction risks, patients with CKD are often excluded from clinical trials, but being such a common population segment, the benefit of therapies is later extrapolated to them in clinical practice.

Kidneys are vulnerable to injury due to high concentration of medication and their metabolites in the renal tubular and interstitial cells<sup>60</sup>. Drug-induced nephrotoxicity accounts for 18-27% of community and hospital-acquired acute kidney injuries (AKI) episodes<sup>61</sup>. Many drugs are known for their nephrotoxic effect (e.g. non-steroidal anti-inflammatory drugs) and can have

severe effects in patients with existing impaired kidney function. Nevertheless, these drugs are likely to be prescribed to CKD patients regardless, due to the poor awareness in society and limited evidence to support clinical guidelines in this population. CKD is a disease that takes a long time to develop and it is a relatively rare event. Therefore, randomized controlled trials (RCTs) are often not powered to capture chronic impairment in kidney function, having too short follow-up time or small sample size. Finally, as mentioned, most RCTs have excluded CKD patients or included only a small proportion that precludes strong conclusion about their safety and effectiveness in this segment of the population<sup>62</sup>. This leaves an important knowledge gap that can have important repercussions in clinical practice.

### **1.5 Mineralocorticoid receptor antagonists (MRA)**

Mineralocorticoid receptor antagonists (MRAs), such as spironolactone and eplerenone, are commonly prescribed to hypertensive and HF patients<sup>63-66</sup>. These classes of drugs are recommended by guidelines because of their capacity to reduce blood pressure and lower the risk of cardiovascular events and mortality, as shown in large RCTs<sup>67-70</sup>. Despite this beneficial effect, the use of this therapy might be limited by the fear of adverse effects, in particular abnormal elevation of serum potassium (hyperkalemia), which can be a life-threatening event<sup>71</sup>. A recent meta-analysis of trials showed that approximately 9% of patients on MRAs experienced hyperkalemia<sup>72</sup>. However, according to the results from routine-care data in the U.S., hyperkalemia incidence may be even higher in the clinical practice<sup>73, 74</sup>. In **Study I**, we assessed hyperkalemia incidence among MRA users selected from a healthcare utilization cohort in Stockholm, Sweden.

The kidneys are actively involved in the long-term maintenance of the potassium homeostasis. Therefore, impairment in kidney function leads to an increment of potassium levels in the blood, predisposing patients with CKD to hyperkalemia. At the same time, routine care data suggests that MRA therapy is often affected by suboptimal dose-titration and monitoring, especially in primary care, which can also increase hyperkalemia incidence and risk of therapy discontinuation<sup>75</sup>.

Current management of hyperkalemia is often based on eliminating modifiable causes: reducing dietary potassium intake, promoting potassium excretion through diuretics use, and/or discontinuing or lowering the dose of hyperkalemia-inducing medications<sup>76</sup>. Stopping MRA, however, might deprive patients of their needed beneficial cardiovascular effects. High risk populations, such as patients with CKD, can be especially affected by this therapeutic compromise. Despite recommendations from clinical guidelines to stop MRA temporarily when potassium levels exceeds 6 mmol/L<sup>77</sup>, in the reality of clinical practice, MRA is often discontinued even with mild hyperkalemic events (>5 mmol/L)<sup>78</sup>. Evidence on the consequences of stopping vs continuing these medications after hyperkalemia is limited and has been poorly investigated in previous observational studies<sup>79-82</sup>. Differences in safety and effectiveness of these two treatment strategies have been investigated in **Study II**.

## 1.6 Glucagon-like peptide-1 receptor agonists (GLP-1 RA)

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are novel glucose-lowering treatments prescribed in patients with type II diabetes<sup>83</sup>. GLP-1 RAs have been also proposed as candidates for use in patients with diabetes at high risk of cardiovascular disease because of their capacity to reduce systolic blood pressure, inflammation, and lipid concentrations as well as eliciting significant reduction in body weight<sup>84</sup>. While all of the trials showed cardiovascular safety (i.e., non-inferiority) compared to standard of care, some trials<sup>85-88</sup> but not all<sup>89-91</sup> observed a significant reduction in the risk of major adverse cardiovascular events (MACE) outcome (cardiovascular mortality, non-fatal myocardial infarction (MI), and non-fatal stroke).

GLP-1 RA treatment could also be beneficial for secondary prevention in patients with manifest cardiovascular disease, but this issue has been considerably less studied. Results from a post-hoc analysis of the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial showed a cardiovascular benefit of liraglutide use compared to placebo also in patients with history of cardiovascular disease at inclusion (HR: 0.83; 95% CI: 0.74-0.93)<sup>85</sup>. Similar results were also observed in a population-based study by Svanström *et al.*<sup>92</sup> showing a 19% lower MACE risk in new users of liraglutide with a history of cardiovascular disease. In subsequent analyses, results were not always consistent among trials and among classes of GLP-1 RA considered<sup>89, 91</sup>. Whether the cardioprotective effect of GLP-1 RA is different depending on kidney function was not entirely explored and it was investigated in **Study III**.

## 1.7 Direct oral anticoagulants (DOAC)

Direct oral anticoagulants (DOACs) are a new class of drugs that have been recently developed for the treatment of AF. AF is the most common arrhythmia with a population prevalence of above 3% but present in >15% among individuals aged  $\geq 75$  years and is one of the leading causes of ischemic stroke worldwide<sup>93</sup>. Historically, AF patients have been treated with warfarin<sup>94</sup>, a vitamin K antagonist (VKA), which effectively prevents two out of three ischemic strokes compared to placebo<sup>95</sup>, but also increases the risk of bleeding, which can be a minor event or result in a fatal hemorrhage. VKA use is limited by a narrow therapeutic index, which determines that patients have to be frequently monitored, resulting in substantial burden to them. Therefore, new classes of oral anticoagulants (i.e., dabigatran, rivaroxaban, apixaban and edoxaban) have been developed and showed similar or greater efficacy and safety compared to VKA in pivotal RCTs of non-valvular AF populations<sup>96-99</sup> and have progressively substituted VKA in Swedish care<sup>100</sup>. The advantages of DOAC use include less drug and food interactions, more stable anticoagulant effects and reduced need for routine monitoring<sup>101, 102</sup>.

Unlike VKA, all DOACs rely on kidney clearance in some capacity. Therefore, elimination of DOACs is slower in patients with CKD, who can be more prone to drug accumulation and a higher risk of bleeding<sup>103</sup>. Because persons with CKD are particularly susceptible to both stroke and bleeding risk<sup>104</sup>, all RCTs of DOACs included few elderly patients and excluded patients with severe CKD (creatinine clearance  $< 30$  mL/min)<sup>96-99</sup>. Despite this, some DOACs have been

approved for use in patients with severe CKD (i.e., apixaban, rivaroxaban and edoxaban for creatinine clearance 15–30 mL/min)<sup>105</sup>.

Adverse kidney outcomes have been repeatedly associated with VKA use in case reports and observational studies<sup>106-110</sup>. Despite findings from some reports suggesting similar risks with DOAC treatment<sup>111-114</sup>, it was also hypothesized that DOAC use may have lower risk of kidney events due to the better cardiovascular profile and the potential beneficial effect on vascular inflammation. Post-hoc analyses from RCTs and meta-analyses seem to support the idea of lower risk of kidney outcome in patients treated with DOAC compared to VKA, however these studies were often limited in sample size, length of follow-up and definition of kidney outcomes<sup>115-120</sup>. The association between DOAC vs VKA use and cardiorenal outcome has been investigated in **Study IV**.

## **1.8 Observational studies supporting and expanding evidence from randomized trials**

RCTs represent the highest level of evidence when it comes to evaluating whether a treatment is safe and efficacious. However, compared to other disciplines in medicine, there are less RCTs in nephrology and when RCTs are conducted, they are more commonly Phase I and II studies<sup>121, 122</sup>. In addition, and according to a recent review, approximately 85% of late trials have explicitly excluded patients based on kidney function<sup>123</sup>. Even though exclusion of CKD patients might be appropriate (e.g. when the disease is severe or there are concerns on the potential nephrotoxicity and adverse reaction), data have shown that also patient with mild or moderate CKD are often excluded from the trials<sup>123</sup>. Collectively, this limits our understanding of the effect of treatments in patients with CKD<sup>124</sup>.

Although some improvements have been made during the last decade<sup>125</sup>, this lack of representation of patients with CKD in trials poses challenges to the nephrologists when they have to manage patients that are also treated for other comorbidities. Moreover, evidence from RCTs might not always be available for some clinical concerns, especially on long-term effects of medications in detecting rare outcomes (e.g. AKI), due to limited sample size and length of the follow-up. At the same time, RCTs may often be costly, impractical, unfeasible, unethical or conducted in highly selected populations which limits the generalizability of the conclusions.

Carefully-conducted observational studies can complement trial evidence and fill these knowledge gaps. By employing methods from pharmacoepidemiology, it is possible to use existing sources of information more efficiently, apply wider inclusion criteria, evaluate effectiveness and safety of the treatment in routine care settings as well as investigate treatment and monitoring practices.

In **appendix 1**, the interested reader can find a narrative review on how pharmacoepidemiological methods may inform nephrologists on best treatment strategies for their patients. We discuss strengths and limitations of different sources of data, as well as considerations on study designs, methods for drug utilization research and information needed to conduct good pharmacoepidemiological studies.

## 2 RESEARCH AIMS

### 2.1 Overarching aims

The first overarching aim of this thesis is to evaluate the effectiveness and safety of common cardiovascular and antidiabetic medications by using comprehensive routine care data as well as advanced pharmacoepidemiology study designs and methods. The second overarching aim is to investigate whether underlying kidney function alters the risk-benefit of these medications.

### 2.2 Study-specific aims

- **Study I:** Investigate the incidence and clinical predictors of hyperkalemia in patients starting MRA in routine care, as well as describe therapeutic reactions to hyperkalemia in routine clinical practice.
- **Study II:** Evaluate the risk-benefit of two different treatment strategies: stop MRA therapy after hyperkalemia vs. continue MRA.
- **Study III:** Investigate cardiovascular effectiveness of GLP-1 RA in diabetes patients surviving an acute MI.
- **Study IV:** Compare the risk of kidney outcomes (CKD progression or AKI) among patients with non-valvular AF initiating DOAC versus VKA.



## 3 MATERIALS AND METHODS

### 3.1 Data sources

#### 3.1.1 The Stockholm CREATinine Measurement (SCREAM) project

The SCREAM project is the largest healthcare utilization cohort from Sweden, covering Stockholm County<sup>126, 127</sup>. The central component of SCREAM is a repository of laboratory data from Stockholm County Council (SLL acronym in Swedish). SLL unifies all healthcare provided in the region of Stockholm. Three laboratory providers (Aleris (now known as Medylabs), Unilabs and Karolinska) perform the majority of all biochemical laboratory tests of the region, including primary, hospital and private healthcare.

The first linkage of SCREAM included data from 2006-2011 (SCREAM-1) of individuals undergoing creatinine testing in healthcare. The second linkage included healthcare data from 2006-2019 (SCREAM-2) of the complete population of Stockholm, with laboratory data for anyone undergoing creatinine or albuminuria testing. In addition to the creatinine and albuminuria testing, the only inclusion criteria for entering SCREAM is residency in Stockholm with a valid personal identifying number. Several laboratory tests, taken or not in concomitance with creatinine, were also extracted. Each laboratory test was accompanied by the Swedish personal identification number<sup>128</sup> of the patient that received it, along with the date of testing, time of the day, method and units. Inter- and intra-laboratory variation is considered minimal, with the three laboratories being frequently audited for harmonization.

The dataset was then linked to regional and national administrative databases including the health data registry of this region (Vårdanalysdatabasen, VAL). VAL contains information on all healthcare consultations in primary, specialist outpatient care and hospitalizations. For each resident all available data since 1997 was included. This is the year in which the International Classification of Diseases Version 10 (ICD-10) coding system was implemented. Each visit record is accompanied by the date, the center and medical department accessed, therapeutic procedures undertaken and established diagnoses. VAL also provides information on patients' demographics, which include: sex and date of birth (month/year), migration procedures (to and from the county), and ascribed municipality of residency.

The SCREAM dataset was then enriched with linkage with national registries provided by the National Board of Health and Welfare as well as Statistics Sweden. Not all of these linkages have been used in this thesis (**Figure 2**):

*The Swedish Prescribed Drug Registry* is a nationwide registry instituted in 2005 which collects information on all prescription drugs dispensed at Swedish pharmacies. This registry present almost complete coverage (>99.7%) of all dispensed drugs. Other available information are: practice (e.g. primary healthcare) and specialty of the prescriber, generic name of the drug, Anatomical Therapeutic Chemical (ATC) code, number of doses dispensed and the costs (both reimbursed expenditure and patient co-payment). Information on actual prescribed dosage is available as unstructured text. This registry did not contain information on over-the-counter,

ambulatory or in-hospital care drugs and it did not completely cover drugs used at nursing homes or vaccines.

*The Swedish Population Registry* records, on a monthly basis, information on vital status for each Swedish citizen with virtually no loss to follow-up. In case of death, the reported cause of death was recorded as well.

*The Swedish Medical Birth Registry* contains data on all births in Sweden since 1973, including mother's age, country of birth, county of residence and infant details including singleton, multiple births and stillbirths, infant sex, and neonatal diagnoses coded using the ICD classification system. The Swedish Medical Birth Registry includes 98–99% of all births in Sweden with high quality data<sup>129</sup>.

*Swedish Cancer registry*, established in 1958, contains information about all malignant tumors and certain benign tumors diagnosed in Sweden. The diagnosis can be done based on clinical examination, morphological examination, surgery, autopsy, or other laboratory examination<sup>130</sup>.

*The Longitudinal Integration Database for Health Insurance and Labour Market Studies* (LISA by Swedish acronym) contains socio-economic data collected from different sociodemographic population registries. Variables included country of birth, educational level, occupational status and income level.

Additionally, SCREAM was linked with a variety of quality registries of national coverage, such as:

*The Swedish Renal Registry* includes Swedish patients referred to a nephrologist and diagnosed with CKD. All hospitals are encouraged to include patients from CKD Stage 3b and it is considered mandatory from the first diagnosis of CKD Stage 4. The registry contains patients' information collected at regularly scheduled visits from the non-dialysis phase until initiation of kidney replacement therapy (first chronic dialysis or kidney transplantation). Additionally, it records data on primary kidney disease, specific laboratory tests (e.g. uric acid) and in-hospital provided drugs for kidney diseases treatment.

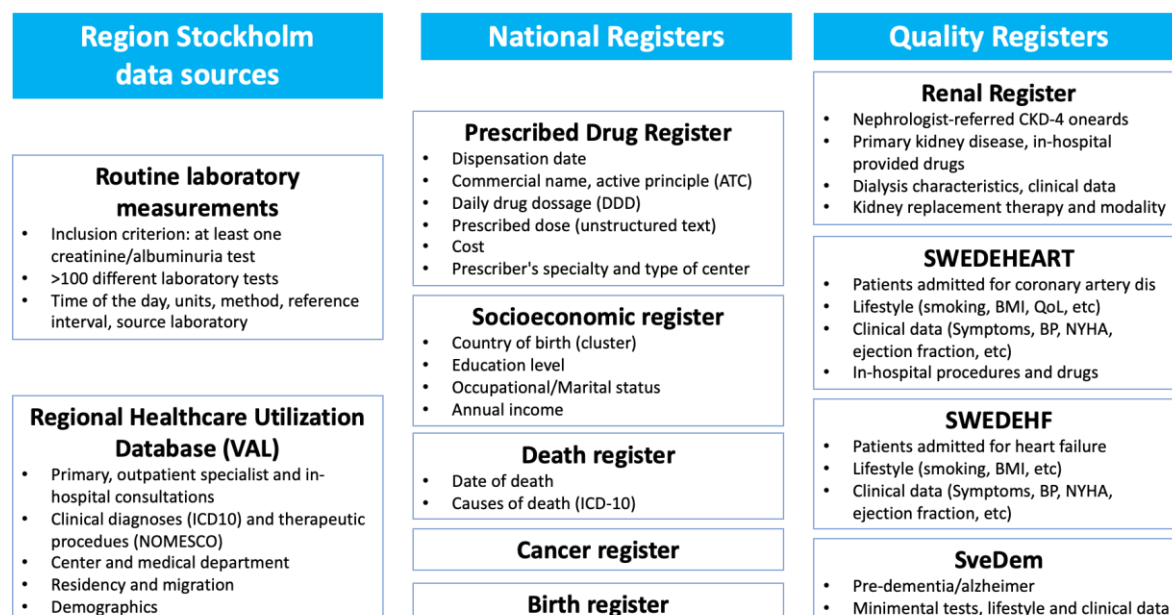
The cardiology registries *Swedish Web-system for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies* (SWEDEHEART) and *Swedish Heart Failure* (SWEDEHF), with information on patients admitted to healthcare with an acute coronary syndrome (ACS) or HF, respectively. A more detailed description of the SWEDEHEART registry is provided in the next section.

*The Swedish Dementia* (SweDem) registry, a quality registry that was initiated in 2007 to monitor the quality of diagnostic and treatment of dementia patients in Sweden. The registry contains information on the content and duration of the examination and diagnosis process, type of housing, dementia diagnosis, cognitive ability, drug therapy, and support provided by county councils and municipalities.

After linkage, the patient's identification number was replaced by a random identifier at the Government offices, safeguarding patient privacy and confidentiality. Only then was the dataset shared with the researchers.

SCREAM-1 was used for **Study I**, while SCREAM-2 was the dataset used for **Study II and IV**.

**Figure 2. Available linkages and key information in the SCREAM project 2006-2019.**



### 3.1.2 The Swedish Web-system for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies (SWEDEHEART) registry

For the purpose of **Study III**, we used all available data collected nationally in SWEDEHEART. This registry contains information of patients hospitalized for suspected ACS or undergoing coronary or valve intervention. SWEDEHEART covers around 90% of patients with ACS treated in hospitals in Sweden. Monitoring of the collected data is performed regularly and reaches elevated agreement (~95%) on important variables between the registry and electronic health records. Comprehensive information are collected prospectively, including patient demographics, past medical history, medications before admission, electrocardiographic changes, body mass index (BMI), smoking, ejection fraction, clinical investigations, medical treatment in hospital, interventions, hospital outcomes, diagnoses, and medications at discharge. Data for **Study III** included the period 2010-2017 and was enriched via linkage with the Swedish Prescribed Drug Registry and the Swedish Population Registry. Similar to SCREAM, the personal identification number was substituted by a random identifier by Statistics Sweden, and de-identified data was made available to the researchers preserving anonymity and allowing the waiving of informed consent.

## 3.2 Study designs applied in this thesis

### 3.2.1 New user study design

A research question that is commonly investigated in pharmacoepidemiological studies is whether a treatment should be initiated or not to treat or prevent a certain outcome. In this setting, the most appropriate approach is to focus on a new-user or incident user design. When we apply this study design, we center our analysis on individuals who initiate treatment in a certain period and we follow them until the end of the study period. Thus, the start of the follow-up coincide with the start of the treatment, or time zero ( $T_0$ )<sup>131</sup>. This design resembles the approach used in RCTs, where the  $T_0$  is the time of randomization, which is usually right before the treatment initiation. New users can be identified by new pharmacy fills, not preceded by other dispensations of the same drug during a specific window of time prior to the dispensation of the drug. The length of this period depends on the research question, the availability of data, pattern of use and pharmacokinetics<sup>132, 133</sup>.

In **Study I**, we selected all adults ( $\geq 18$  years old) that initiated MRA therapy in Stockholm between 1st January 2007 and 31st December 2010, irrespective of indication, and that had at least one creatinine and a potassium ( $K^+$ ) measured at treatment initiation or within the year prior. These tests were then used to estimate their baseline level of  $K^+$  and kidney function. New users of MRA were defined as first time users of MRA in the study period, with no previous dispensation recorded for at least one year prior. In **Study II**, we extended the same definition but applied it to a more contemporary dataset (end of eligibility period was 31<sup>st</sup> December of 2018).

In **Study IV**, we identified all adults that started oral anticoagulants (OACs) in the period between 1<sup>st</sup> January 2011 and 31<sup>st</sup> December 2018, with a diagnosis of AF in the preceding 5 years. New users of OAC were identified as individuals with a first prescription of DOAC or VKA drugs with no previous dispensations of any OAC registered in the Prescribed Drug Registry. The date of OAC initiation was defined as index date and start of the follow-up ( $T_0$ ).

### 3.2.2 Active comparator design

The active comparator design compares the treatment of interest with another treatment that has a similar indication<sup>134, 135</sup>. This design is a better choice than comparing users of the treatment with patients that are not treated (non-users) as it reduces confounding by indication. A non-treated group can include, compared to those that were treated, patients with very different medical history, concomitant medications and prognosis, thus increasing the chances for both measured and unmeasured confounding.

Choosing an active comparator instead of a non-users group also defines a research question more relevant for clinical practice. The interest on safety and effectiveness of a treatment usually relies on the potential benefit or harm compared to another treatment that could be prescribed for the same medical condition. The information from an active comparator design

can then be used by physicians and patients to make an informed decision on what will be the best treatment strategy among all the available alternatives.

In **Study I**, we defined a parallel cohort of new users of beta-blockers to compare the incidence of hyperkalemia during the first year of follow-up. This cohort was selected because it had to some extent, a similar indication as MRA use but less presumed hyperkalemia risk.

In **Study III**, the main analysis was focused on evaluating the risk associated with use of GLP-1 RA vs non-users among diabetes patients surviving a MI. Because this drug was only recently introduced in the market, the comparator (non-use) referred to standard of care (same control group used in pivotal trials of GLP-1 RA). However, we performed an additional analysis using sulfonylurea as an active comparator to reduce the possibility of unmeasured confounding.

In **Study IV**, we focused on patients with AF and compared the effectiveness and safety of two medications with the same indication, VKA and DOAC.

### 3.2.3 Target trial emulation design

The target trial emulation framework is a recently developed methodology to explore causal inference in observational studies<sup>136</sup>. The idea is to consider the ideal randomized trial that we would design to answer our research question and use observational data to emulate it. Some of the advantages of this approach include prevention of unwanted biases such as prevalent user and immortal time bias<sup>137-139</sup>, as well as easier comparison between results from observational studies and findings from RCTs<sup>140</sup>. Similar to trials, the first step is to define a study protocol that should include: eligibility criteria, the definition of the treatment strategies that we want to compare, the assignment procedure among selected individuals, the length of follow-up, outcomes of interest and the statistical analysis.

An example of a target trial protocol is provided in the online supplemental material of **Study II**. In summary, we wanted to compare, among new users of MRA who experienced incident hyperkalemia, the strategies of “Stop MRA within the first 6 months after hyperkalemia” vs “Continue MRA for at least 6 months after hyperkalemia”. The 6 months grace period was necessary due to the data available in SCREAM regarding treatment decision. In this registry-based study, we did not have information on the actual decision made by the physician as consequence of the adverse event. The only information available in the dataset was whether the patient received a new dispensation of the treatment or not. Therefore, we could only assume what the physician decided by assessing presence or absence of a new dispensation after the event. Thus, we needed to identify a period after hyperkalemia during which we could determine whether the patient received a new dispensation or not. Based on common MRA treatment patterns, we decided for a 6 months window. All patients were on treatment at the beginning of the follow-up (index hyperkalemia) but some of them continued after the event, while others stopped.

Comparing treatment strategies that vary over time requires adjustment methods that properly account for time-varying confounding, such as parametric G-formula or cloning, censoring and

weighting<sup>136, 141</sup>. In order to deal with confounding associated with changes in the treatment strategy in **Study II**, the latter was applied (**Figure 3**).

The approach consists in three steps:

*Step 1: Cloning and assignment of treatment strategies to each replicate*

A consequence of using a grace period is that, for the duration of this time window, an individual might be consistent with more than one strategy. In **Study II**, an individual who received a new dispensation at the end of the 6 months was consistent with both strategies up to that point. Had a patient died before the end of the grace period, it would have been impossible to know which decision was taken after the hyperkalemic event. One possibility to overcome this problem is to create an exact copy (clone) of individuals and assign each clone to a different strategy. The dataset for the analysis will then contain all the information twice, including comorbidities, medications and outcomes. By design, the baseline characteristics of the two clones will be exactly the same and no baseline confounding will be present.

*Step 2: Censoring when clones deviate from the assigned treatment*

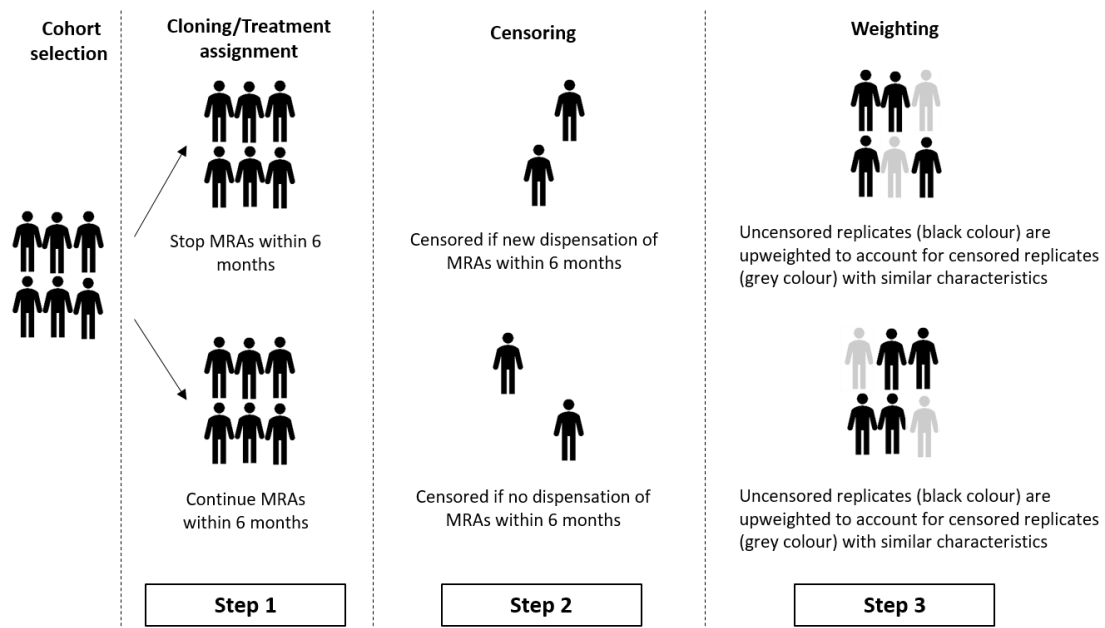
The clones included in the dataset will not always adhere to the assigned strategy. In order to estimate the effect on the outcomes associated with the specific strategy, we need to censor clones whenever they deviate from the assigned treatment.

In **Study II**, we split the grace period in weekly intervals and determined whether the specific clone was adherent to the assigned treatment at each of these time intervals. Clones assigned to stop the MRA treatment within 6 months from hyperkalemia were censored if a new dispensation of MRA was recorded before the end of the grace period. Those assigned to continue the treatment after hyperkalemia were censored if there was no additional dispensation before the end of the grace period.

*Step 3: Inverse probability weighting*

The artificial censoring of clones during grace period is likely informative. Several reasons can determine the continuation or stop of the treatment: an adverse event, the age of the patient, use of other medications, patient's medical history, etc. In order to avoid selection bias, all these components were adjusted for by using an inverse probability weighting method. Briefly, a weight was assigned to the uncensored clones and it was equal to the inverse of the probability of remaining uncensored, conditional on all components that can influence the treatment decision. Intuitively, uncensored clones are up-weighted to account for censored clones that have similar characteristics. Using this method, the bias introduced by informative censoring is removed by creating a pseudo-population in which censoring no longer depends on measured characteristics.

**Figure 3. Schematic representation of the cloning, censoring and weighting method (Study II)**



Abbreviation: MRA, mineralocorticoid receptor antagonist

In **Study II**, the estimated inverse probability of censoring weights were obtained by fitting a pooled logistic regression model with being uncensored as the outcome and including demographics, medical history, concomitant medication, kidney function, previous MRA duration and severity of index hyperkalemia as independent variables. Two models were fitted separately for each treatment strategy arm to account for different censoring patterns between arms. We then used the estimated probabilities to construct the inverse probability of censoring weights as shown in **Table 1**. Finally, the weights at each weekly interval were obtained as the cumulative product of all weights up to that time point.

**Table 1. Contribution to the weights at each time point by MRA treatment strategy (Study II)**

Assigned strategy	Time interval	Contribution to weights
Stop MRA within 6 months	$0 \leq t \leq 6$ months	$1/p$
	$t > 6$ months	1
Continue MRA for at least 6 months	$0 \leq t < 6$ months	1
	$t = 6$ months	$1/p$
	$t > 6$ months	1

Abbreviation: MRA, mineralocorticoid receptor antagonist

\*  $t$  is the time since index hyperkalemia and  $p$  is the probability of remaining uncensored conditional on all baseline and time-varying covariates. The weights are assigned to 1 after the grace period of 6 months because the interest is only in the initial decision after hyperkalemia (within the first 6 months). In addition, in the continuation arm clones can be censored only if they did not receive a new dispensation of MRA during the initial 6 months. Therefore, censoring can only be assessed at the end of the grace period and the weights will have contribution 1 until then.

### 3.3 Adjustment for confounding in observational studies

Absence of treatment randomization in pharmacoepidemiological studies means that we need to account for confounding in the statistical analysis. The first critical aspect is to select the appropriate set of confounders to adjust for in the analysis<sup>142</sup>. In general, it is not recommended to solely rely on statistical approaches to identify potential confounders, such as only including statistically significant covariates (i.e. backward and forward methods) or retaining variables that modify the regression coefficient of the treatment variable<sup>143-145</sup>. These methods are strongly influenced by sample size and data availability. More importantly, they are not able to distinguish confounders from other covariates that are associated with the exposure and the outcome, such as mediators and colliders, for which adjustment may be undesirable or harmful<sup>146</sup>. Instead, a method called “disjunctive cause criterion” selects any pre-exposure covariate that is a cause of the exposure, or the outcome, or both. This approach is deemed to be the most appropriate to adjust for confounding with the least potential biases<sup>142, 147</sup>. However, this method requires a pre-existing knowledge on the biological plausibility of an association between these variables and the exposure and/or the outcome.

Once the confounders have been identified, several methods can be employed to adjust for them. These include: multivariable regression, exact matching, standardization, and methods based on propensity scores (PS, i.e. matching, weighting stratification and adjustment). If the models are correctly specified and the study is performed in a time-fixed setting (i.e. the treatment group is defined at a specific point in time), all these methods provide appropriate adjustment for measured confounding, although the interpretation of the estimates might depend on the method used. In particular, methods such as multivariable adjustment (and PS adjustment) estimate a conditional effect, while matching and weighting provide an estimate of a marginal causal effect. The conditional effect is the effect that applies at specific levels of the covariates<sup>148</sup>. The marginal effect is the average effect (over covariate levels). In a setting in which the estimated effects are not collapsible (i.e. hazard ratio) the conditional and marginal effect might not be the same, so the proper method should be selected based on the effect of interest.

Methods based on PS have become a cornerstone of adjustment for confounding in pharmacoepidemiological studies. These methods combine all confounder information in a single score, which formally represent the probability of receiving the specific treatment conditional on the patient’s measured confounders<sup>149</sup>. The PS is usually estimated using a logistic regression with the treatment as dependent variable and all the potential confounders as independent variables:

$$PS = p(x) = P(T = 1|X = x) = \frac{\exp(\alpha + \beta X)}{1 + \exp(\alpha + \beta X)}$$

where  $p()$  is the probability,  $T$  is the treatment (1 = treated, 0 = untreated),  $X$  is the vector of all confounders,  $\alpha$  and  $\beta$  are the parameters of the logistic model that need to be estimated.



After estimation, these scores can be used for confounding adjustment in different ways: adjustment as covariate in a multivariable regression model, matching or weighting. The simplest approach is to add the PS as a regressor in the model with only the treatment and the outcome. This method provides an advantage compared to a multivariable regression when the outcome is rare. In settings where the number of events is very low, including all covariates in the model can cause problems with convergence and statistical power. This is less of a problem when only the PS is included in the model, however, an incorrectly specified PS model can also introduce bias in the estimates.

In PS matching, each patient in the treated group is matched with an untreated patient that has a similar PS value. The most used methods to combine individuals are the one-to-one or one-to-many nearest neighbor matching. Using these matching methods, each treated patient is matched to one or more untreated patients that have a PS within a pre-specified maximum distance (caliper)<sup>150</sup>. The quality of the balancing can be assessed using absolute standardized differences, calculated as the difference in sample means (or proportions for dichotomous variables) divided by the pooled standard deviation. Usually, a standardized difference <0.1 is used to indicate acceptable balance between treatment groups<sup>151</sup>.

Finally, PS can be used to create weights as the inverse probability of receiving the study treatment  $p(x)$ . Specifically, the weight is equal to  $1/p(x)$  for the treated and  $1/(1-p(x))$  for the untreated patients. This method is known as inverse probability of treatment weighting (IPTW). Applying the IPTW approach, we create a weighted population, or pseudo-population, in which the measured confounders are evenly distributed between treated and untreated patients. Similar to the PS-matching, the quality of this balance can be assessed using standardized differences.

Extreme weights are commonly observed whenever the PS is close to 0 for the treated patients and close to 1 for the untreated. One solution is to pre-specify the maximum value allowed (e.g. 99<sup>th</sup> percentile) and “truncate” any weight exceeding it. Alternatively, we can define stabilized weights, which use the marginal probability of treatment instead of 1 as numerator:

$$SW_{treated} = \frac{P(T = 1)}{P(T = 1|X = x)}$$

and

$$SW_{untreated} = \frac{1 - P(T = 1)}{1 - P(T = 1|X = x)}$$

where  $P(T=1)$  is obtained from a logistic regression model with the treatment as dependent variable and no confounders.

In **Study I**, we evaluated the mortality risk associated with hyperkalemia occurrence using a time-dependent Cox model. The follow-up was split at the time of incident hyperkalemia after MRA initiation and all covariates were time-updated. Adjustment for confounding was performed including all covariates in the multivariable Cox model. In a sensitivity analysis, we

also evaluated the incidence of hyperkalemia in a parallel cohort of new users of beta-blockers. In order to balance baseline characteristics between cohorts, we created a 1:1 propensity score matched beta-blockers group using the nearest neighbor approach with a caliper of 0.05. The propensity score was calculated with a multivariable logistic regression including all available confounders.

As mentioned in the previous section, in **Study II** we have applied a target trial emulation approach. The discrete-time HR for stopping MRA on the study outcome was estimated using a weighted Cox proportional hazard model and weighted cumulative incidence curves. The use of weights allowed to balance the distribution of confounders between treatment strategies. In case of no unmeasured confounding, the weighted cumulative incidence curves provide the hypothetical cumulative incidence that would have been observed had all patients followed that specific treatment strategy<sup>152</sup>.

In **Study III**, we estimated the association between GLP-1 RA use and cardiovascular outcomes using a multivariable Cox regression, adjusting for all measured confounding. To assess robustness of our finding, we have also matched users and non-users of GLP-1 RA using exact and PS matching. In the first analysis, we created a 1:5 matched cohort where users were matched with non-users that had the same age, sex and category of eGFR, which were considered the main confounding of the treatment-outcome association. In the second analysis, applying the same 1:5 matching ratio, we matched based on the PS and a nearest neighbor matching without replacement using a caliper of 0.01. All confounders used in the multivariable adjustment were also included in the logistic model for the PS.

Finally, in **Study IV**, we applied IPTW to adjust for confounding of the association between use of DOAC and study outcomes by balancing 50 clinical characteristics between groups. The weights were calculated employing a multivariable logistic regression to estimate the probability of receiving DOAC vs. VKA. We used stabilized weights to minimize extreme values. In the per-protocol analysis we also applied inverse probability of censoring weights to account for informative censoring.

### **3.4 Assessing robustness and consistency of findings**

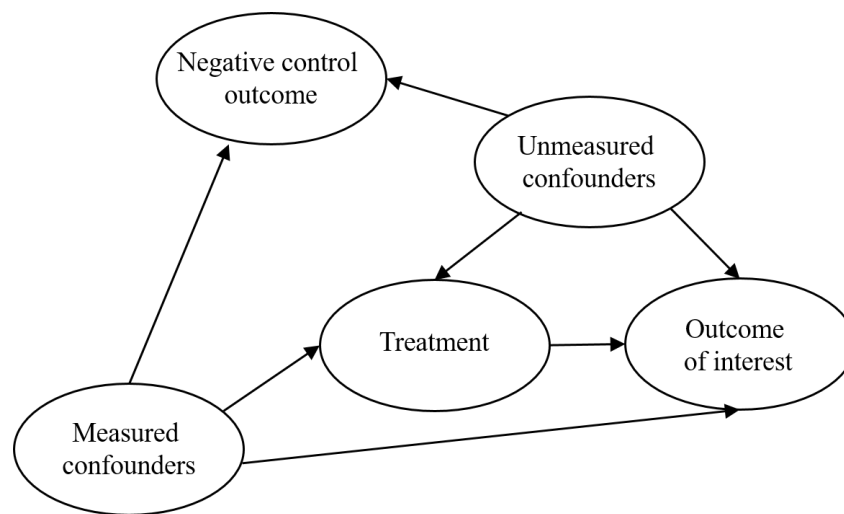
#### **3.4.1 Negative control outcomes**

Despite extensive efforts to adjust for measured confounders of the treatment-outcome association, the risk of residual unmeasured confounding is unavoidable in observational studies. Lack of randomization and limited data on patients' characteristics are the main reason why it is important to consider the possibility of unmeasured confounding that could explain the observed association. One of the methods applied to investigate the presence of unmeasured confounding is the use of negative control outcomes<sup>153</sup>.

Ideally, a negative control outcome should have the same set of confounders (measured and unmeasured) as the treatment-outcome association under investigation but for which the treatment has not direct causal effect (i.e. no arrow between treatment and negative control

outcome, **Figure 4**). The basic idea of this approach is to evaluate whether an association between the exposure and the negative control outcome appears, even after accounting for all measured confounding. If that is the case, it means that there is some common unmeasured confounding that could also potentially bias the association with the outcome of interest. In practice, the set of confounders will only approximately overlap, at best, and it is possible that unmeasured confounding between the exposure and the negative control outcome still exists. This means that this sensitivity analysis can only provide an indication on the existence of potential residual confounding and an unexpected association between exposure and the negative control outcome still does not prove that the treatment-outcome association is biased.

**Figure 4. Causal diagram showing an ideal negative control outcome**



In **Study II**, we have investigated the robustness of our results using fracture risk as negative control outcome. We hypothesized that the decision of stopping or continuing MRA after hyperkalemia should not have an effect on the risk of fracture. The association between treatment strategy and negative control outcome was estimated using the same weighted model applied in the main analysis. In **Study IV**, we selected two negative control outcomes, pneumonia and cataract surgery, which have been commonly used in previous research on the same topic<sup>154, 155</sup>. An observed association between DOAC use and pneumonia or cataract surgery would be an indication for potential residual confounding.

### 3.4.2 Accounting for changes in the treatment pattern over time: per-protocol design

In pharmacoepidemiological studies patients are usually followed from treatment initiation until the occurrence of the study outcome or the end of the follow-up. The latter is commonly defined by administrative censoring such as end of the data coverage, end of observation study, loss to follow-up (e.g. emigration) or death (when it is not the outcome of interest). The association with the outcome is often based on the treatment assigned at baseline, without accounting for possible changes in the treatment strategy during follow-up (e.g. treatment discontinuation, switch, etc.). This approach is comparable to assess the intention-to-treat effect in a RCT, in which the comparison is made between the randomized treatments at baseline.

However, especially in a real-world setting, deviation from the “protocol” are likely and they should be accounted for in the analysis. This is especially relevant in studies in which the therapeutic effect of the treatment is no longer relevant shortly after the treatment discontinuation.

Intuitively, a possible approach would be to exclude non-adherent individuals. However, this method will lead to unbalancing between groups, selection bias and reduction in study power<sup>156</sup>. Instead, a common approach used in pharmacoepidemiology is to censor patients when they no longer comply with the “assigned” treatment. In this way, we only consider events that occur while patients are on-treatment and they are still compliant with the treatment assigned at baseline.

In **Study I**, we performed a sensitivity analysis censoring the cohort if they discontinued MRA treatment during the first year of follow-up. Discontinuation was defined based on pills supply, which was calculated as number of pills dispensed divided by the prescribed daily dose plus a lag-time of 30 days to account for stockpiling and hospitalizations. The treatment was considered discontinued whenever there was no additional dispensation before the end of the pills supply.

In **Study II**, the main results were complemented with a supplemental analysis accounting also for changes in the assigned treatment strategy that occurred after the initial grace period of 6 months. Discontinuation was defined whenever there was no subsequent dispensation or it was >6 months from the previous one. Applying this approach, we estimated the effect of “always continue MRA after hyperkalemia” vs. “stop MRA within 6 months after hyperkalemia and never restart”.

In **Study IV**, we censored patients at treatment discontinuation, defined as absence of a refill before the end of the estimated pill supply plus a lag-phase of 120 days, or switch from DOAC to VKA or vice versa. In this setting, it was hypothesized a different rate of discontinuation or switch depending on the initial treatment assignment. Therefore, censoring due to discontinuation/switch was considered informative and we used inverse probability of censoring weights to account for differential loss to follow-up between groups<sup>157</sup>.

### **3.4.3 Subgroup analyses**

In analyses of RCTs and observational studies is common to report, together with the overall results, subgroup or subset analyses. The treatment-outcome association is investigated among patients with similar realization of a specific characteristic (e.g. only men) or can be compared with the others (e.g. men vs women). The main goal is to investigate differences within groups in terms of treatment effect, since patients can be affected differently by dispensed treatments. These results can then be used in routine care to choose the best treatment based on the individual characteristics of the patient. The subgroups should be identified a priori and be supported by a biological rationale.

When the focus is on the effect modification, it has been argued that results from the subgroup analyses should report the relative rather than absolute effects<sup>158, 159</sup>. Differences in terms of absolute effects are often observed even when the relative effect (e.g. hazard ratio) is similar between groups. This occurs because the underlying risk might be quite different in each group (e.g. men have higher risk of cardiovascular events than women), thus, the same relative change will result in substantial difference on the absolute scale.

In **Study I and II**, we repeated the main analyses in the subgroup of patients with history of HF, a main indication for MRA use. Additionally, in **Study II** we assessed the consistency of our findings in subgroup analyses by age (<70 or ≥70 years), sex, eGFR category (eGFR <60 or ≥60 mL/min/1.73m<sup>2</sup>) and index hyperkalemia severity [mild (K<sup>+</sup> >5.0–5.5 mmol/L) or moderate/severe (K<sup>+</sup> >5.5 mmol/L)].

In **Study III**, effect modification was investigated in subgroups defined a priori: age (<70 or ≥70 years), sex, eGFR category (eGFR <60 or ≥60 mL/min/1.73m<sup>2</sup>) and ST-segment elevation MI (STEMI, yes/no).

Finally, in **Study IV**, we assessed whether the effect of DOACs compared to VKA was different across strata of age (<75 or ≥75 years), sex and eGFR category (eGFR <60 or ≥60 mL/min/1.73m<sup>2</sup>). The main analysis was also performed in the restricted population of patients with CHA<sub>2</sub>DS<sub>2</sub>-VASc ≥ 2 (more strict indication for OAC use).

### 3.5 Ethical considerations

The presented work was conducted entirely using laboratory and registry-based databases. In accordance with the Personal Data Act in Sweden and with the European General Data Protection Regulation, personal data was anonymized at the Swedish Board of Health and Welfare before being sent to the researchers and safely stored in encrypted servers at the department of Medical Epidemiology and Biostatistics. Informed consent is not deemed necessary for anonymized registry-based data accordingly to Swedish law. All the presented studies have been approved by the Regional Ethical Review Board in Stockholm.

## 4 RESULTS

### 4.1 Study I

For this study, we selected adults ( $\geq 18$  year) who initiated MRA treatment in routine care between 2007 and 2010 and had available information on eGFR and  $K^+$  at baseline. At the start of MRA therapy, patients' median age was 73 years and 53% were women. The median  $K^+$  level was 3.9 (Interquartile range (IQR): 3.6-4.2) mmol/L. The most common comorbidities were hypertension (64%), HF (46%), CKD (28%, eGFR $<60$  ml/min/1.73 m<sup>2</sup>), diabetes mellitus (25%) and history of MI (18%).

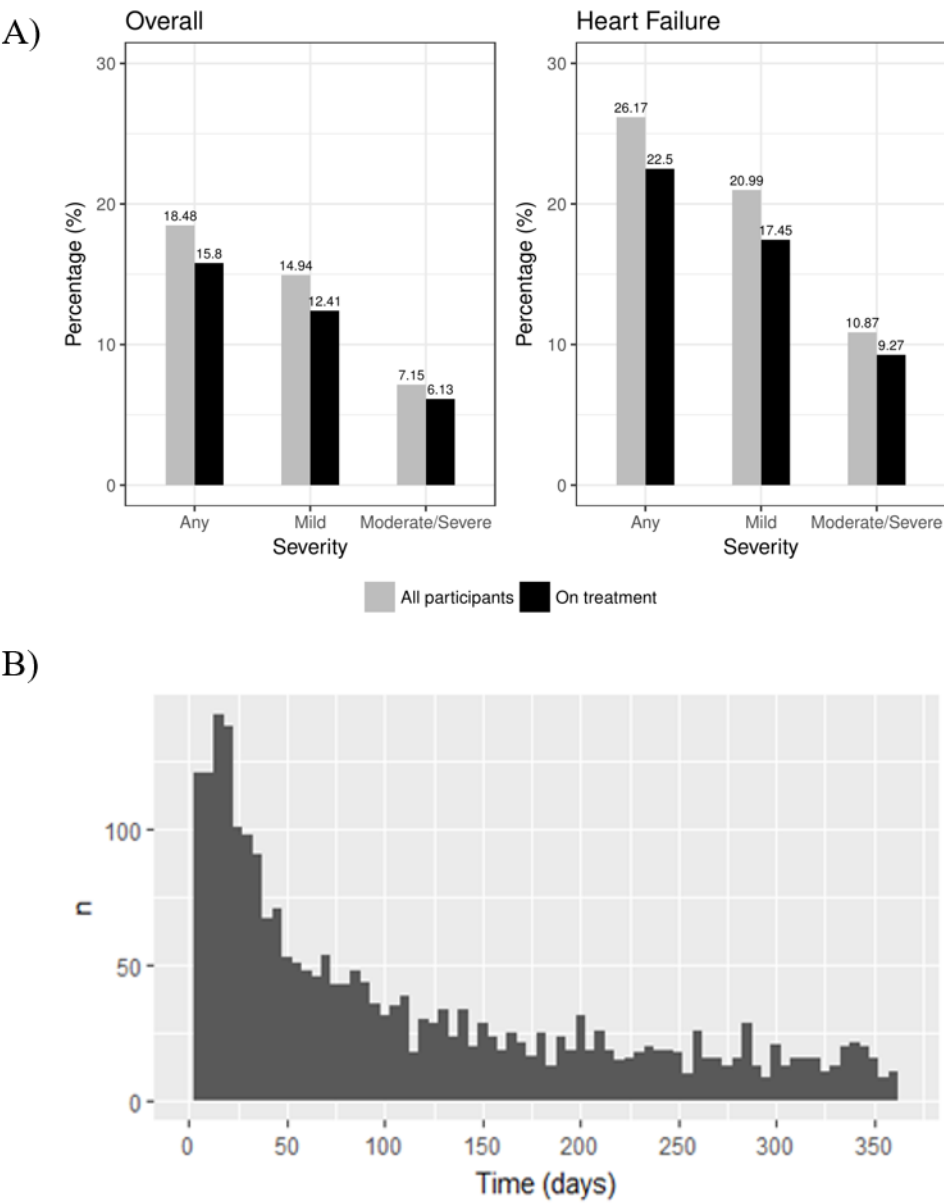
During the year after MRA initiation, 2536 (18%) experienced at least one hyperkalemia episode. Mild hyperkalemias were more common than moderate/severe hyperkalemias (15% vs. 7% respectively) (**Figure 4.1.1**). The proportion of hyperkalemia cases was higher in the subpopulation of patients with HF where 26% of patients experienced at least one detected hyperkalemia, and 11% experienced moderate/severe hyperkalemia. The distribution of time to first detected hyperkalemia showed that the majority of events occurred quite early during therapy, in particular within the first three months (**Figure 4.1.1**).

The main predictors at MRA initiation of incident hyperkalemia included increasing age, lower kidney function and elevated baseline  $K^+$  level. In general, predictors of mild or moderate/severe events were similar, but with some differences in magnitude of the associations.

Of the 2536 patients that experienced hyperkalemia, 2169 (85%) had the event while still on MRA therapy. Of those, 408 patients (18%) died as result of or shortly after hyperkalemia (within 4 months). Among the remaining 1761 patients, 53% continued the MRA therapy after the event and 47% stopped (**Table 4.1.1**). Patients who continued the therapy were more often prescribed the same MRA dose (10%) as before the hyperkalemic event. As many as 23% of patients also discontinued angiotensin-converting-enzyme inhibitor (ACEi) or angiotensin receptor blockers (ARBs), while 45% received de novo dispensation of diuretics and 1.6% started sodium polystyrene sulfonate. Severity and timing of hyperkalemia influenced prescription patterns. Patients who experienced a more severe event, compared to a mild event, were more likely to stop the MRA therapy (58% vs 43%). At the same time, events that occurred less than 3 months since MRA initiation were more often followed by MRA discontinuation or dose reduction.

Early ( $<3$  months) and more severe hyperkalemic events ( $K^+ > 5.5$  mmol/L), together with lower kidney function, were strongly associated with higher odds of MRA discontinuation.

**Figure 4.1.1 Proportion of hyperkalemic events overall and in the subpopulation with heart failure (Panel A) and time-to-event distribution (Panel B) during one year from mineralocorticoid receptor antagonists (MRA) initiation.**



**Table 4.1.1 Matrix of drug prescription patterns after hyperkalemia: overall, by event severity and by time since therapy initiation.**

	Overall	By event severity		By timing	
	(N = 1,761)	Mild hyperkalemia (N = 1,277)	Moderate/severe hyperkalemia (N = 484)	<3 mo. of therapy (N = 1084)	>3 mo. of therapy (N = 677)
<b>MRA continuation</b>	934 (53%)	731 (57%)	203 (42%)	535 (49%)	399 (59%)
-Same dose	842 (90%)	668 (91%)	174 (86%)	475 (89%)	367 (92%)
-Reduced dose	92 (10%)	63 (9%)	29 (14%)	60 (11%)	32 (8%)
<b>MRA cessation</b>	827 (47%)	546 (43%)	281 (58%)	549 (51%)	278 (41%)
<b>Discontinuation of ACE/ARBs*</b>	282 (23%)	191 (22%)	91 (26.8%)	194 (25%)	88 (20%)
<b>Prescription of new diuretics**</b>	255 (45%)	171 (42%)	84 (53.2%)	133 (47%)	122 (44%)
<b>Prescription of new SPS</b>	28 (1.6%)	10 (0.8%)	18 (3.7%)	19 (1.8%)	9 (1.3%)

\* Proportions based on the number of individuals that were consuming ACE/ARBs at time of event (n=1220).

\*\* Proportions based on the number of individuals not consuming diuretics at time of event (n=562).

Mild hyperkalemia: K 5.0-5.5 mmol/L; Moderate/Severe hyperkalemia: K>5.5 mmol/L; ACEi, angiotensin-converting-enzyme inhibitor; ARBs, angiotensin receptor blockers; SPS, sodium polystyrene sulfonate

## 4.2 Study II

For this study, we selected a cohort of adults ( $\geq 18$  years) who started MRA treatment in the period 2007-2018 and survived the first detected hyperkalemia. Out of the 39,518 patients included, 7,366 survived a hyperkalemia episode while on-treatment. Among those, at the time of the hyperkalemic event, the median age was 76 (IQR: 68-84), 45% were women, the median eGFR was 49 (IQR: 35-68) ml/min/1.73 m<sup>2</sup>, and the majority (58%) had been on MRA for less than three months. Common comorbidities were HF (69%), hypertension (77%) CKD (66%, eGFR <60 ml/min/1.73 m<sup>2</sup>) and diabetes (38%). Concomitant use of beta-blockers (78%), ACEi/ARBs (77%) and diuretics (76%) was also common. The majority of index hyperkalemia episodes (75%) were mild, 18% were moderate, and 7% were severe.

Patients who stopped MRA after hyperkalemia had a higher 2-year absolute risk of the composite outcome compared to those who continued, corresponding to an adjusted HR of 1.10 (95% CI 1.06-1.14) (**Table 4.2.1**). In contrast, the 2-year absolute risk of recurrent hyperkalemia was lower among those who stopped MRA (50.1%, 95% CI 48.2-52.3%) compared to those that continued the treatment (63%, 95% CI 61.4-64.6%), which correspond to an adjusted HR of 0.75 (95% CI 0.72-0.79).

The observed associations between treatment strategy and outcomes were consistent across strata of age, sex, eGFR, severity of index hyperkalemia (**Figure 4.2.1**) and in the restricted subpopulation of patients with history of HF.



**Table 4.2.1 Two-year risks of study outcomes associated with stopping vs. continuing MRA after hyperkalemia (n=7,366).**

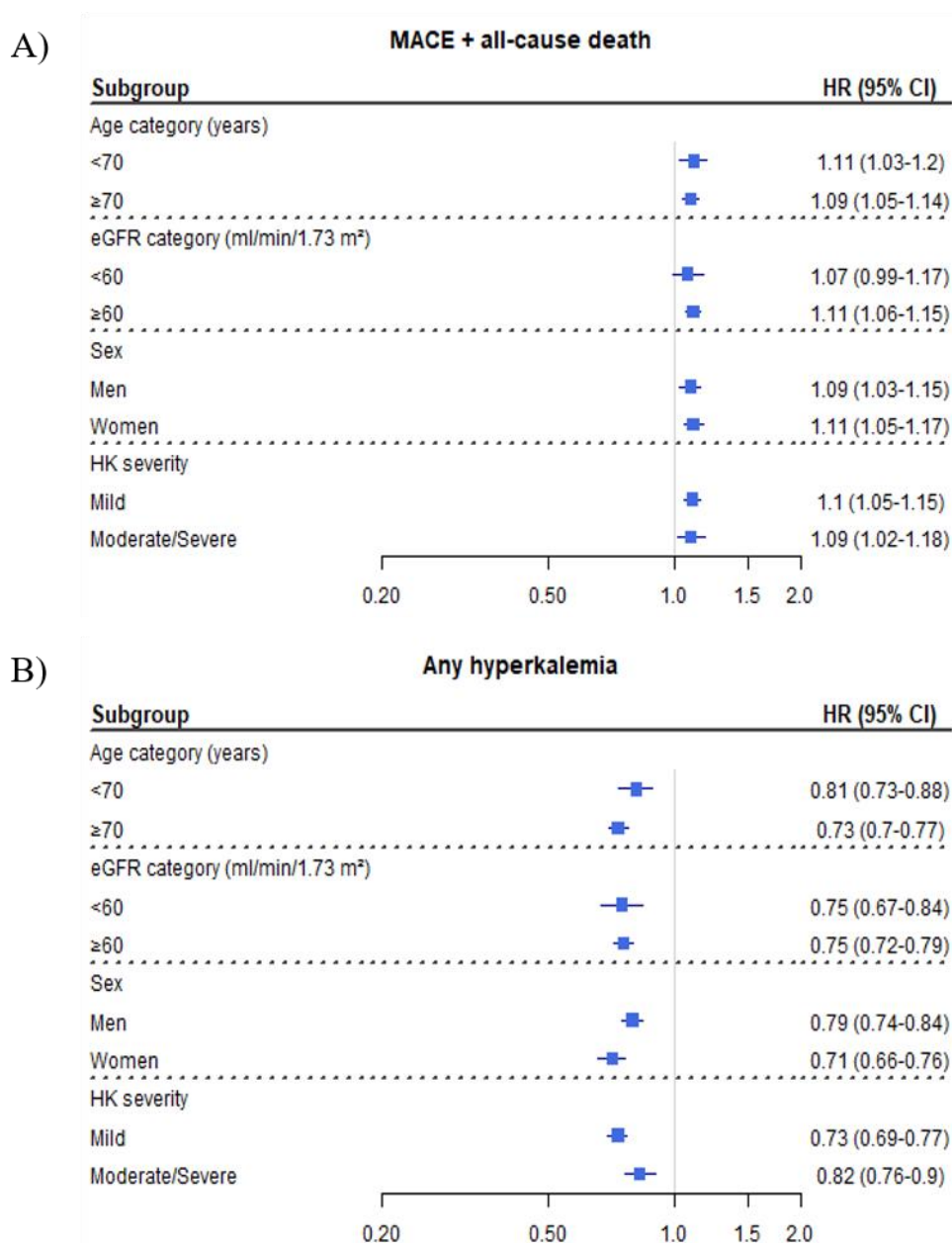
	<b>Absolute risk % (95% CI)</b>	<b>Risk ratio (95% CI)</b>	<b>Hazard Ratio (95% CI)</b>
<b>Composite of death, myocardial infarction, stroke and heart failure</b>			
Continue MRA	60.0 (58.7 - 61.6)	Reference	Reference
Stop MRA	63.6 (62.0 - 65.4)	1.06 (1.03-1.09)	1.10 (1.06-1.14)
<b>All-cause death</b>			
Continue MRA	38.7 (37.2 - 40.1)	Reference	Reference
Stop MRA	42.0 (40.2 - 43.8)	1.09 (1.03-1.14)	1.11 (1.05-1.17)
<b>Major adverse cardiovascular events (MACE)</b>			
Continue MRA	48.6 (47.0 - 50.2)	Reference	Reference
Stop MRA	51.3 (49.4 - 53.3)	1.05 (1.01-1.1)	1.08 (1.03-1.13)
<b>Recurrent hyperkalemia (potassium &gt; 5.0 mmol/L)</b>			
Continue MRA	63.0 (61.4 - 64.6)	Reference	Reference
Stop MRA	50.1 (48.2 - 52.3)	0.80 (0.76-0.83)	0.75 (0.72-0.79)
<b>Recurrent moderate/severe hyperkalemia (potassium &gt; 5.5 mmol/L)</b>			
Continue MRA	32.6 (31.0 - 34.1)	Reference	Reference
Stop MRA	24.0 (22.4 - 25.9)	0.74 (0.68-0.80)	0.73 (0.67-0.78)

Abbreviations: MRA, mineralocorticoid receptor antagonist.

MACE is defined as composite of cardiovascular death, myocardial infarction, stroke and heart failure

Results derived from the inverse-probability weighted Cox proportional hazard model. The weights are calculated including: age, sex, estimated glomerular filtration rate, severity and timing of baseline hyperkalemia, comorbidities (myocardial infarction, hypertension, heart failure, peripheral vascular disease, cerebrovascular disease, diabetes mellitus) and medications (angiotensin converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARBs), beta-blockers, thiazide or loop diuretics, sodium polystyrene sulfonate, non-steroidal anti-inflammatory drugs, other blood pressure-lowering drugs)

**Figure 4.2.1 Association between treatment assignment and risk of the composite event (Panel A) and recurrent hyperkalemia (Panel B) in age, estimated glomerular filtration rate (eGFR) category, sex and severity of index hyperkalemia strata.**



Abbreviations: MACE, major adverse cardiovascular event; eGFR, estimated glomerular filtration rate; HK, hyperkalemia  
Mild hyperkalemia ( $K^+ > 5.0$ - $5.5$  mmol/L), moderate/severe hyperkalemia ( $K^+ > 5.5$  mmol/L)

Results from inverse-probability weighted Cox proportional hazard model (continuing MRA is the reference group). The weights are calculated including: age, sex, estimated glomerular filtration rate, severity and timing of baseline hyperkalemia, comorbidities (myocardial infarction, hypertension, heart failure, peripheral vascular disease, cerebrovascular disease, diabetes mellitus) and medications (angiotensin converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARBs), beta-blockers, thiazide or loop diuretics, sodium polystyrene sulfonate, non-steroidal anti-inflammatory drugs, other blood pressure-lowering drugs)

### 4.3 Study III

We here identified a cohort of adult (>18 years) diabetes patients who survived an MI event during the period 2010-2017 and were discharged with a dispensation of glucose-lowering drugs. Among the 17,868 patients selected, 365 (2%) received GLP-1 RA at discharge. Compared with non-users (standard care), they were generally younger (median age 65 vs. 71 years), more frequently ex-smokers or obese. GLP-1 RA users more often had hypertension, history of percutaneous coronary intervention, slightly lower proportion of CKD and were less commonly using ACEi/ARBs.

During a median follow-up time of 2.98 years, 5,634 patients experienced MACE. The incidence rate was lower among GLP-1 RA users compared to non-users (97.9 vs. 148.7 per 1000 person-years, respectively) (**Table 4.3.1**). The adjusted HR showed a significant 28% relative risk reduction associated with GLP-1 RA use (HR 0.72, 95% CI 0.56-0.92). A similar direction of the association was also observed among the single components of the composite MACE outcome. There was no suggestion of effect modification among the strata of age, sex, eGFR category or STEMI/NonSTEMI (**Figure 4.3.1**).

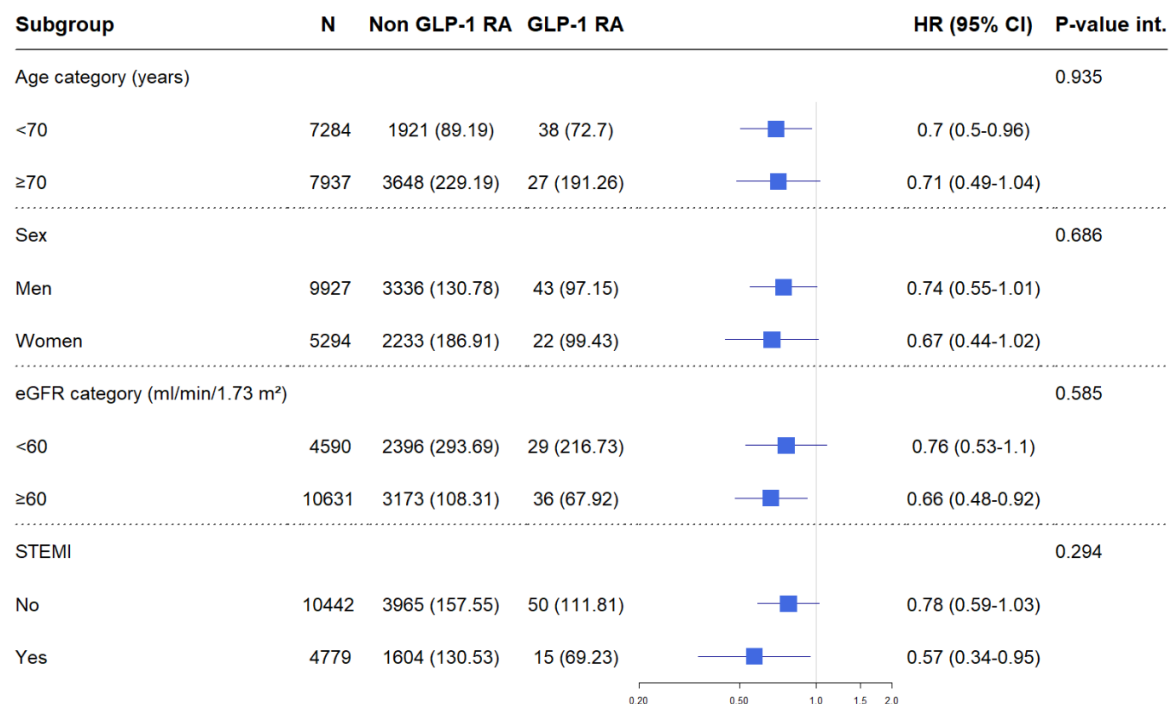
**Table 4.3.1 Risk of cardiovascular events associated with GLP-1 RA use vs. non-use**

	N events (IR per 1000 PY)	Non GLP-1 RA (IR per 1000 PY)	GLP-1 RA (IR per 1000 PY)	Adjusted HR (95% CI)
<b>MACE (composite)</b>	5634 (147.8)	5569 (148.69)	65 (97.91)	0.72 ( 0.56 - 0.92 )
<b>Single components of MACE</b>				
<b>Stroke</b>	860 (17.45)	855 (17.63)	5 (6.39)	0.42 ( 0.18 - 1.02 )
<b>Heart failure</b>	3577 (82.99)	3535 (83.37)	42 (60.25)	0.81 ( 0.60 - 1.10 )
<b>Myocardial re- infarction</b>	2437 (54.53)	2409 (54.8)	28 (38.34)	0.71 ( 0.49 - 1.04 )
<b>CV death</b>	1354 (26.56)	1344 (26.78)	10 (12.7)	0.73 ( 0.39 - 1.36 )

Abbreviations: GLP-1 RA, GLP-1 receptor agonist; IR, Incidence rate; PY, person-years; HR, hazard ratio; CI, confidence interval; MACE, major adverse cardiovascular event; CV, cardiovascular

Model adjusted for: age, sex, smoking, body mass index, eGFR category, comorbidities (heart failure, cancer, hypertension, percutaneous coronary intervention, coronary artery bypass grafting, stroke, peripheral vascular disease, atrial fibrillation, killip, ST-segment elevation myocardial infarction) and cardiovascular medications (aspirin, statins, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta blockers, P2Y12 inhibitors)

**Figure 4.3.1 Subgroup analyses: Risk of major adverse cardiovascular events (MACE) among users vs non-users of GLP-1 RA by age, sex, ST elevation myocardial infarction (STEMI) and estimated glomerular filtration rate (eGFR) category.**



Abbreviations: HR, hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; STEMI, ST-segment elevation myocardial infarction

Model adjusted for (when relevant): age, sex, smoking, body mass index, eGFR category, comorbidities (heart failure, cancer, hypertension, percutaneous coronary intervention, coronary artery bypass grafting, stroke, peripheral vascular disease, atrial fibrillation, killip, ST-segment elevation myocardial infarction) and cardiovascular medications (aspirin, statins, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta blockers, P2Y12 inhibitors)

#### 4.4 Study IV

We here identified a cohort of adults (age  $\geq 18$  years) who initiated OAC treatment during the period 2011-2018 and had a diagnosis of AF. Out of the 32,699 patients selected, 18,323 (56%) started DOAC and 14,376 (44%) started VKA treatment. Their median age was 75 years (IQR: 68-83) and 45% were women. The median eGFR was 73 (IQR: 59-85) ml/min/1.73m<sup>2</sup> and 27% of the participants had an eGFR  $< 60$  ml/min/1.73m<sup>2</sup>. The most common comorbidities were hypertension (72%), vascular disease (30%), history of cancer (26%) and congestive heart failure/left ventricular dysfunction (25%). The median CHA<sub>2</sub>DS<sub>2</sub>-VASc score was 3 (IQR: 2-5), the median Modified-CHADS<sub>2</sub> score was 5 (IQR: 3-7) and the median HAS-BLED score was 2 (IQR: 2-3). Patients were also commonly prescribed  $\beta$ -blockers (80%), renin-angiotensin-aldosterone system inhibitor (RAASi, 56%), aspirin (44%) and statins (36%).

During a median follow-up time of 3.8 (IQR: 2.1-5.8) years, we observed occurrence of CKD progression among 1208 individuals in the DOAC group and 2244 in the VKA group. The incidence rates was 30.4 and 36.3 per 1000 person-years, respectively (**Table 4.4.1**). Compared to VKA, the adjusted HR showed a 13% relative risk reduction for CKD progression among DOAC users (HR: 0.87, 95% CI 0.78-0.98).

During the same period, 1825 patients in the DOAC group and 3277 patients in the VKA group experienced an AKI event. The corresponding incidence rates were 46.7 and 54.5 per 1000 person-years respectively. Compared to VKA, DOAC use was associated with a 12% AKI risk reduction, with an adjusted HR of 0.88 (95% CI 0.80-0.97).

In terms of cardiovascular outcomes, no differences were observed for the composite outcome of ischemic stroke and systemic embolism, and the single components of ischemic stroke. A protective effect of DOAC use was observed for major bleeding and intracranial bleeding but no difference for gastrointestinal and other type of bleedings. No association was observed for all-cause and cardiovascular death.

Finally, there was no evidence of effect modification across eGFR strata for the risk of CKD progression, AKI and bleeding, while there was suggestion of differential effects between groups for the stroke systemic embolism, ischemic stroke and cardiovascular mortality (**Figure 4.4.1**).

**Table 4.4.1. Number of events, incidence rates and adjusted hazard ratios for the association between DOAC vs. VKA initiation and outcomes.**

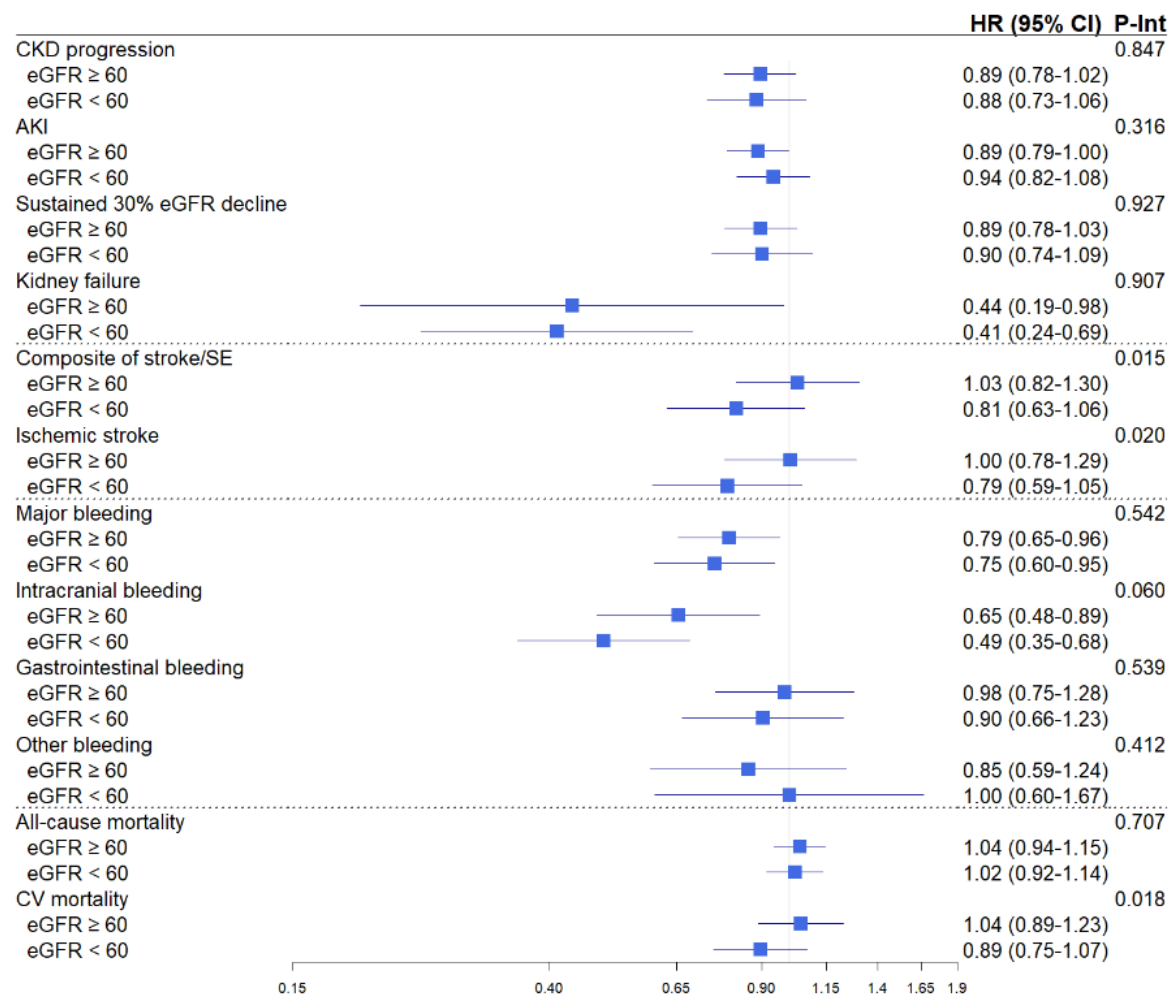
	<b>VKA: No of Events (IR/ 1000 person- years)*</b>	<b>DOAC: No of Events (IR/1000 person- years)*</b>	<b>Adjusted HR DOAC vs. VKA (95% CI)**</b>
<b>Kidney outcomes</b>			
CKD progression	2244 (36.3)	1208 (30.4)	0.87 (0.78-0.98)
Sustained 30% eGFR decline	2205 (35.7)	1202 (30.3)	0.88 (0.78-0.98)
Kidney Failure	196 (3.0)	42 (1.0)	0.43 (0.25-0.73)
AKI	3277 (54.49)	1825 (46.7)	0.88 (0.80-0.97)
<b>Cardiovascular outcomes</b>			
Composite of stroke and systemic embolism	1118 (15.3)	734 (13.3)	0.93 (0.78-1.11)
Ischemic stroke	991 (13.2)	658 (11.9)	0.88 (0.73-1.06)
<b>Bleeding outcomes</b>			
Major bleeding	1414 (19.5)	808 (14.7)	0.77 (0.67-0.89)
Intracranial bleeding	635 (8.5)	316 (5.6)	0.59 (0.47-0.75)
Gastrointestinal bleeding	615 (8.3)	398 (7.1)	0.96 (0.79-1.17)
Other bleeding	311 (4.2)	170 (3.0)	0.88 (0.66-1.18)
<b>Mortality</b>			
All-cause mortality	4842 (64.1)	3222 (57.1)	1.04 (0.95-1.14)
CV death	2351 (31.1)	1467 (26.0)	0.99 (0.84-1.17)

Abbreviations: VKA, vitamin K antagonist; DOAC, direct oral anticoagulants; IR, incidence rate; HR, hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury; CV, cardiovascular

\* Number of events, incidence rates were calculated in the original, unweighted population.

\*\* Analyses were adjusted for the following 50 variables: age, sex, calendar year, numbers of primary healthcare visits, numbers of outpatient specialist visits, numbers of diagnoses issued, numbers of procedure codes, education, estimate glomerular filtration rate, hypertension, anemia, liver disease, renal disease, alcohol abuse, prior bleeding, stroke/transient ischemic stroke/embolism, stroke, myocardial infarction, heart failure, congestive heart failure, vascular disease, chronic obstructive pulmonary disease, rheumatoid arthritis, diabetes, diabetic complications, cancer, deep vein thrombosis, knee/hip surgery, percutaneous coronary intervention, venous thromboembolism, fracture, risk scores (CHA2DS2-VASc, modified CHADS<sub>2</sub>, HAS-BLED), concomitant use of: aspirin, clopidogrel, non-steroidal anti-inflammatory drugs, other antiplatelet, corticosteroids, diuretics, beta blockers, calcium channel blockers, renin-angiotensin-aldosterone-system inhibitors, statin, insulin, other antidiabetic medications, antidepressants, digoxin, nitrate, proton-pump inhibitors using inverse probability of treatment weighting.

**Figure 4.4.1. Association between direct oral anticoagulants (DOAC) use and vitamin K antagonists (VKA) by estimated glomerular filtration rate (eGFR) category.**



Abbreviations: DOAC, direct oral anticoagulant; VKA, vitamin K antagonist; eGFR, estimated glomerular filtration rate; HR, hazard ratio; CI, confidence interval; P-int, p-value interaction; AKI, acute kidney injury; SE, systemic embolism; CV, cardiovascular.

Analyses were adjusted for: age, sex, calendar year, numbers of primary healthcare visits, numbers of outpatient specialist visits, numbers of diagnoses issued, numbers of procedure codes, education, estimate glomerular filtration rate, hypertension, anemia, liver disease, renal disease, alcohol abuse, prior bleeding, stroke/transient ischemic stroke/embolism, stroke, myocardial infarction, heart failure, congestive heart failure, vascular disease, chronic obstructive pulmonary disease, rheumatoid arthritis, diabetes, diabetic complications, cancer, deep vein thrombosis, knee/hip surgery, percutaneous coronary intervention, venous thromboembolism, fracture, risk scores (CHA2DS2-VASc, modified CHADS<sub>2</sub>, HAS-BLED), concomitant use of: aspirin, clopidogrel, non-steroidal anti-inflammatory drugs, other antiplatelet, corticosteroids, diuretics, beta blockers, calcium channel blockers, renin-angiotensin-aldosterone-system inhibitors, statin, insulin, other antidiabetic medications, antidepressants, digoxin, nitrate, proton-pump inhibitors using inverse probability of treatment weighting

## 5 DISCUSSION

### 5.1 Main findings

#### 5.1.1 CKD is common among patients with history of cardiovascular diseases and diabetes

Results from the presented thesis illustrate that CKD is a very common comorbidity among patients with history of hypertension, HF, AF and diabetes. In **Study I and II**, we observed that 28% of patients selected in the cohort had an eGFR  $< 60$  ml/min/1.73 m<sup>2</sup> at the time of MRA initiation. In **Study III**, we reported that CKD was also a common comorbidity (31%) among diabetes patients who experience a MI. These results are comparable with findings from a previous study that observed a prevalence of 25% in the diabetic population<sup>160</sup>. Finally, in **Study IV** we observed a CKD prevalence of 27% among patients with a diagnosis of AF and treated with OAC. This is consistent with results from another healthcare utilization cohort<sup>161</sup>.

The high prevalence of CKD in the selected cohorts is not surprising when we consider the strong relationship between impaired kidney function and the diseases considered. Hypertension is one of the most important risk factors for CKD, due to the detrimental effect of elevated blood pressure in the glomerular vascularization<sup>162</sup>. High blood pressure forces the vessels to stretch to manage the increased blood flow. If this condition persists, blood vessels become weaker and harder, resulting in impairment of kidney function. Recent findings from the atherosclerosis risk in communities (ARIC) study showed that patients with elevated blood pressure were more likely to develop CKD compared to those with normal blood pressure<sup>163, 164</sup>. Diabetes mellitus is another leading risk factor for CKD worldwide<sup>165</sup> and it is the only diabetic complication that continuous to grow<sup>166</sup>. CKD in patients with diabetes is referred to as Diabetic Kidney Disease (DKD). In the development of DKD, the renin-angiotensin system (RAS) is likely the most important contributor<sup>167</sup>, together with hyperglycemia. The mediated effect of high blood sugar results in oxidative stress, and the release of proinflammatory and profibrotic mediators<sup>168-170</sup>. Finally the decline in kidney function is strongly associated with higher risk of HF<sup>23-25</sup> and AF<sup>28, 29</sup>, especially in more severe stages.

#### 5.1.2 Patients with CKD are at increased risk of adverse events and treatment discontinuation

Among patients initiated with MRA, we observed that impaired kidney function was strongly associated with increased risk of hyperkalemia and treatment discontinuation (**Study I**). These results are consistent with findings from post-hoc analyses of the Randomized Aldactone Evaluation Study (RALES) trial and Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) trial<sup>171, 172</sup>. Vardeny *et al.*<sup>172</sup> observed that among patients with reduced baseline eGFR ( $<60$  ml/min/1.73m<sup>2</sup>) hyperkalemia occurred more frequently among spironolactone users compared to placebo (26% vs 9% respectively,  $p < 0.001$ ). These percentage were more elevated than among patients with normal kidney function (15% vs 6%), showing a significantly higher risk of hyperkalemia among patients with CKD (odds ratio: 1.53, 95% CI 1.16-2.02). Rossignol *et al.*<sup>171</sup>, showed that 8.9% of patients in



the eplerenone group experience a serum potassium elevation ( $K^+ > 5.5$  mmol/L) during follow-up. Compared with patients with normal kidney function, those with CKD had 26% and 73% higher risk of mild and moderate hyperkalemia (HR 1.26, 95%CI 1.10-1.44 and HR 1.73, 95%CI 1.33-2.25) respectively.

Several factors contribute to the increased risk of hyperkalemia among patients with CKD. First, as CKD progresses, kidney damage reduces potassium excretion, which tends to accumulate in the body. Second, comorbidities common in CKD patients could also contribute to facilitating the occurrence of hyperkalemia (e.g. hypertension, diabetes, older age)<sup>173</sup>. Finally, CKD patients are often prescribed medications that provide cardiorenal protection (e.g. ACEi and ARBs) but that increase the risk of hyperkalemia<sup>174, 175</sup>.

In **Study I**, we also showed that patients with CKD are more likely to discontinue MRA therapy after hyperkalemia. This result confirms previous evidence from post-hoc analyses of RCTs and observational studies<sup>79, 80</sup>. In a post-hoc analysis from the PROTECT trial, Beusekamp *et al.*<sup>79</sup> investigated differences in treatment patterns in patients who experienced hyperkalemia during a hospitalization for acute HF. They observed that patients discharged with no treatment or down-titration of MRA had a lower eGFR compared with those that received a constant dosage or up-titration. Rossignol *et al.*<sup>80</sup> investigated the association between hyperkalemia and RAASi discontinuation in a cohort of patients with HF enrolled in a multicenter, prospective observational study. The authors reported that renal dysfunction was strongly associated with MRA discontinuation.

In observational studies, it is not always possible to assess the reasons behind a clinician's decision to continue or stop the medication after an adverse event because this information is rarely registered in the available data sources. However, we can hypothesize that the strong association between CKD and treatment discontinuation is likely a consequence of the physicians' concern over the increased risk of hyperkalemia among CKD patients.

In line with the evidence from RCTs and observational studies, current clinical guidelines suggest stopping MRA therapy temporarily when  $K^+$  exceeds 6 mmol/L<sup>77</sup>. This is supported by the findings of **Study II**, where we observed that patients who continued with the therapy had a lower risk of MACE and all-cause death but higher risk of recurrent hyperkalemia.

### **5.1.3 Patients with CKD may similarly benefit from recommended therapies**

The results presented in this thesis suggest that cardioprotective and antidiabetic medications may have a similar risk/benefit profile in patients with CKD compared with patients with normal kidney function, and these medications should not be denied to them.

In **Study II**, our results suggested that patients who stopped MRA treatment after hyperkalemia were, compared to those who continued, at lower risk of recurrent hyperkalemia but at higher risk of cardiovascular events. Similar results were also observed in patients with different baseline eGFR category ( $<$  or  $\geq 60$  ml/min/1.73m<sup>2</sup>). The main findings were consistent with previous observational studies<sup>79-82</sup>, but our study overcame a number of limitations and biases

that characterized these studies, such as small sample size, short follow-up, immortal time and prevalent user bias.

In **Study III**, we observed that the beneficial effect of GLP-1 RA treatment was consistent across CKD stages ( $\text{eGFR} < \text{or} \geq 60 \text{ ml/min/1.73m}^2$ ). This finding is in accordance with evidence from RCTs and meta-analyses<sup>85, 176-178</sup>. In a recent meta-analysis including 60,080 patients from 8 RCTs, Sattar *et al.*<sup>110</sup> showed that patients with and without CKD ( $\text{eGFR} < \text{or} \geq 60 \text{ ml/min/1.73m}^2$ , respectively) had a similar protective effect of GLP-1 RA compared to placebo (HR 0.88, 95% CI 0.77-1.01 vs HR 0.83, 95% CI 0.74-0.93,  $p = 0.52$ ). We expanded this evidence by providing an assessment of the effectiveness of GLP-1 RA across levels of kidney function in patients followed in routine clinical practice and who survived a recent MI.

Finally, results presented in **Study IV** showed that initiation of DOAC vs VKA treatment was associated with more favorable cardiorenal outcomes in both CKD and non-CKD groups. These results are comparable with findings from post-hoc analyses of the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTELE) trial and studies performed in healthcare utilization cohorts<sup>116, 155, 179-184</sup>. In a large U.S. cohort of nonvalvular AF patients, Yao *et al.*<sup>155</sup> reported that the risk associated with DOAC use, compared with VKA treatment, was consistent across levels of kidney function for both AKI and eGFR decline. Ashley *et al.*<sup>184</sup> observed that DOAC use in the period 2009-2016 had a similar effect on the risk of cardiovascular events, mortality or bleeding compared to VKA use among patients with  $\text{eGFR} < 30$ , 30-59 and  $\geq 60 \text{ mL/min/1.73m}^2$ . Our study provided supporting evidence to these results in a cohort with larger sample size and longer follow-up.

These findings emphasize, one more time, the importance of accounting for kidney function in pharmacoepidemiological studies. Future research that aims to expand current knowledge on effectiveness and safety of medications for cardiorenal protection should provide detailed information on the treatment effects among patients with different CKD stages. In particular, observational studies with appropriate information on kidney function can play an important role in providing additional insight in this high-risk population that it is often understudied in RCTs.

## 5.2 METHODOLOGICAL CONSIDERATIONS

### 5.2.1 From randomized controlled trials to observational studies

Assessing the safety and effectiveness of drugs in both trials and routine care is necessary for advancing patients' treatment in healthcare. A well-designed RCT can provide information on benefit and harm of a drug in term of causality, which is pivotal for the definition of appropriate guidelines for physicians. However, RCTs are often very expensive, apply very strict inclusion/exclusion criteria that limit the generalizability of the results and cannot answer all research questions since many exposures cannot be randomized due to ethical or practical reasons<sup>185, 186</sup>.

Because of these limitations, the number of observational studies that use healthcare data is growing. This can be explained by the increased availability of large datasets obtained from routinely collected data and by advanced statistical methods that can help draw causal conclusions from them. Observational studies can also provide evidence in populations with wider range of inclusion/exclusion criteria, longer periods of observation and different treatment indication, which increase the generalizability of the results.

However, observational studies may be prone, among other limitations, to confounding, immortal time bias and prevalent user bias, which can all be minimized through carefully selected study designs, statistical methods and proper selection (and availability) of confounders. As discussed earlier, application of new users design, use of an active comparator and target trial emulation methods can help reduce these biases and prevent unnecessary flaws<sup>187</sup>.

Absence of randomization represents a major limitation in observational studies. In an RCT the treatment is assigned at random. Therefore, patient's characteristics do not influence the probability of receiving one treatment or the other. Thus, in large samples, all characteristics are balanced between groups and the treatment effect can be directly estimated without accounting for them in the statistical model. However, in observational studies, the decision of prescribing a medication is based on specific characteristics of the patient (e.g. medical history, concomitant medications, age, etc.), so they will not be balanced between groups. Commonly used methods to balance these characteristics, such as multivariable adjustment, matching and methods based on the propensity score, should be carefully selected depending on the number of events, the time-setting in which we assess the exposure (time-fixed or time-varying), the number of confounders and the effect that we want to estimate (i.e. marginal or conditional).

Apart from preventing unnecessary biases, the decision on the study design will also depend on the specific research question. While case-control designs are more suitable for investigating rare-outcomes and perhaps multiple exposures, they may not be the best choice when we are interested in estimating the association between the treatment and multiple outcomes (in that case, we should use a cohort design instead). On the other hand, if the interest is on acute events of transient treatments, one may want to design a self-controlled case series study<sup>188</sup>.

### **5.2.2 Data availability is crucial in observational studies**

Any decision regarding study designs and statistical methods depends on the available data. In trials all needed data are pre-defined before starting the trial and are then collected at each planned visit. In contrast, observational studies are performed using data from disease-specific cohorts, registries, healthcare utilization cohorts, insurance or reimbursement datasets. Each of these data sources have their own advantages and disadvantages that should be taken into consideration when planning the study. For example, disease specific registries will have more detailed and frequent information regarding important factors associated with the specific disease (e.g. ejection fraction in HF registries) but it will lack data on measurements between scheduled visits and it will be less generalizable than healthcare utilization cohorts.

In pharmacoepidemiological studies, the most critical data source is the dataset containing information on prescribed or dispensed medications. While patients are closely monitored in RCTs, healthcare data do not provide a clear assessment of the time on and off-treatment, which can only be estimated using the available data on drug prescriptions/dispensations. In all the studies presented in this thesis, we emphasized the advantage of having access to the Prescribed Drug Registry, which provides almost complete coverage of all medications dispensed in Swedish pharmacies<sup>126</sup>. Using this registry made it possible to estimate compliance and adherence to the treatment by looking at the number of pills supplied and presence/absence of new dispensations instead of simply relying on prescriptions (which do not provide clear information whether and when the treatment was collected by the patient at the pharmacy).

With this thesis, we also illustrate the importance of laboratory measurements when studying safety and effectiveness of medications. When laboratory data are not available, the assessment of comorbidities or events, such as CKD or hyperkalemia, is performed using diagnostic codes (e.g. ICD-10 codes). These codes are often characterized by high specificity but low sensitivity, especially when the events are mild or the disease does not have clear symptoms<sup>14, 189-191</sup>. Therefore, when available, use of laboratory measurements can improve the definition of these comorbidities and outcomes.

However, working with laboratory tests can be challenging. First, the number of tests is often correlated with patient health status and physicians' decision-making process. The sicker the patients, more frequently they will be tested. At the same time, each medication might have a different indication based on laboratory values (e.g. antihypertensive medications and kidney function) and can require different level of monitoring (e.g. MRA and K<sup>+</sup> monitoring), thus the number of tests will vary depending on which medications are prescribed. Second, not all laboratory measurements provide reliable information on the actual level of the biomarker. For example, creatinine values can be quite variable over time and can be influenced by acute illness, hospital procedures or even measurement errors<sup>192</sup>.

All these elements should also be taken into consideration when the aim is to analyze changes of kidney function over time. One of the most relevant endpoints in kidney research is CKD progression. Several definitions have been used in observational studies, including diagnosis-related endpoints (e.g. KRT<sup>193</sup>), laboratory-based changes of eGFR (e.g. sustained decline >30%<sup>194</sup>), doubling of creatinine<sup>195</sup>, CKD diagnosis, or a combination of the above. While KRT would be the preferred outcome in observational studies, it is usually very rare and takes a long time to develop, thus requiring large sample sizes and long follow-up to be sufficiently powered. Therefore, definitions of kidney outcomes should combine diagnosis of kidney events (e.g. KRT and AKI) with information from the laboratory data on changes in creatinine. However, as frequency of testing and variability of creatinine can be affected by factors not associated with the exposure of interest, this poses a risk for outcome ascertainment bias<sup>196, 197</sup>. For example, similar to the approach used in RCT, sustained 30% decline in eGFR is often used in observational studies to identify CKD progression. This endpoint is identified whenever a value of eGFR during follow-up is  $\geq 30\%$  lower than the baseline value. However, this “two-

point” method is susceptible to transient variations in eGFR (e.g. a single drop in eGFR due to in-hospital surgery) that may misclassify the outcome, since it requires only one measurement below the threshold to identify a new event.

In **Study IV**, we tried to overcome these limitations by applying an approach proposed by Zee *et al.*<sup>198</sup>, which defines sustained eGFR decline based on linear interpolation between all available values of eGFR during follow-up. The estimated parameters of the model are then used to predict the point in time when eGFR will cross the 30% threshold, instead of looking at each test separately. Using simulation and real-world data, the authors reported that the regression method was more accurate than the two-point approach, especially with high eGFR variability and more missing data, which is often the case in healthcare utilization datasets. Moreover, in cohort studies, the regression model also identified a less rapid decline compared to the two-point method, which was influenced by transient reduction in eGFR.

Observational studies might still fail to achieve complete reproducibility of the findings from RCTs, even when the study is designed properly, comprehensive data sources are used or in the absence of biases<sup>199</sup>. Registries and healthcare databases present intrinsic limitations that make an exact emulation of RCTs impossible. Often inclusion and exclusion criteria from the trials can only be emulated to a certain extent and lack of clinical details limits the definition of important comorbidities and outcomes, which negatively affect the agreement between observational studies and RCTs. However, research using routine care data is essential and researchers should attempt to find the best approaches to overcome these limitations. An important work has been recently started with the RCT-DUPLICATE project<sup>200</sup>, that aims to identify processes for proper and transparent development of observational studies. Interim findings from this project confirmed that using appropriate methods to deal with biases enhances the validity of findings from observational studies and they should be always considered in future research that aim to investigate treatment-outcome associations in routine clinical practice.

## **6 CONCLUSIONS**

### **6.1 Overarching conclusions**

The work presented in this thesis emphasizes the importance of pharmacoepidemiology in routine care to expand trial evidence on the safety and effectiveness of medications in real-world settings.

This thesis also highlights the key role of underlying kidney function in assessing the risk – benefit of medications and the importance of evaluating variation in laboratory values to better ascertain adverse drug events. Patients with CKD were common, were often at higher risk of adverse outcomes but could similarly benefit from recommended treatment.

### **6.2 Study-specific conclusions**

1. Hyperkalemia was common among patients initiating MRA in clinical practice. This adverse event was often followed by discontinuation of the treatment, especially when the event was moderate/severe and occurred early after therapy initiation. Patients with CKD were at high risk of hyperkalemia and MRA discontinuation.
2. Compared with patients who continued MRA after hyperkalemia, those who stopped had a lower risk of recurrent hyperkalemia, but a higher risk of cardiovascular events and death.
3. In patients with diabetes surviving MI, use of GLP-1 RA, compared with standard care, was associated with lower risk of subsequent cardiovascular events.
4. Compared to VKA, DOAC use in AF patients was associated with lower risk of CKD progression, AKI and major bleeding. The risk of the composite of stroke/systemic embolism and mortality was similar between both therapies.

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## **APPENDIX 1**