AARHUS UNIVERSITY

Epidemiology of Nephrotic Syndrome

- Occurrence and Prognosis in a Nationwide Cohort of Adults

PhD thesis

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List of papers

I. **Vestergaard SV**, Birn H, Hansen AT, Nørgaard M, Nitsch D, Christiansen CF. Comparison of patients with hospital-recorded nephrotic syndrome and patients with nephrotic proteinuria and hypoalbuminemia: a nationwide study in Denmark.R1 [ACCEPTED]

II. **Vestergaard SV**, Birn H, Jensen SK, Sørensen HT, Nitsch D, Christiansen CF. 40-year trends in incidence and mortality of nephrotic syndrome – a population-based cohort study. [SUBMITTED]

III. **Vestergaard SV**, Birn H, Darvalics B, Nitsch D, Sørensen HT, Christiansen CF. Risk of thromboembolic and bleeding events in patients with nephrotic syndrome: a population-based cohort study. [IN PREPARATION]

List of abbreviations

ACE inhibitors: Angiotensin-converting enzyme inhibitors

ATE: arterial thromboembolism

CI: confidence interval

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

eGFR: Estimated glomerular filtration rate

ICD: International Classification of Diseases

IQR: interquartile range

HR: Hazard ratio

MeSH: Medical subject headings

NS: Nephrotic syndrome

P-albumin: Plasma albumin

VTE: Venous thromboembolism

Contents

THESIS STRUCTURE

The thesis is based on three studies examining nephrotic syndrome (NS) in Danish medical databases. The studies are referred to by their Roman numerals (I-III). The first study (I) explores the identification of adult NS patients in the Danish National Patient Registry and compares these to patients identified with biochemical features of NS in corresponding laboratory databases. The second and third studies (II and III) elucidate the occurrence and prognosis of hospital-diagnosed NS in Denmark by examining the incidence and mortality of NS, and the risk of thromboembolic and bleeding complications in patients with NS.

The thesis consists of nine chapters. The first chapter provides a brief introduction to NS, as well as the epidemiology of NS based on the existing literature, with a focus on the questions addressed in Studies I-III. The following three chapters describe the methodology we used and our main findings from Studies I-III, followed by my discussion of our findings in the context of the existing literature, limitations of the methods we applied, and on future aspects. In the final chapters, I provide a summary of the thesis in English and Danish, list the references, and finally, I provide the manuscripts in full length.

INTRODUCTION

History of nephrotic syndrome

The first known description of proteinuria was attributed to Hippocrates more than two thousand years ago, when he observed that bubbles on the surface of urine were associated with a prolonged course of disease.[1] That heavy proteinuria can lead to dropsy (early term for edema) was not described before more than a thousand years later, when the clinical picture of the nephrotic syndrome (NS) was described. This description is found in a pamphlet of disease in children from the 14th century, where the Flemish physician, Cornelius Roelans, suggested that herbs cooked in white wine could cure the swelling of the body that was attributed to imbalanced humors.[1] Despite that Belgian wine at that time was low in alcohol due to difficulties with ripening the grapes,[2] it is unlikely that this cure would pass a phase 3 trial as a treatment of childhood NS today. During the 18th and 19th centuries, the clinical features of NS were connected to the kidneys. Severe edema was proposed to be due to excess protein in the urine, and the low protein level in the blood was quantified.[1, 3] The term NS was introduced by the physician Henry Christian in 1947 and was widely accepted in the following years.[3, 4] In parallel with the quantum leaps seen in other areas of medicine during the 20th century, major advances were made in describing and treating NS. Pathologists went from examining only kidneys from deceased persons, to examining the histopathology of kidney disease in living kidneys after percutaneous kidney biopsy was introduced in 1944.[5] The introduction of steroids as a treatment of childhood NS in 1950 paved the way for a treatment of NS that in modified versions has persisted in treatment of both childhood and adult NS.[3]

Definition of nephrotic syndrome

Today, it is widely accepted that NS is characterized by two biochemical features and a clinical observation including 1) nephrotic proteinuria (excessive loss of protein to the urine), 2) hypoalbuminemia (low blood albumin level), and 3) peripheral edema (accumulation of fluid in peripheral tissue).[6] In addition to these key features, the syndrome often encompasses hyperlipidemia, lipiduria, metabolic imbalances, hypertension, and complications including thromboembolism, infection, and acute or chronic renal impairment.[7, 8] The clinical presentation of NS can be similar to other conditions (e.g. preeclampsia in pregnant women, heart failure, and allergic reactions), and differential diagnoses must be considered in the diagnostic workup of NS.[7, 9] NS can develop in patients with primary glomerular disease (primary NS) or

systemic conditions (secondary NS) such as medication use, allergens, infections, neoplasms, and metabolic disease.[8] In recent years, the discoveries of specific underlying causes have elucidated the heterogeneity of conditions and mechanism behind NS.[3, 8, 10] However, given the shared features of heavy proteinuria and hypoalbuminemia and the potential complications, the term NS is still considered clinically useful.[8, 11]

It is widely accepted that nephrotic proteinuria is defined by a daily loss of 3 grams of protein to the urine.[6] This rather arbitrary cutoff value is based on a study from 1958 including 45 patients with glomerular disease.[4] Today, protein loss is quantified by measuring either albumin or total protein in the urine. Quantifying daily protein or albumin loss by 24-hour urine collection can be tedious and prone to errors, and the easier and more accessible assessment of albuminuria in spot urine tests is increasingly used in clinical practice.[6, 9] No single standard cutoff level for hypoalbuminemia in the definition of NS is used. Different reviews and guidelines have proposed different cutoffs, including any hypoalbuminemia (plasma albumin [p-albumin] lower than reference limit),[4, 8] p-albumin <30 g/L,[9] or p-albumin <25 g/L.[7, 9] Finally, while the presence of edema is widely accepted in the definition of NS,[6] it has been proposed to be removed from the criteria essential of NS as not all patients develop edema.[4]

Pathophysiology of nephrotic syndrome

While the underlying pathology of the disease leading to NS can vary a lot, some pathophysiological elements are shared in the development of NS. Below, I briefly describe the mechanism of the key features of NS, including nephrotic proteinuria, hypoalbuminemia, and edema. The human kidneys produce urine by filtrating the blood to ensure homeostasis in the body.[8] The filtration starts in thousands of glomeruli where an ultra-filtrate is created, and through the tubular system the filtrate is concentrated to urine. The glomeruli serve as filters and allow fluids and smaller solubles to pass. Larger molecules including large proteins are in the main restricted from passing, partly due to the interdigitating food processes of cells called podocytes. When the podocytes are damaged, proteins can pass into the filtrate, and opposed to water, only small amounts of protein can be reabsorbed through the tubular system. Consequently, podocyte damage leads to loss of protein to the urine, proteinuria.[12] Albumin is the most abundant of proteins in the blood. In addition to heavy proteinuria, an insufficient increase in albumin production by the liver and an increase in albumin catabolism contributes to a low albumin level in the blood, hypoalbuminemia.[8] Albumin contributes to the oncotic pressure in the blood, and loss of oncotic pressure

and subsequent intravascular volume depletion may contribute to edema formation. In addition, changes in vascular permeability, reduction in the capillary-interstitial oncotic pressure gradient, and most importantly, salt and water retention in the distal tubules of the kidneys can contribute to edema formation in NS patients.[13]

The clinical course of nephrotic syndrome

Several elements in the clinical course affect the outcome of NS as illustrated in Figure 1 (a modified version of a figure by Sackett et al.).[14]

Behind every patient is a person, and every person contracting NS has an age, a biological sex, a lifestyle with exercise, a diet, leisure habits, etc. Before turning ill with NS, patients may also have encountered other diseases (comorbidities). All these factors in individuals with NS affect the other elements of the clinical course that decide the clinical outcome.

As previously mentioned, NS may appear in patients with primary glomerular disease (primary NS) or systemic conditions (secondary NS) such as underlying diabetes or cancer. The diagnostic workup in NS patients includes a detailed medical history, measurement of blood pressure, pulse rate, weight and BMI, quantification of proteinuria/albuminuria in spot urine (and possibly 24-hour urine), urine dipstick, and blood tests of: p-albumin, estimated glomerular filtration rate [eGFR], glucose and hemoglobin A1c, hemoglobin, platelets, leukocytes, C-reactive protein, electrolytes, liver and lipid panels.[7, 9, 15] Radiological examination may include ultrasound and/or computed tomography (CT) of the kidneys and urinary tract, and depending on the symptom picture also chest x-ray or CT, cardiac echo, and abdominal ultrasound. In adults with NS and no apparent cause, kidney biopsies are used to examine the histopathology which can guide clinicians in choice of treatment and prognosis.[7, 9] Among the wide range of primary glomerular diseases, some are more commonly associated with NS in adults including membranous nephropathy, minimal change disease, and focal segmental glomerulosclerosis.[8]

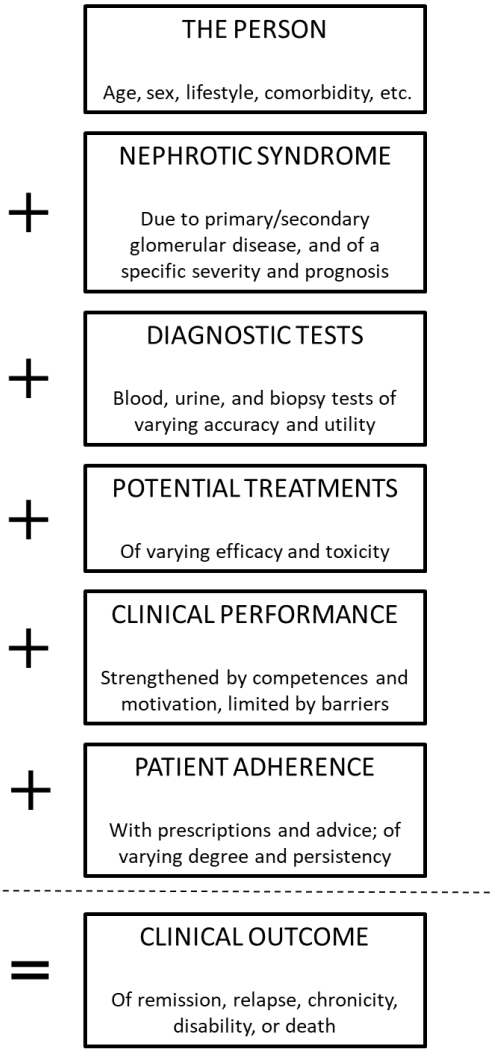

Figure 1. Model describing the steps in the clinical course of an illness that can affect the clinical outcome (modified version of figure by Sackett et al.). [14]

The treatment of NS depends on the cause of NS. If secondary, the underlying condition should be treated to see if this leads to remission. If primary, the recommended treatment depends on the type of the underlying glomerular disease with first-line treatments including corticosteroids and immunosupressants.[6] In addition, patients with NS may receive treatment targeting proteinuria (e.g. angiotensin-converting enzyme [ACE] inhibitors), edema (e.g. restricting sodium intake, and diuretics), and hyperlipidemia (e.g. statins).[10] The systematic use of anticoagulant drugs for prevention of venous thromboembolism (VTE) in NS is continuously debated.[16, 17] The most recent guidelines recommend an evaluation of the benefits and detriments of anticoagulant prevention in each individual patient, with suggestions based on “low” and “very low” quality of evidence.[6]

The clinical performance depends on the competence and motivation of the clinical staff. The clinical performance is likely improved by nephrological specialized care and clinical guidelines based on high quality evidence. The most recent international guideline on the treatment of glomerular disorders including NS was mostly based on low and very low quality of evidence and it noticed a scarcity in evidence from randomized controlled trials.[6]

Good adherence to treatment relies on development of a good understanding of the condition in individuals with NS, which enables patients to take part in the treatment decisions. However, patient information about NS is sparse, possibly due to the rarity and complexity of NS and the general lack of high-quality evidence regarding NS. Therefore, close communication between clinical caregiver and patients with NS is needed to ensure adherence to the treatment, which is critical for the clinical outcome.[18]

The clinical outcome after NS can vary between remission with no sequela and death. Complications such as thromboembolism, infections, or reduced kidney function may increase the risk of disability or death. Only some patients experience complete remission of the first episode of NS, and they may experience subsequent relapses yielding chronic kidney disease that in worst-case can progress to end-stage kidney disease.[8]

In summary, high quality evidence can contribute to improved clinical outcomes of patients with NS. Research studies can address questions at different steps of the clinical course (Figure 1), and observational studies can provide valuable information for more reasons.[19] First, data on the incidence of NS are necessary to identify risk groups, which potentially enables prevention of NS. Moreover, data on absolute and relative risk of clinical outcomes in patients with NS can improve the understanding of disease processes including identification of modifiable factors that affect the prognosis. Finally, the current guideline of treatment of NS calls for better evidence on treatment of NS, and risk data of clinical outcomes are needed for designing high-quality clinical trials.

Literature review

To obtain an overview of the existing literature on the epidemiology of NS, I searched in Medline using the medical subject headings (MeSH) and Boolean operators (AND/OR/NOT). The search queries used for each study are listed in Box 1, and every search was most recently repeated on 25 April 2021. Initially, I reviewed

titles and abstracts of all search results to select potentially relevant papers to be reviewed in full length. I searched for additional relevant papers in the reference lists, and finally, I used CoCite to search for additional papers based on the paper most relevant to my studies.[20] I extracted and tabulated information from the papers I found to be relevant for my studies.

Box 1. Medline search queries for literature search in Studies I-III.

STUDY I - Characteristics of patients with nephrotic syndrome	
("completeness" OR "PPV" OR "positive predictive value" OR "sensitivity" OR "specificity" OR "validation" OR "validity") AND "nephrotic syndrome" AND ("hospital-diagnosis" OR "hospital diagnosis" OR "hospital code" OR "diagnosis code") AND ("adult" OR "adults")	0 hits
("nephrotic syndrome" OR "nephrotic proteinuria" OR "nephrotic range proteinuria" OR "nephrotic-range proteinuria") AND ("cohort") AND ("adult")	344 hits
(nephrotic syndrome[MeSH Terms]) AND (adult[MeSH Terms]) AND (observationalstudy[Filter]) AND (english[Filter])	21 hits
(nephrotic syndrome[MeSH Terms]) AND (adult[MeSH Terms]) AND ("observational" OR "registry" OR "register" OR "database") AND (english[Filter])	89 hits
STUDY II – Incidence and mortality of nephrotic syndrome	
nephrotic syndrome[MeSH Terms] AND Epidemiology[MeSH Subheading] AND adult[MeSH Terms] AND english[Filter]	367 hits
"incidence" AND "nephrotic syndrome" AND ("adult" OR "adults")	469 hits
"mortality" AND "nephrotic syndrome" AND ("adult" OR "adults")	325 hits
STUDY III - Risk of bleeding and thromboses in nephrotic syndrome	
"Nephrotic Syndrome"[Mesh] AND "Adult"[Mesh] AND "Hemorrhage"[Mesh]	267 hits
"Nephrotic Syndrome" AND "Adult" AND ("Hemorrhage" OR "bleeding")	118 hits
"Nephrotic Syndrome"[MeSH Terms] AND ("Adult" OR "Adults" OR "Adult"[MeSH Terms]) AND ("Thromboembolism"[MeSH Terms] OR "Thrombosis"[MeSH Terms] OR "Myocardial Ischemia"[MeSH Terms] OR "Stroke"[MeSH Terms]) NOT (casereports[Filter])	216 hits
"Nephrotic Syndrome" AND ("Adult" OR "Adults" OR "Adult") AND ("Thromboembolism" OR "Thrombosis" OR "Myocardial Ischemia" OR "Stroke") NOT (casereports[Filter])	259 hits

Table 1. Summary of literature, study I.

STUDY I - Characteristics of patients with nephrotic syndrome						
First author, journal, year	**Design, data sources, country, period**	**Inclusion criteria**	**Size study pop.**	**Age, sex, and ethnicity**	**Proportion with specific glomerular pathology**	**Baseline characteristics: Medication use, prior comorbidity, and laboratory test results**
Haas,[21] American Journal of Kidney Diseases, 1997	Cross sectional, Record review, USA, 1976-1979 and 1995-1997	Sampled NS patients from 1000 adults who underwent kidney biopsies,	n=233	Age: Mean age ± SD White = 49.2 ± 15.9 Black = 43.8 ± 16.0	1995-1997: MCD: n=35 (15%) MN: n=77 (33%) FSGS: n=81 (35%) Amyloid: n=9 (4%) MPGN: n=5 (2%) Chronic GN: n=1 (<1%) IgAN: n=20 (9%) Focal GN: n=0 (0%) Fibrillary: n=2 (1%) Other GN: n=2 (1%)	
Waldman,[22] Clinical Journal of the American Society of Nephrology, 2007	Cohort study, Record review, USA, 1990-2005	Biopsy verified MCD	n=95	Mean age ± SD: 45.1 ± 1.6 (interval: 19-78) Sex: 42% Men		Hypertension 42.9% Mean eGFR ± SD: 71.7 ml/min/1.73m^2 ± 4.0 Mean s-albumin ± SD: 22.1 g/L ± 0.08 Mean u-protein ± SD: 9.93 g/day ± 0.71
Mahmoodi,[23] Circulation, 2008	Cohort study, Electronic patient registry, The Netherlands, 1995-2044	Outpatient, verified proteinuria	n= 298	Age: Mean age ± SD= 42y ± 18 Sex: 59% Men	MCD: n=49 (16%) MN: n=72 (24%) FSGS: n=36 (12%) MPGN: n=26 (9%) DN: n=32 (11%) NOS: n=83 (28%)	Hypertension: n= 182 (61%) Hyperlipidemia: n= 221 (92%) Diabetes: n= 42 (14%) Prior VTE/ATE: n=10 (10%)
Dumas de la Roque,[24] Journal of Clinical Medicine, 2018	Cohort study, Record review, France, 2007-2014	Biopsy verified MCD or FSGS in patients with NS	n=165 By type: MCD: n=97 FSGS: n=68	MCD patients: Median age [IQR]: 47y [27.5-64] Men: 57% FSGS patients: Median age [IQR]: 57y [42.2-66.7] Men: 71%		MCD patients: Hypertension: 48.8% Prior venous thromboembolism: 3.1% Prior arterial thromboembolism: 3.1% FSGS patients: Hypertension: 46.3% Prior venous thromboembolism: 1.5% Prior arterial thromboembolism: 7.3%

Yamamoto,[25] Clinical and Experimental Nephrology, 2020	Cohort study, Japan renal biopsy registry (J-RBR), Japan, 2009-2010	Primary NS, biopsy verified,	n=374	MCD patients: Median age [IQR]: 41y 26, 61 Men: n=90 (58%) MN patients: Median age [IQR]: 66y (59, 74) Men: n=83 (56%) FSGS patients: Median age [IQR]: 62y (29, 73) Men: n=25 (66%) Other patients: Median age [IQR]: 58y (46, 71) Men: n=19 (58%)	MCD: n= 155 (41.4%) MN: n=148 (39.6%) FSGS: n=38 (10.2%) Other: n=33 (8.8%)	In total: 32% used RAS blockader, 37% used statins, and 4% used antidiabetics. MCD patients: RAS blockers: 13.5% Statins: 27.1% Antidiabetics: 4.5% MN patients: RAS blockers: 45.9% Statins: 48.0% Antidiabetics: 2.7% FSGS patients: RAS blockers: 42.1% Statins: 55.3% Antidiabetics: 5.3% Other patients: RAS blockers: 45.5% Statins: 15.2% Antidiabetics: 9.1%
Shinkawa,[26] Nephrology Dialysis Transplantation, 2020	Cohort study, Hospital patient database, Japan, 2008-2017	Patients with hospital-recorded NS without prior VTE	n=7,473	Age Median age [IQR]: 69y [53-79] Sex Men: n=104 (47%)	Any biopsy: n=3,418 (46%) MCD: n=1315 (18%) FSGS: n=181 (2%) MN: n=829 (11%) MPGN: n=73 (1%) DN: n=1064 (14%)	Diabetes: n=222 (30%) Cancer: n=501 (7%) Medication Diuretics: n=2158 (29%) Anticoagulants: n=607 (8%)
Kolb,[27] Kidney International Reports, 2021	Cohort study, National biopsy registry, Scotland, 2014-2017	Patients with NS and kidney biopsy,	n=522 Type Primary NS: n=372 Secondary NS: n=150	Any NS patients: median age [IQR]: 63.0y [49.8-72.4] 54% men Primary NS patients: median age [IQR]: 63.5y [49.6-72.5] 55% men Secondary NS patients: median age [IQR]: 62.1y [50.5-72.1] 52% men		Any NS patients: eGFR ml/min, median (IQR) = 62 (35-91) UACR mg/mmol, median (IQR) = 538 (327-802) p-alb g/l, median (IQR) = 22.0 (16.0-26.0) Primary NS patients: eGFR ml/min, median (IQR) = 70 (41-93) UACR mg/mmol, median (IQR) = 572 (280-829) p-alb g/l, median (IQR) = 21.0 (15.0-25.8) Secondary NS patients: eGFR ml/min, median (IQR) = 46 (28-77) UACR mg/mmol, median (IQR) = 464 (336-680) p-alb g/l, median (IQR) = 23.0 (18.8-27.2)

Abbreviations: ; DN, Diabetic nephropathy; DNPR, Danish National Patient Registry; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; IgAN, Immunoglobulin A nephropathy; IQR, interquartile range; J-RBR, Japan renal biopsy registry ; MCD, Minimal change disease; MN, membranous nephropathy; MPGN, Membranoproliferative glomerulonephritis; NOS, not otherwise specified; RAS, Renin-angiotensin system; s-albumin, serum albumin; SD, standard deviation

Table 2. Summary of literature, study II, incidence

STUDY II – incidence of nephrotic syndrome						
Author, journal, year	**Design, country, period**	**Data sources, inclusion criteria**	**Outcome measure**	**Participants, Age and sex**	**Results**	**Comments**
Sharpstone,[28] British Medical Journal, 1969	Cohort study, England, 1966-1968	Census: adult population of n=~2,770,000 Survey in patients >14 years with kidney biopsy and clinical NS and without systemic disease that could lead to NS (NOS)	Incidence rate of biopsy-proven PGD indicated by NS	Biopsy cohort: n=56 22 (39%) Men	Annual rate of biopsy indicated by NS: 0.9 per 100,000 PYs	- Only primary NS with biopsy in context of NS. - Not restricted to patients with first-time biopsy - Lack uncertainty estimates
Tiebosch,[29] Kidney International, 1987	Cohort study, The Netherlands, 1978-1985	Adult population serviced by hospital, n= ~1,500,000 Renal biopsies from patients 16-65 years without diabetes mellitus, liver cirrhosis, ankylosing spondylitis, multiple myeloma, generalized arteriosclerosis), drug-associated NS, chronic hypertension, Alport	Incidence rate of biopsy-proven PGD indicated by NS	Biopsy cohort: n=45	Annual rate of biopsy indicated by NS: 2.7 per 100,000 PYs	- Only primary NS with biopsy in context of NS - Not restricted to patients with first-time biopsy - Lack uncertainty estimates
Autuly,[30] New Therapeutic Strategies in Nephrology, 1991	Cohort study, France, 1976-1989	Adults population of ~400,000 adults. Biopsy registry: Patients >15 years with biopsy in context of NS	Incidence rate of biopsy-proven PGD indicated by NS	Biopsy cohort: n=186 Age: 15-64 years: n=128 65+ years: n=28 Male/female ratio: 1.85:1	Annual rate of biopsy indicated by NS: 15-64 years: 4.2 per 100,000 population 65+ years: 7.1 per 100,000 population	- Only primary NS with biopsy in context of NS - Not restricted to patients with first-time biopsy - Lack uncertainty estimates
Simon,[31] Kidney International, 1994	Cohort study, France, 1976-1990	Adults population of 410,644 adults. Renal biopsies from patients 10-79 years with first-time kidney biopsy	Incidence rate of biopsy-proven PGD indicated by NS	Biopsy cohort: n=219 Mean age ± SD: 1976-80: 47y ±14 1981-85: 50y ±14 1986-90: 52y ±14 Male/female ratio: 1976-80: 1.3:1 1981-85: 1.5:1 1986-90: 1.6:1	Annual rate of biopsy indicated by NS: 3.8 per 100,000 PYs By time period: 1976-1980: 3.6 per 100,000 PYs 1981-1985: 3.7 per 100,000 PYs 1986-1990: 4.0 per 100,000 PYs	- Only primary NS with biopsy in context of NS - Lack uncertainty estimates
Stratta,[32] American Journal of Kidney Disease, 1996	Cohort study, Italy, 1970-1994	Census: adults >15 years ~3.7 mil. Pop Renal biopsies from patients >15 years without systemic disease (i.e. Lupus, vasculitis, diabetes, Goodpasture syndrome, Alport) with first-time kidney biopsies	Incidence rate of biopsy-proven PGD indicated by NS	Biopsy cohort: n= 1,926 Mean age ± SD: 1990-1994: 47y ±18y	Annual rate of biopsy indicated by NS: Overall = 1.74 per 100,000 PYs By age group: 15-24 y: 0.31 per 100,000 PYs 25-34 y: 1.47 per 100,000 PYs 35-44 y: 1.30 per 100,000 PYs 45-54 y: 1.48 per 100,000 PYs 55-64 y: 2.61 per 100,000 PYs 65-74 y: 3.47 per 100,000 PYs 75+ y: 1.71 per 100,000 PYs	- Only primary NS with biopsy in context of NS - Lack uncertainty estimates

Covic,[33] Nephrology Dialysis Transplantation, 2006	Cohort study, Romania, 1995-2004	Census: 1995: 6.4 mil. population 2004: 6.2 mil. population Biopsy registry: Renal biopsies on adults >18 years	Incidence rate of biopsy-proven PDG Proportion of biopsies indicated by NS	Biopsy cohort: n= 635 Mean age ± SD = 38.5y ±15.2 51.5% males	Rate of renal biopsy in year 2004: 11.3 p.m.p./year Proportion of biopsies indicated by NS: 52.3% *Annual rate of biopsy indicated by NS: 11.3 per 1000,000 PYs*0.523= 0.59 per 100,000 PYs	- Only primary NS with biopsy in context of NS. - Not restricted to patients with first-time biopsy. - Lack uncertainty estimates -*I calculated the NS rate similarly to similar studies
Wirta,[34] Nephrology Dialysis Transplantation, 2008	Cohort study, Finland, 1976-2000	Census: ~423,689 population Renal biopsy records: All kidney biopsies in patients without kidney abnormalities or known kidney damage	Incidence rate of biopsy-proven PDG Proportion of biopsies indicated by NS	Biopsy cohort: n= 2,567 median age = 44.0 y male/female ratio: 1512/1055	Rate of renal biopsy: 254 p.m.p./year Proportion of biopsies indicated by NS: 16.4% *Annual rate of biopsy indicated by NS: 254 per 1000,000 PYs*0.164 = 4.2 per 100,000 PYs	- Only primary NS with biopsy in context of NS. - Not restricted to adults. - Not restricted to patients with first-time biopsy. - Lack uncertainty estimates -*I calculated the NS rate similarly to similar studies
Naumovic,[35] Nephrology Dialysis Transplantation, 2009	Cohort study, Serbia, 1987-2006	Census: ~7.5 mil. population Review of renal biopsy records in adults without kidney transplantation	Incidence rate of renal biopsies Proportion of biopsies indicated by NS	Biopsy cohort: n= 2,362 Mean age ± SD: 39y ±14 Men: 51.2% males NS cohort: n=872 Mean age ± SD: 35.5y ±13.4 Male/female ratio: 1.1	Rate of renal biopsy: 10.8 p.m.p./year Proportion of biopsies indicated by NS: 53.6% *Annual rate of biopsy indicated by NS: 10.8 per 1000,000 PYs*0.536 = 0.58 per 100,000 PYs	- Primary or secondary NS with biopsy in context of NS. - Not restricted to patients with first-time biopsy - Lack uncertainty estimates -*I calculated the NS rate similarly to similar studies
Jegatheesan,[36] Nephrology, 2016	Cohort study, Australia, 2002-2011	Census: 2002: ~2.7 mil. pop 2011: ~3.4 mil. pop Review of electronic kidney biopsy records on adults >18 years without kidney transplantation	Incidence rate of biopsy-proven glomerular disease indicated by NS	Total biopsy cohort: n= 2,048 Mean age ± SD: 48y ±17 ~60% males	Annual rate of biopsy indicated by NS: 2.28 per 100,000 PYs	- Primary or secondary NS with biopsy in context of NS - Lack uncertainty estimates
Kolb,[27] Kidney International Reports, 2021	Cohort study, Scotland, 2014-2017	All data obtained from Scottish Renal Biopsy Registry, except mortality data from National Records of Scotland. Included all non-kidney transplanted adults with kidney biopsy indicated by NS.	Incidence rate of biopsy-proven glomerular disease indicated by NS	n=522 Median age [IQR]: 63y [50-72] 54% men	Annual rate of biopsy indicated by NS: 2.42 per 100,000 PYs	- Primary or secondary NS with biopsy in context of NS - Lack uncertainty estimates

Abbreviations: mil., million; NOS, not otherwise specified; NS, nephrotic syndrome; PGD, primary glomerular disease; p.m.p., per million population; PYs, person-years; SD, standard deviation; y, years

Table 3. Summary of literature, study II, mortality

STUDY II - mortality of nephrotic syndrome					
Author, journal, year	**Design, country, period, Data sources**	**Inclusion criteria**	**Participants, Age, sex**	**Results**	**Comments**
Nolasco,[37] Kidney International, 1986	Prospective cohort study, England, 1963-1982, Primary data collection	Adult patients with nephrotic syndrome and minimal change glomerular lesion in kidney biopsy.	n=89 Baseline: mean age ± SD: 42y ±19 Hypertension: n=27 (30%)	n=11 (12%) died within 3 years	-Restricted to NS patients with biopsy-proven minimal change disease. -Did not report loss to follow-up - Lack uncertainty estimates
Eriguchi,[38] Nephrology Dialysis Transplantation, 2009	Prospective cohort study, Japan, 1988-2005, Primary data collection	Adult patients with biopsy-proven idiopathic membranous nephropathy with NS, and without secondary causes of membranous nephropathy, coexisting other glomerular disease, previously treated iMN.	n=103	n=14 patients died. Cumulative mortality risk 5-year: 7.1% 10-year: 12.8% 20-year: 27.1%	-Restricted to NS patients with biopsy-proven iMN - Lack uncertainty estimates
Chou,[39] Clinical Journal of the American Society of Nephrology, 2012	Retrospective cohort study, Taiwan, 1993-2006, Patient records (medical, pathology, laboratory)	Adults (>18y) without kidney transplants undergoing kidney biopsy for NS, renal failure, or persistent urinary abnormalities. Restricted to glomerular pathology: MCD, MN, FSGS, IgAN	n=580 Baseline: mean age ± SD: 44.4y ±16.8 Men 58.5% Diabetes in 7.9% Hypertension in 32.5% Proteinuria >3.5 g/day in 71.3% GN-type: MN: n=209 FSGS: n=132 IgAN: n=130 MCD: n=109	Median follow-up time: 5.9 years (IQR: 5.7) Mortality during follow-up Overall all-cause deaths: n=65 (11.2%) Death by GN-type: MN: n=36 (17.2%) FSGS: n=19 (14.4%) IgAN: n=6 (4.6%) MCD: n=4 (3.7%)	-Restricted to patients with biopsy-proven MCD, MN, FSGS, IgAN. -Not restricted to patients with NS (yet 71.3% has nephrotic proteinuria). - Did not report maximum length of follow-up or loss to follow-up. - Lack uncertainty estimates
McQuarrie,[40] Nephrology Dialysis Transplantation, 2012	Cohort study, Scotland, 1997-2008, Renal electronic patient records	Adults with membranous nephropathy, and nephrotic proteinuria and hypoalbuminemia not secondary to hepatitis, systemic lupus erythematosus, or underlying malignancy.	n=95 Baseline: Mean age ± SD: 61.4y ±14.0 74.7% male sex	1-year mortality risk = 9.6% 5-year mortality risk = 16.8%	-Restricted to NS patients with biopsy-proven membranous nephropathy - Lack uncertainty estimates
Chen,[41] Journal of Nephrology, 2014	Cohort study, China, 2002-2011, Primary data collection	Adult patients with biopsy-proven idiopathic membranous nephropathy with nephrotic-range proteinuria (>3.5 g/day) and chronic kidney disease (CKD) stages 2–4.	n=129 Baseline: Median age [IQR]: 58y [50-66] Men: 82%	n=11 (8.5%) patients died within 1 year	-Restricted to NS patients with biopsy-proven membranous nephropathy - Lack uncertainty estimates

van den Brand,[42] Journal of the American Society of Nephrology , 2014	Cohort study, The Netherlands, 1995-2009, medical records/correspondence	Patients with biopsy-proven iMN, i.e. without prior use of medication (NSAID, gold, penicillamine), autoimmune disorders, infections, malignancy. Patients not treated restrictively were excluded.	n=254 Baseline: Mean age (SD): 53y (14) 68% Men Nephrotic Syndrome in 89% 8% died before initiating	Median follow-up 57 months (IQR:32-90). 20 patients lost to follow-up. Mortality risk, % (95%-CI): 1-year: 0% (0-2) 3-year: 3% (1-6) 5-year: 6% (3-10) 10-year: 10% (5-17)	-Not restricted to patients with NS (89% has NS). -Restricted to NS patients with biopsy-proven membranous nephropathy -Additional effort to follow those lost to follow-up found 7/20 had died. -Worst case scenario sensitivity analysis: 5-year mortality: 11% (8-17) 10-year mortality:17% (11-25) - Lack uncertainty estimates
Rankin,[43] Clinical Practice, 2017	Cohort study, Scotland, 2008-2013, Renal Biopsy Registry	Adults with a first NS-indicated biopsy showing primary glomerulonephritis in the Glasgow Renal and Transplant Unit, excluding patients with systemic disease (diabetes, hepatitis, lupus, amyloidosis)	n=206 Mean age ± SD: 55y ± 19 Male sex: 60% Mean eGFR ± SD: 72 ml/min ± 40 Pathology: iMN: n=79 (38%) MCD: n=54 (26%) FSGS: n=37 (18%) IgAN: n=22 (11%) Mesangiocappillary GN: n=14 (7%)	Median follow-up [IQR]: 2.9 years (1.6–4.7). Deaths overall: n=39 (19%) Deaths by pathology type: iMN: n=20 (25%) MCD: n=3 (7%) FSGS: n=8 (22%) IgAN: n=6 (27%) Mesangiocappillary GN: n=2 (15%)	- restricted to primary NS with kidney biopsy - States follow up of up to 3 years, but reports follow-up >3 years. - Did not report loss to follow-up - Did not consider censoring in cumulative risk estimates. – The crude mortality risk (%) by pathology type was calculated as one minus crude survival probability - Lack uncertainty estimates
Yamamoto,[25] Clinical and Experimental Nephrology, 2020	Cohort study, Japan, 2009-2015, the Japan Nephrotic Syndrome Cohort Study (JNSCS).	Prospectively included 455 patients with primary nephrotic syndrome and kidney biopsy (2009-2010). Excluded 81 patients with no kidney biopsy in entry period, secondary NS, sclerosing glomerulonephritis with unknown etiology, incomplete informed consent, duplicate registrations, or unknown reason.	n=374 Type of GN, n, median age [IQR]: MCD, n= 155, 41y [26-61] MN, n= 148, 56y [59-74] FSGS, n= 38, 62y [29-73] Other, n=33, 58y [46-71]	23/374 (6%) patients died during 5-year follow-up Death by type of GN: MCD: 8/155 (5%) MN: 12/148 (8%) FSGS: 1/38 (3%) Other: 2/33 (6%)	-Restricted to patients with biopsy in context of primary NS -Uncertainty estimates only provided for mortality rates, and not for mortality risks
Kolb,[27] Kidney International Reports, 2020	Cohort study, Scotland, 2014-2017, Renal Biopsy Registry, death from National Records of Scotland	Included all non-kidney transplanted adults with kidney biopsy indicated by NS. Followed from 2014 through 2018.	Any NS patients: n=522 median age [IQR]: 63.0y [49.8-72.4] 54% men Primary NS patients: n=372 median age [IQR]: 63.5y [49.6-72.5] 55% men Secondary NS patients: n=150 median age [IQR]: 62.1y [50.5-72.1] 52% men	Median follow-up [IQR]: 866 days [524-1264] 110 deaths in total. 3-year mortality risk of 21% 3-year mortality risk: <u>Primary NS:</u> <60 years: 2% ≥60 years: 24% <u>Secondary NS</u> <60 years: 15% ≥60 years: 54%	-Restricted to patients with biopsy in context of NS -Leading cause of death in 110 events: Cardiovascular, 23 (21%); Renal (other), 8 (7%); Cancer, 21 (19%); Infection, 11 (10%); NS, 5 (5%); ESKD, 0 (0%); Bleeding, 1 (1%); VTE, 1 (1%); Other, 40 (36%)

Abbreviations: CKD, chronic kidney disease; ESKD, end-stage kidney disease; FSGS, focal segmental glomerulosclerosis; IgAN, Immunoglobulin A nephropathy; iMN, idiopathic membranous nephropathy; JNSCS, Japan Nephrotic Syndrome Cohort Study; MCD, Minimal change disease; MN, membranous nephropathy; NSAID, Non-steroidal anti-inflammatory drug; VTE, venous thromboembolism; IQR, interquartile range

Table 4. Summary of literature, study III

STUDY III - risk of bleeding and thromboses in nephrotic syndrome							
Author, journal, year	**Design, country, period, Data sources**	**Inclusion criteria, Outcome criteria**	**Outcome measures**	**Study cohort size**	**Baseline characteristics**	**Results**	**Comments**
Ordonez,[44] Kidney International, 1993	Matched cohort study, USA, 1961-1981, Medical records from The Kaiser Permanente Medical Care Program of Northern California (KPMCP)	- Patients with hospital-diagnosed NS, aged >15 years, without prior coronary heart disease (CHD) - Comparisons matched 1:1 by age and sex among patients without hospital-diagnosed NS - Outcomes: From medical records: CHD i.e. myocardial infarction (MI), angina pectoris (AP), coronary insufficiency, probable or definite ischemia in ECG	Incidence rate of CHD per 1000 PYs Hazard ratio of CHD	n=142	Mean age : 34.7y Men: 62.7% Current smoker: 43.6% History of hypertension: 29.8%	Mean follow up for CHD: 5.6 years MI events: n=11, rate of 14.9 per 1,000 PYs HR of MI in NS vs comparisons: HR adjusted*: 5.5 (95%-CI: 1.6-18.3) CHD events: n=18, rate of 25.2 per 1,000 PYs HR of CHD in NS vs comparisons: HR adjusted*: 2.3 (95%-CI: 1.0-5.2)	- Limited information on loss to follow-up. - Restricted to persons without previous coronary heart disease or diabetes. - *HRs adjusted for hypertension and smoking
Mahmoodi,[23] Circulation, 2008	Cohort study, The Netherlands, 1995-2004, Electronic medical records	Patients with NS followed ≥6 months in outpatient clinic, ≥18 years, without acute life-threatening disease. Symptomatic thromboembolisms verified by radiology/ECG/ biochemistry/clinic	Annual incidence rates of VTE and ATE	n=298	Mean age ± SD= 42y ± 18 Men: n=177 (59%) Pathology: MCD: n=49 (16%) MN: n=72 (24%) FSGS: n=36 (12%) MPGN: n=26 (9%) DN: n=32 (11%) NOS: n=83 (28%) Comorbidities Hypertension: n= 182 (61%) Hyperlipidemia: n= 221 (92%) Diabetes: n= 42 (14%) Prior VTE/ATE: n=10(10%)	<u>VTE</u> Mean follow-up ± SD for VTE: 10y ±8y VTE events: n=29 Average annual risk of VTE = 1.02% (95%-CI: 0.68-1.46) Type of first VTE: PE (38%), DVT (34%), PE+DVT (10%), PE+RVT (10%), RVT (3%), Mesenteric VT (3%) <u>ATE</u> Mean follow-up ± SD for ATE: 10y ±9y ATE events: n=43 Average annual risk of ATE = 1.48% (95%-CI: 1.07-1.99) Type of first ATE: MI (44%), UAP (14%), PAD (14%), Ischemic stroke (12%), TIA (12%), Amaurosis fugax (2%), aorta thrombosis (2%)	-Not clear if restricted to first-time NS. - Not clear if started follow-up on NS admission or 6 months thereafter. -No information on number of excluded patients due to followed for <6 months. -No direct comparison group. - Some patients classified by type of pathology from clinical context without biopsy (72% of DN, and 54% of NOS). -Counted 15 (35%) patients with ATE before NS diagnosis as outcomes

Li,[45] Thrombosis Research, 2012	Cross sectional study, China, 2009-2010, Prospective follow-up and examination	-Inclusion Patients with biopsy-verified MN, nephrotic proteinuria, hypoalbuminemia, eGFR >40 ml/min and without: Lupus, antiphospholipid syndrome, hepatitis B, hepatitis C, cancer, major surgery, prolonged immobilization, use of oral contraceptives, or anticoagulation. -Outcome All patients examined by CT angiography and compression venous ultrasonography for VTE (DVT, RVT, PE)	Prevalence of VTE (%)	n=100	Overall Age range: 18-73 years Men: n=80 (80%)	Prevalence of VTE overall: n=36 (36%) Prevalence of VTE by type: RVT: n=33 (33%) PE: n=17 (17%) Prevalence of symptomatic VTE by type: RVT: n=7 (7%) PE: n=9 (9%)	- Restricted to pts with MN - Restricted to patients without systemic diseases or known risk factors for thromboses - Radiological examination allows identification of asymptomatic VTE. - Cross-sectional design allows no detection of VTE after initial assessment. - Prevalence of DVT overall not reported. -no reported uncertainty for risk estimates (e.g. confidence intervals)
Christiansen,[46] Journal of Thrombosis and Haemostasis, 2014	Case-control study, Denmark, 1980-2010, Danish National Patient Registry (DNPR)	-Cases First-time recorded VTE (ICD-8/ICD-10) among in-/outpatients without prior kidney transplantation from the DNPR. -Controls Matched to cases on age and sex among persons without prior kidney transplantation from the Civil Registration System -Exposure Hospital-recorded in-/outpatient kidney disease before VTE in the DNPR. NS is one of exposure groups.	Odds ratio (OR) of VTE after exposure to NS	Cases: n=128,096 Controls: n=642,426	Cases: NS: n=127 (0.1%) Cancer: n=23,320 (18.2%) Surgery: n= 24,520 (19.1%) Fracture/Trauma: n=11,504 (9.0%) Pregnancy: n=1140 (0.9%) Heart Failure: n=9,212 (7.2%) COPD: n=12,460 (9.7%) Diabetes: n=7,695 (6.0%) Controls: NS: n=176 (0.0%) Cancer: n=46,188 (7.2%) Surgery: n=26,101 (4.1%) Fracture/Trauma: n=9,992 (1.6%) Pregnancy: n=1,181 (0.2%) Heart Failure: n=19,055 (3.0%) COPD: n=29,542 (4.6%) Diabetes: n=22,988 (3.6%)	OR (95%-CI) of VTE after NS overall: Unadjusted: 3.60 (2.86-4.52) Adjusted*: 2.89 (2.26-3.69) OR (95%-CI) by follow-up period <u>0-90 days:</u> Unadjusted: 24.49 (9.44-63.55) Adjusted*: 23.23 (8.58-62.89) <u>91-365 days:</u> Unadjusted: 9.29 (4.85-17.78) Adjusted*: 6.92 (3.48-13.79) <u>1-5 years:</u> Unadjusted: 2.83 (1.86-4.32) Adjusted*: 2.25 (1.42-3.54) <u>>5 years:</u> Unadjusted: 2.16 (1.50-3.10) Adjusted*: 1.70 (1.15-2.51)	-Case-control study design does not allow estimating absolute risk measures. -Because of risk set sampling the ORs can be interpreted as unbiased incident rate ratios. -*ORs adjusted for known potential confounders (i.e. cancer, surgery, fracture/trauma, pregnancy, myocardial infarction, congestive heart failure, hypertension, chronic pulmonary disease, liver disease, diabetes and psychiatric disease)

Zhang,[47] Radiology, 2014	Cross-sectional study, China, 2010-2011, Prospective enrollment and clinical examination at single center.	- Inclusion: Clinical Nephrotic syndrome with nephrotic proteinuria and hypoalbuminemia, and s-creatinine level <2 mg/dL (176.8 mmol/L) or >2 mg/dL if in maintenance dialysis. Excluded: Clinically unstable patients and those with prior reaction to iodinated contrast media. - Outcome PE or RVT confirmed by CT interpreted by two radiologists blinded to patient characteristics	Prevalence (%) of PE and RVT	n=512	512 patients in the study cohort Men: n=331 Mean age ± SD: 37 y ±17 Age range: 9–81 years CT pulmonary angiography: n=458 Renal CT venography: n=505	Prevalence of any PE or RVT: n=180 (35%) PE events: n=153 Prevalence of PE (95%-CI): 33% (28-39) Of all PE events, n=25 (16%) were symptomatic. RVT event: n=112 Prevalence of RVT (95%-CI): 22% (18-27) Of all RVT events, n=15 (13%) were symptomatic.	-Not restricted to adults. n=80 (16%) were <18 years - Included patients who declined radiologic examination when describing baseline characteristics.
Li,[48] Clinical and Experimental Nephrology, 2016	Cross-sectional study, China, 2012-2014, Single-center primary data collection	-Inclusion Patients with biopsy-verified FSGS, nephrotic proteinuria and hypoalbuminemia, accepting screening with CT angiography. Excluded patients with obesity, systemic hypertension, HIV, or risk factors for VTE (e.g. cancer, major surgery, or prolonged immobilization), use of oral contraceptives or anticoagulants. -Outcome: Scanned all patients. PE and RVT detected by CT pulmonary angiography and CT venography. DVT detected by compression venous ultrasonography.	Prevalence of VTE (%)	n=120	By outcome Thrombus group: n=12 Mean age ± SD= 30.3 ± 19.4 Men: n=9 (75%) No thrombus group: n=108 Mean age ± SD= 33.5 ± 16.4 Men: n=80 (74.1%)	VTE events: n=12 (10%) PE events: n=8 (6.7%) RVT: n=4 (3.3%) DVT events: n=3 (2.5%)	- Restricted to NS patients with FSGS and eGFR>40 ml/min - Restricted to persons with primary FSGS - Screened for VTE – i.e. some patients were asymptomatic. - No information on reason for not accepting CT angiography in 15 patients of 135 who refused. - Difference in examination periods, i.e. n=70 (58%) had CT scan at initial NS episode,n=50 (42%) had CT during relapse. - Patient characteristics only reported stratified by outcome - No reported uncertainty for risk estimates (e.g. confidence intervals)

Lee,[49] Kidney International, 2016	Cohort study, USA, 1980-2011, The Glomerular Disease Collaborative Network (GDCN)	Patients with biopsy-verified MN, >18 years, and without prior viral hepatitis or cancer, and not treated with anticoagulants. Outcomes From medical records: arterial thromboembolic event (ATE) incl.: acute coronary syndrome (myocardial infarction, or unstable angina), thrombotic ischemic stroke, peripheral artery disease	Cumulative risk (%) of ATE considering death and ESKD competing risks	n=404	From the GDCN: mean age ± SD= 51.4 ± 15.5 Men: 60% Diabetes: 9% Smoking: Ever: 33% Current: 21% History of CVE: 12% Mean eGFR ± SD: 68.9 ml/min ± 33.5 Nephrotic syndrome: 88%	Median follow-up [IQR]: 24.3 months [9.9–52.7] Number of ATE events overall: n=31 ATE risk overall: 1-year risk of CVE: 4.4% 2-years risk of CVE: 5.4% 3-years risk of CVE: 8.2% Number of ATE events by type: acute coronary syndrome: n=22 thrombotic ischemic stroke: n= 8 peripheral artery disease: n=1	- restricted to patients with primary MN in biopsy -Not restricted to pts with NS (88% of patients had NS at presentation) - Restricted to patients not treated with anticoagulant drugs - excluded 29 patients with insufficient follow-up information. - Considered competing risk of death. -no reported uncertainty for risk estimates (e.g. confidence intervals)
Rankin,[43] Nephron, 2017	Cohort study, Scotland, 2008-2013, The Scottish Renal Biopsy Registry	Adults with a first NS-indicated biopsy showing primary glomerulonephritis in the Glasgow Renal and Transplant Unit, excluding patients with systemic disease (diabetes, hepatitis, lupus, amyloidosis)	Risk (%) of VTE Risk (%) of major bleeding	n=206	Mean age ± SD: 55y ± 19 Men: 60% Mean eGFR ± SD: 72 ml/min ± 40 Pathology: iMN: n=79 (38%) MCD: n=54 (26%) FSGS: n=37 (18%) IgAN: n=22 (11%) Mesangiocappillary GN: n=14 (7%)	Median follow-up [IQR]: 2.9 years (1.6–4.7). VTE events overall: n=14 (6.8%) VTE events by pathology: iMN: n=7 (8.9%) MCD: n=5 (9.3%) FSGF: n=1 (2.7%) IgAN: n=1 (4.5%) Mesangiocappillary GN: n=0 Major bleeding events overall: n=7 (3.4%)	- restricted to primary NS with kidney biopsy - included VTE occurring up to 1 year before biopsy included as outcome. - States follow up of up to 3 years, but reports follow-up >3 years. - No information on loss to follow-up - Did not consider censoring or competing risk of death in cumulative risk estimates. -no reported uncertainty for risk estimates (e.g. confidence intervals)

Gyamlani,[50] Nephrology Dialysis Transplantation, 2017	Cohort study, USA, 2004-2013, Nested in cohort study (the Racial and Cardiovascular Risk Anomalies in CKD study) using Veteran Affairs research database	Inclusion Patients with ICD-9-CM coded NS in-/outpatient during 2004-2006, without VTE at first encounter with NS, and with available s-albumin data. Outcomes Followed up to 10 years for incident VTE (RVT, DVT, PE) recorded with ICD-9-CM in-/outpatient	Absolute risk of VTE (%) and rate per 1000 person-years	n=7,037	Mean age ± SD= 57 ± 11 Men: n=6,728 (96%) African-American race: n=2,177 (32%) Mean eGFR ± SD: 83 ml/min ± 17 Prevalent comorbidity: Diabetes: n=4,245 (60%) Hypertension: n=5,929 (84%) Cardiovascular disease: n=1,240 (18%) Cancer: n=802 (11%) Anticoagulation use: n=3,884 (55%)	Median follow-up time [IQR]: 8.1 years [6.4–8.6] VTE events: n=158 VTE, Risk of VTE: 2.25% Rate of VTE: 3.17 per 1000 PYs (95%-CI: 2.72–3.71)	- Nested in study population of US veterans with eGFR>60 ml/min at baseline of inclusion -Not restricted to first-time NS (i.e. included prevalent NS patients) -Included few (4%) women - No information on loss to follow-up - Restricted to persons with available s-albumin at baseline - Comorbidities information obtained during 2004-2006, i.e. not specified if assessed only during 2 years after NS in some patients. - Used Kaplan Meier method for cumulative risk of VTE, i.e. not accounting for competing risk of death.
Huang,[51] Journal of Stroke and Cerebrovascular Diseases, 2019	Matched cohort study, Taiwan, 2000-2008, Electronic hospital records	-First-time hospital recorded NS (at 1 admission or 3 clinical visits), aged 18-110 years, without prior stroke, with complete demographic data -Matched comparisons 1:4 on age, sex, CCI-score, index-date -Hospital-diagnosed stroke (ischemic or hemorrhagic)	Incidence rate of stroke HR of stroke compared to matched comparisons	n=3,496	Mean age ± SD: 48.6y ±15.2y Men: n=2,007 (57.4%) By CCI-score: CCI=0: n= 2,027 (58%) CCI=1: n= 755 (22%) CCI=2: n= 373 (11%) CCI≥3: n= 341 (10%) Comorbidity: Ischemic heart disease: n= 240 (7%) Heart Failure, n= 37 (1%)	Follow-up period mean± SD: 9.1y ±2.9y Incidence rate of ischemic stroke in NS group: 9.93 per 1000 PYs HR of stroke overall: Crude HR (95%-CI): 1.39 (1.23-1.56) Adjusted HR (95%-CI): 1.37 (1.21-1.54) HR of ischemic stroke: Crude HR (95%-CI): 1.40 (1.23-1.59) Adjusted HR (95%-CI): 1.38 (1.21-1.57) HR of hemorrhagic stroke: Crude HR (95%-CI): 1.33 (0.89-1.98) Adjusted HR (95%-CI): 1.26 (0.84-1.88)	-Restricted to patients without prior stroke only in NS group and not in controls -No information on exclusion due to missing demographic data. -No information on washout period when identifying first-time NS -No information on time of end of follow-up -Absolute risk/rate of stroke only reported for selected (statistically significant increased) outcomes

Kelddahl,[52] BMC Nephrology, 2019	Cohort study, Denmark, 2006-2012, Review of medical records	Inclusion: NS (nephrotic proteinuria and hypoalbuminemia) and biopsy-verified glomerular disease, >16 years, without prior diabetes, anticoagulant use, or RRT. Outcome: VTE or bleeding described in medical records	Number of thrombo-embolic (PE, RVT or DVT, stroke) or bleeding (minor or major) events (n)	n=79 Exposed: n= 44 Non-exposed: n=35	Exposed: Median age [IQR]: 43y [17-78] Men: n (%): 26 (59%) Non-exposed: Median age [IQR]: 52y [22-84] Men: n (%): 13 (37) Pathology, overall: MCD: n=35 (44%) MN: n=19 (24%) FSGS: n=7 (9%) Other: n=18 (23%)	Median follow-up time [IQR]: Exposed: 92w [34-178] Non-exposed: 49w [19-98] Overall VTE events: n=4 (5%) Overall bleeding events: n=7 (9%) Major bleeding events: n=2 (2.5%)	- Small sample size - No information on loss to follow-up -Restricted to bioptized patients with primary NS - Not specified if restricted to first-time NS - Did not compute cumulative risk estimates accounting for censoring or competing risk of death
Shinkawa,[26] Nephrology Dialysis Transplantation, 2020	Observational cohort study, Japan, 2008-2017, Hospital-registry	First-time hospital-diagnosed NS, >18 years, no prior VTE Recorded VTE and imaging procedure in hospital-registry	Absolute risk (%) of VTE during hospitalization	n=7,473	Median age [IQR]: 69y [53-79] Sex: n=104 (47%) men Pathology MCD: n=1315 (18%) FSGS: n=181 (2%) MN: n=829 (11%) MPGN: n=73 (1%) DN: n=1064 (14%) Comorbidities Diabetes: n=222 (30%) Cancer: n=501 (7%) Medication Diuretics: n=2158 (29%) Anticoagulants: n=607 (8%)	VTE events overall (risk, %): n=221 (3.0%) Type of VTE events (risk, %) PE events (risk, %): n=14 (0.2%) RVT events overall (risk, %): n=11 (0.1%) DVT events overall (risk, %): n=198 (2.6%)	- Assessed risk of VTE only during initial hospitalization. - No reported information on loss to follow-up -No reported uncertainty for risk estimates (e.g. confidence intervals)

Abbreviations: AP, angina pectoris; ATE, arterial thromboembolism; CAD, coronary artery disease; CCI, Charlson Comorbidity Index; CHD, Coronary heart disease ; COPD, Chronic obstructive pulmonary disease; CT, Computed tomography; DN, Diabetic nephropathy; DNPR, Danish National Patient Registry; DVT, deep vein thrombosis; ECG, Electrocardiography; ESKD, end-stage kidney disease ; FSGS, focal segmental glomerulosclerosis; GDCN, The Glomerular Disease Collaborative Network; GN, glomerulonephritis; ICD, International Classification of Diseases; IQR, interquartile range; HIV, human immunodeficiency virus; HR, Hazard ratio; MCD, Minimal change disease; MI, myocardial infarction ; MPGN, Membranoproliferative glomerulonephritis; MN, membranous nephropathy; NOS, not otherwise specified; NS, nephrotic syndrome; OR, Odds ratio; PAD, peripheral artery disease; PE, pulmonary embolism; PYs, person-years; RRT, renal replacement therapy; RVT, Renal vein thrombosis; s-creatinine, serum creatinine; SD, standard deviation; SLE, Systemic Lupus Erythematosus; TIA, transient ischemic attack; UAP, unstable angina pectoris; VTE, venous thromboembolism

Identification of patients with nephrotic syndrome (Study I)

NS is a rare condition, and secondary data sources are useful for identifying NS patients to increase the sample size in studies of NS.[53] Previous observational studies of NS were based on cohorts identified in clinical care, hospital registries, or most commonly in pathology registries (Table 1).[21, 23, 25-27] However, pathology registries include only patients referred to kidney biopsy, and these may only comprise a subset of patients with NS.[54] Therefore, the available evidence of NS may only consider a selected subset of patients with NS.

Patient registries may be used to identify a less selected group of patients with NS than biopsy registries by including patients based on hospital discharge diagnoses.[55] However, the clinical presentation of NS may be confused with symptoms of heart failure, liver disease, or allergic reactions, and patients may visit other care providers before receiving help from specialized hospital doctors.[7] Moreover, alternative diagnoses for example describing the underlying glomerulonephritis (International Classification of Diseases, 10th revision [ICD-10]: N03, chronic glomerulonephritis) instead of the code specific to NS (ICD: N04) may be recorded. Thus, recording of NS in hospital registries can be influenced by coding practices, incomplete reporting, and selected referral to kidney biopsies. Another alternative data source is laboratory databases, in which patients with fundamental biochemical features of NS can be identified.[4] This approach may be less specific but more sensitive than using either pathology or hospital registries, and therefore the characteristics of patients in the resulting cohorts may differ.

Incidence of nephrotic syndrome (Study II)

Data on the incidence of NS in adults are scarce, and the only available evidence comes from studies based on biopsy registries reporting rates of biopsies indicated by NS. These studies have reported varying incidence of NS in adults from 0.58 to 4.2 per 100,000 person-years (Table 2).[27-36] The large variation in previous estimates of NS incidence may reflect the greatly varying biopsy rates between countries.[54] Moreover, not all adult patients with NS have a kidney biopsy, and consequently the reported incidence of NS may be underestimated.

In recent decades, there has been a demographic shift of age in western countries and this trend is expected to continue. The worldwide proportion of persons who are above 60 years of age was 9% in 1990

and 12% in 2013, and the proportion is expected to increase to 21% in 2050.[56] A French study from 1991 reported higher incidence of NS in elderly than in young adults.[30] Additionally, the prevalence of the most common risk factor for secondary NS, i.e. diabetes, has increased over the past decades.[57]

In summary, the current data on the incidence of NS regard a selected group of patients, and the influence of the increasingly aging population with increasing prevalence of risk factors on the incidence of NS over the past decades remains unclarified.

Mortality of nephrotic syndrome (Study II)

The mortality of NS may have changed over the past decades. In general, the mortality increases with age and higher comorbidity burden.[58] As described above, the global population has aged. The increase in aging combined with unhealthy lifestyle (western diet, lack of physical exercise, smoking, etc.) contributes to a worldwide increase in prevalence of non-communicable disease.[58-60] These changes may also have changed the distribution of age and comorbidity burden of patients with first-time NS, and in turn led to increased mortality of patients with NS.

On the other hand, diagnostic tools, diagnostic activity, and treatment of NS and its complications have improved during the last decades. First, guidelines have recommended screening of patients with diabetes and hypertension for kidney damage by spot urine tests. Moreover, advancement in immunopathology combined with new treatment regimens for subtypes of NS may have improved mortality in selected patient groups.[12, 61] Finally, prophylaxis and treatment of thromboembolic disease have improved considerably, potentially improving the mortality of NS, as thromboembolism may be a severe complication related to NS.[16, 62] These factors may have led to a decrease in the mortality of NS.

Similar to studies of incidence of NS, most studies of the mortality of NS have focused on NS patients with a kidney biopsy (Table 3).[25, 27, 37-42, 63] The few previous studies of mortality of NS that were not restricted to patients with specific subtypes of glomerulonephritis reported an overall mortality during five years after NS ranging from 6% to 21%.[25, 27, 43] All other previous studies focused on patients with NS with specific types of glomerular pathology.[37-42] The existing evidence of mortality of NS may regard only a subset of patients for several reasons: First, in some patient groups there may be a reluctance to perform kidney biopsy (e.g. those with diabetes or the elderly).[54, 64] Furthermore, biopsy may be contraindicated in some patients (e.g.

due to anticoagulant drug use). Finally, patients with secondary NS, e.g. those with diabetes, cancer, or autoimmune disorders, were excluded from most previous studies of the mortality of NS.[25, 38, 40, 42, 43] Therefore, the overall mortality of NS is largely unclarified.

Thromboembolism and bleeding in nephrotic syndrome (Study III)

Thrombotic complications in patients with NS were first described by Pierre Rayer in 1840 reporting two cases of renal vein thromboses in patients with NS.[1] Since then, both arterial thromboembolism (ATE) and VTE have been associated with NS,[44, 46] the latter considered the more common.[11]

The etiology behind thromboembolism in NS is complex, but several contributing factors have been identified including for example loss of antithrombotic proteins to the urine (e.g. antithrombine III, and proteins C and S), increase in procoagulant factors (e.g. fibrinogen, and factors V and VIII), impaired fibrinolysis, and thrombosytosis.[10, 16, 65, 66] In addition, underlying disease (e.g. cancer or diabetes) and immunosuppressive treatment could contribute to increased risk of thromboembolism in NS.[67-71] On the other hand, patients with NS may have increased risk of bleeding due to proteinuria, impaired kidney function, underlying disease (e.g. cancer), or use of medication (e.g. steroids or anticoagulants).[72-76]

While several studies described the absolute risk of VTE in patients with NS, few studies have compared their risk of thromboembolism and bleeding to that in other persons (Table 4). Of these, one focused on ATE only,[44] , one focused on VTE only,[46] one indirectly compared to risk in the general population of same age and sex.[23] Furthermore, previous studies of thromboembolism in NS were limited by small sample sizes, incomplete follow-up, competing risk of death, and they were restricted to patients without systemic diseases.[23, 26, 43, 44, 49, 50]

The risk of bleeding in patients with NS has received little attention. Case reports have described major severe bleeding events in patients with NS;[77-79] two studies have reported major bleeding events in approximately 3% of patients with NS;[43, 52] and one study compared patients with NS to the background population finding a slightly increased risk of hemorrhagic stroke (hazard ratio [HR] of 1.26) in NS patients.[51]

Danish population and patient registries available for research allowed us to examine both absolute and relative risks of thromboembolism and bleeding in patients with NS, with minimal loss to follow-up and

considering competing risk of death.[80] Using a matched cohort study design, we compared the risk to that in persons without NS from the background population and other patients in high risk of these complications (i.e. with chronic kidney disease).[73, 81]

Summary

The existing evidence of the incidence, mortality, and risk of thromboembolism and bleeding in patients with NS is scant, as most of the data regard only a subset of patients with kidney biopsies in context of NS. Also, NS is a rare condition which limited previous studies by small sample sizes, and in settings without population-based registries incomplete follow-up data may have led to loss to follow-up. These shortcomings can be minimized in studies based on population-based Danish registries. In Studies I-III, we examined cohorts of patients with hospital-recorded NS, patients with biochemical features of NS, and patients with biopsy in context of hospital-recorded NS.

Aims

Study I

- To explore if first-time hospital diagnosis of NS reflected new events of NS.
- To examine if patients with hospital-recorded NS are comparable to all patients with nephrotic proteinuria and hypoalbuminemia in laboratory databases regarding age, sex, comorbidity, medication use, and renal histopathology.

Study II

- To examine trends in incidence, histopathology, and mortality of NS over the past 40 years.
- To examine the age- and sex-specific incidence of NS and the age-specific distribution of histopathological findings and mortality of NS.

Study III

- To examine the absolute risk of ATE or VTE and bleeding in patients with NS, and in subgroups of patients.
- To examine if NS is associated with a long-term increased risk of these complications.

METHODS

Setting

Studies I-III were all based in the Danish population (~5.8 million population in 2021). Denmark is a northern European country with a long history of nationwide population-based registries.[82] The healthcare system in Denmark is universally tax-funded with equal access for residents to public hospitals. Public hospitals provide the majority of hospital services in Denmark, including all specialized care of patients with NS. High quality data from hospital contacts are available for research.[83] Denmark is currently divided in five administrative regions, and in some registries without nationwide coverage, (e.g. laboratory databases, regional data are available for research (Figure 2).[84]

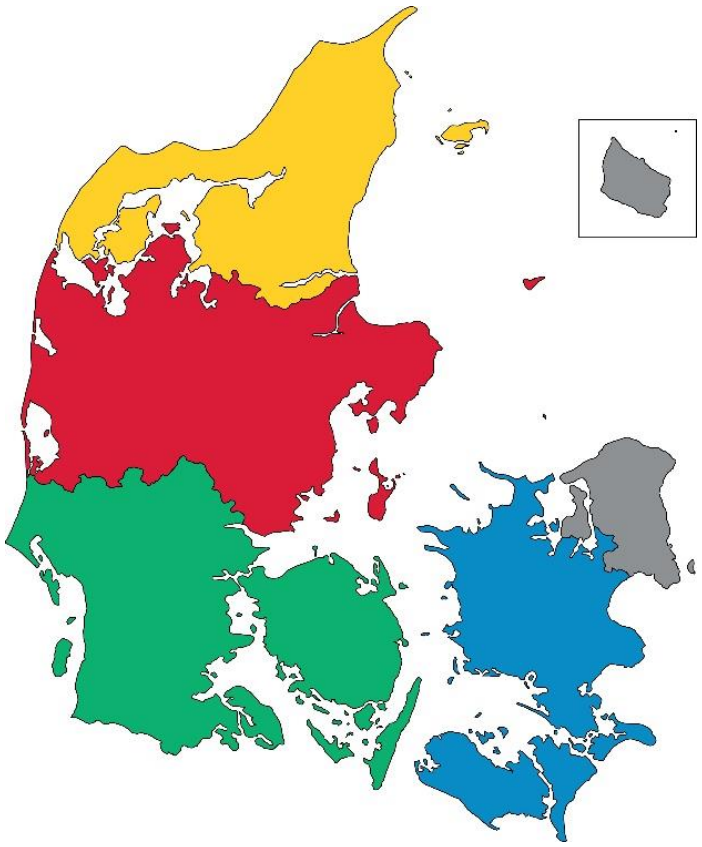

Figure 2. Map of Denmark and its administrative regions (Yellow, Northern Denmark; Red, Central Denmark; Green, Southern Denmark; Blue, Zealand; and Grey, Capital).

Data sources

For this PhD project, we applied for data to establish a comprehensive dataset including multiple data sources to study kidney diseases:

The Danish Civil Registration System (Studies I-III)

The Danish Civil Registration System holds date of birth, sex, vital status, and place of living, as well as personal identification number (i.e. a unique 10-digit number for every Danish citizen given at birth or to residents upon immigration) enabling identification of each individual across the nationwide healthcare registries.[83]

The Danish National Patient Registry (Studies I-III)

The Danish National Patient Registry holds information on all inpatient hospital contacts since 1977 and all outpatient or emergency room contacts since 1995. The data include details about dates of admission and discharge, procedures and operations, and one or more discharge diagnoses recorded using the ICD-8 before 1994 and the ICD-10 thereafter.[55]

The Clinical Laboratory Information System Research Database at Aarhus University (Studies I and III)

The Clinical Laboratory Information System Research Database at Aarhus University contains detailed information on all tests from general practice, outpatient clinics, emergency rooms, or in-hospital that are analyzed in hospital laboratories. It covers the Central and North Denmark Regions since 1992 and is considered geographically complete from 2005 (Figure 2).[84] In Figure 3, the number of proteinuria tests from the registry is plotted by calendar year. It appears that proteinuria tests are more or less completely recorded since year 2000~2002, which is similar to what has previously been described.[85]

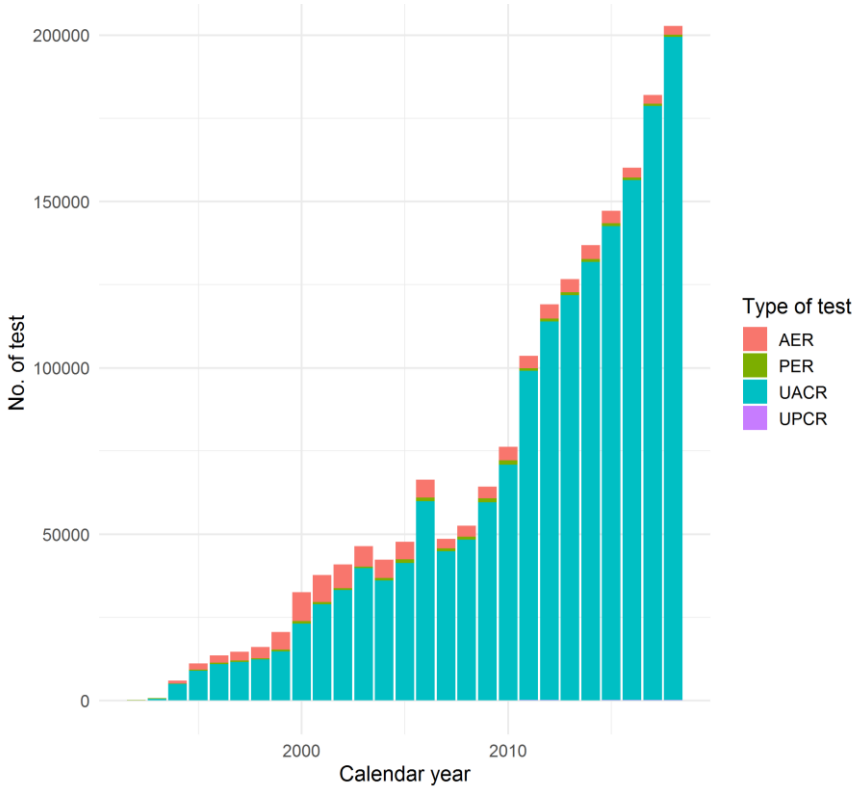

Figure 3. Number of proteinuria tests over time in the Central and North Denmark Regions from 1992 through 2018.

Abbreviations: AER, albumin excretion rate; PER, protein excretion rate; UACR, urine albumin-creatinine ratio; UPCR, urine protein-creatinine ratio

The Register of Laboratory Results for Research (Studies I and III)

The Register of Laboratory Results for Research contains detailed information on all tests from general practice, outpatient clinics, emergency rooms, or in-hospital that are analyzed in hospital laboratories. It covers the regions of Southern Denmark, North Denmark, Zealand, and the Capital since 2008 and is considered geographically complete from July 2015 (Figure 2).[84]

The Danish National Pathology Registry (Studies I-III)

The Danish National Pathology Registry holds pathological descriptions from all pathological examinations at every Danish hospital since 1999. Yet, the registry contains recordings of kidney biopsies as far back as 1972, and the majority of hospitals contributed with data since the early 1980s.[86]

The National Prescription Registry (Studies I and III)

The Danish National Prescription Registry includes nationwide detailed data on all prescriptions filled at Danish outpatient pharmacies since 1995. Drug types are classified according to the Anatomical Therapeutic Chemical classification system.[87]

The Danish Cancer Registry (Study II)

The Danish Cancer Registry was established in 1943 and it includes date of cancer diagnosis, cancer type, and stage. Cancer type is classified according to the ICD-10 from 1978 and onwards.[88]

The Danish Register of Causes of Death (Study II)

The Danish Register of Causes of Death holds information on causes of death as registered in death certificates since 1943, and since 1994 the causes are recorded using the ICD-10 system.[89]

Study designs

Studies I-III were all cohort studies based in the Danish population. In Study III, we used a matched cohort study design, matching patients with NS to persons without NS from the general population. In Study I, we used a cohort design instead of a cross-sectional design, as it allowed us to follow patients in either cohort up to 1 year after the index date. In Studies II and III, the cohort study design was preferred over a case-control design, as it enabled us to estimate both absolute and relative risks of the outcomes using time-to-event analyses.[90]

Study populations

In Study I, we established two potentially overlapping cohorts in the Central and Northern Denmark Regions during 2004-2018, and in the Southern Denmark, Zealand, and Capital Regions during 2016-2018 (Figure 2). The different time periods within different regions were chosen to ensure complete coverage of laboratory data in the entire study period with sufficient wash-out periods. Of note, the large increase in recorded urine albumin-creatinine ratio tests since 2009, as seen in Figure 3, does not reflect increasing completeness, but rather we expect it to reflect a guideline-driven increase in screening for kidney damage in patients with diabetes and hypertension. The first cohort included patients with first-time hospital-recorded NS, and the second included patients with first-time nephrotic proteinuria and hypoalbuminemia measured no more than one day apart. Patients with ongoing pregnancy were excluded from each cohort,

as nephrotic proteinuria and hypoalbuminemia could reflect preeclampsia in pregnant women instead of NS. The flow of inclusion for Study I is illustrated in Figure 4.

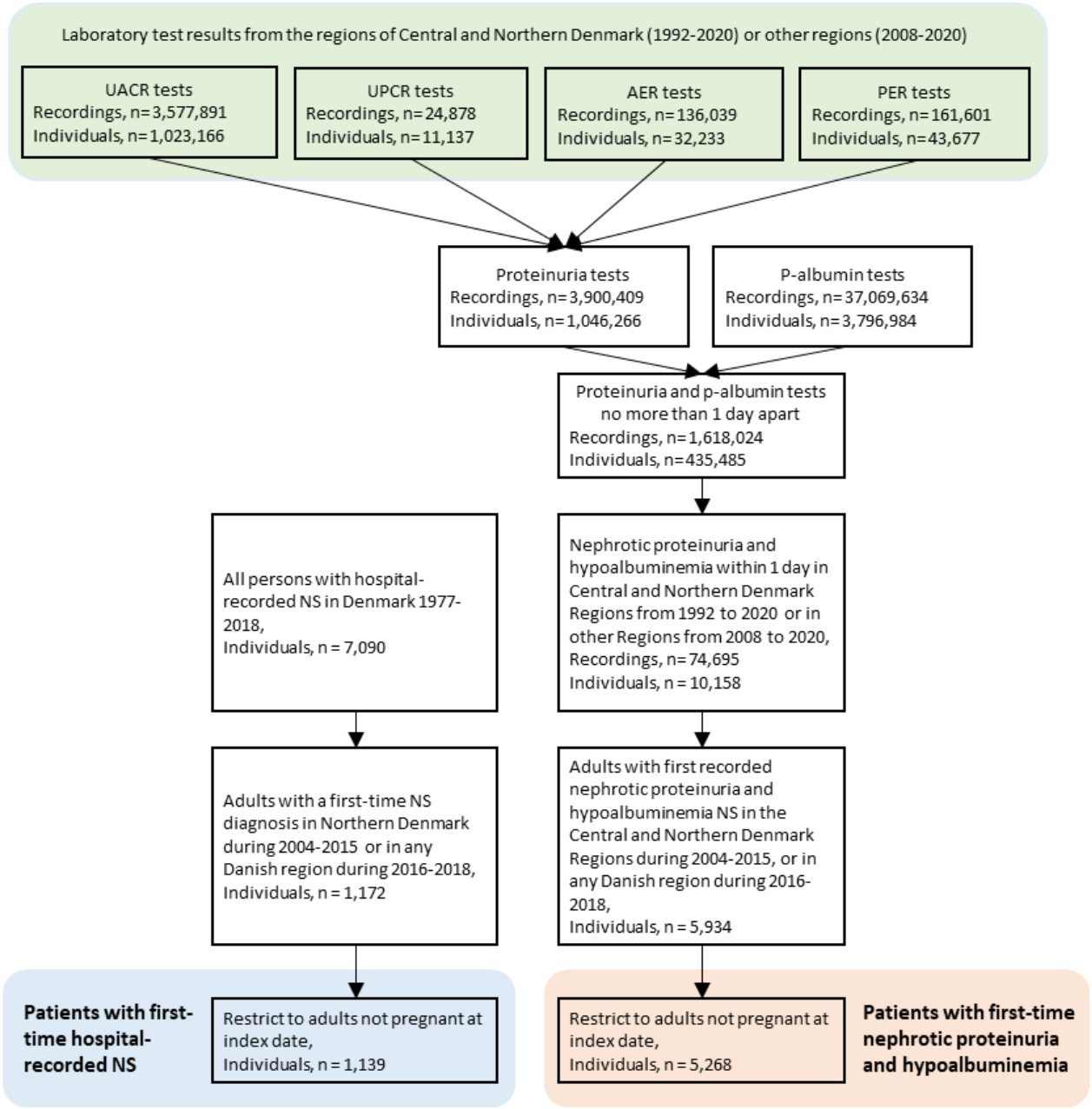

Figure 4. Flowchart of cohort sampling (Study I) from all the available laboratory tests in Denmark during 1992-2020 (in top) to the final cohorts (in bottom).

Abbreviations: UACR, urine albumin-creatinine ratio; UPCR, urine protein-creatinine ratio; AER, albumin excretion rate; PER, protein excretion rate; P-albumin, plasma albumin; NS, nephrotic syndrome

In Studies II-III, we identified patients with first-time hospital-recorded NS. In Study II, patients were included from 1979 through 2018 (Suppl. Figure 1, Appendix II), whereas in Study III, patients were included from 1995 through 2018 (Suppl. Figure 1, Appendix III). The later starting point in Study III was chosen to ensure that outcomes recorded in outpatient clinics or emergency rooms would be captured. In Study III, we stablished a comparison cohort including ten comparisons from the general population for each NS patient matched on age (in years) and sex. We sampled comparisons with replacement from the general population without prior NS and without kidney transplantation before the matching date.[91] For comparisons, the matching date was used as the index date.

Exposure

In Studies I-III, first-time hospital-recorded NS was identified based on hospital discharge diagnoses specific to NS (ICD-10: N04) recorded in the Danish National Patient Registry. We used diagnoses recorded as primary or secondary. Even though diagnoses were recorded at discharge, we used the first date of the hospital contact as index date, because outpatient courses can go on for years. Thus, using date of discharge could cause a considerable delay in between the actual debut of NS and the index date.

In Study I, in laboratory records, nephrotic proteinuria was defined as spot urine albumin-creatinine ratio >220 mg/mmol, or urine albumin excretion rate >2.2 g/day, or spot urine protein-creatinine ratio >350 mg/mmol, or urine protein excretion rate >3.5 g/day. Hypoalbuminemia was defined as plasma albumin (p-albumin) <36 g/L in persons <70 years and <34 g/L in persons ≥70 years. [4, 6, 92]

Outcomes

In Study I, we followed patients with nephrotic proteinuria and hypoalbuminemia for hospital-recorded kidney disease (i.e. specifically NS, any glomerular disease, and any nephropathy) from any time before to 1 year after the index date.[55] Patients with hospital-recorded NS were similarly followed for recorded laboratory tests indicating nephrotic proteinuria, and both nephrotic proteinuria and hypoalbuminemia.[84, 85] Finally, we obtained any histopathological findings in kidney biopsies +/- six months from index date,[86] and laboratory test results +/- 31 days from index date among patients in either cohort.[84, 85]

In Study II, information on death was obtained from the Danish Civil Registrations System.[80]

In Study III, thromboembolic events (myocardial infarction, ischemic stroke, other ATE, pulmonary embolism, deep vein thrombosis, other VTE) and bleeding events (cerebral bleeding, respiratory tract bleeding, gastrointestinal bleeding, urinary tract bleeding) were identified by discharge diagnoses (primary or secondary from inpatients, outpatients, or emergency rooms visitors) in the Danish National Patient Registry.[55]

Covariates

For each study participant we obtained data on vital status, sex and age,[80] on kidney transplant diagnoses or procedures,[55] on hospital-recorded kidney disease, comorbidity, fractures, surgery, or pregnancy,[55, 88] on histopathological findings in kidney biopsies,[86] on laboratory test results,[84, 85] and on recent use of medication.[87] This information was obtained to characterize patients (Studies I-III), to standardize (Study II), to stratify by potential effect-modifiers (Studies II-III), and to adjust for potential confounding (Studies II-III). The most recent eGFR in each patient was computed from plasma creatinine tests not taken during inpatient stays or emergency room visits using the CKD-EPI formula (Studies I and III).[93]

Statistical analyses

Patient characteristics (Studies I-III)

In contingency tables, we characterized study participants with first-time nephrotic proteinuria and hypoalbuminemia (Study I), first-time NS (Studies I-III), and matched comparisons (Study III) at index date. To examine the underlying glomerular pathology and severity of disease, we tabulated the histopathological findings +/- 6 months from index date (Studies I-III), and the highest recorded proteinuria and lowest recorded p-albumin test results +/- 31 days from index date (Study I). We also plotted the proportion of NS patients with kidney biopsies and their histopathological findings by age group and calendar period.

Timing and completeness of NS diagnoses (Study I)

To examine if NS diagnoses were recorded at time of fulfilled biochemical criteria of NS, we plotted the cumulative incidence proportions (risk) of having nephrotic proteinuria, and both nephrotic proteinuria and hypoalbuminemia around index date of NS. To examine how many patients with nephrotic proteinuria and hypoalbuminemia received a diagnosis reflecting NS, or glomerular disease, or any nephropathy, we plotted the risk of these hospital-diagnoses around the index date.

Incidence of NS (Study II)

We estimated crude and standardized incidence rates dividing number of first-time hospital-recorded NS events by 100,000 person-years (1 year per persons alive in the background population on 1 January each year) with 95% confidence intervals (CIs) directly standardized by age in years and sex in year 2000.[94]

Mortality of NS (Study II)

Patients with NS were followed from index date until death, emigration, or 1 January 2020, whichever came first. We estimated 1- and 5-year mortality (1-Kaplan Meier estimate) with 95% CIs by calendar-period and age group. To account for changes in age and sex in the NS cohort over time, we computed directly standardized mortality rates of death per 100 person-years by calendar period (standardized by age group and sex). For patients with NS and kidney biopsies, we computed mortality 1 and 5 years from the index date or date of biopsy (whichever came latest) by type of histopathological findings. In this analysis, follow-up was started only when both NS and a biopsy had been recorded to avoid immortal time bias. Among patient with NS who died from 1994 and onwards, we computed the proportion who died of diseases grouped by ICD-10 chapters.

Risk of thromboembolic events and bleeding in NS (Study III)

Patients with NS and their comparisons were followed from the index date to the first date of thromboembolic or bleeding events, death, emigration, 31 December 2018, or 10 years of follow-up, whichever came first. Patients with a thromboembolic event were censored for subsequent other types of thromboembolism, but continuously followed for bleeding events, and vice versa. We estimated risk of outcome events during 0-1-year and 0-10-year follow-ups, considering death a competing risk, and presented the risk in tables (overall and by subtype) and risk curves (overall). In Study II, we found a considerable risk of death after NS and it was crucial to consider the competing risk of death to avoid overestimating the risks.[95, 96] To compare the risk of the outcomes in patients with NS to that in the general population, we estimated crude and adjusted HRs with 95% CIs using the Cox proportional hazard regression analysis. We confirmed proportionality by inspecting log-log plots and plotted Schoenfeld residuals. In patients with kidney biopsies, we estimated the risk of thromboembolism and bleeding, starting follow-up from the end of the biopsy assessment period (day 180). Even though the type of histopathology is unlikely to change during the biopsy assessment window, we delayed the start of follow-

up in this analysis to avoid conditioning on the future (i.e. by classifying patients according to findings in future biopsies).

All data management, analyses, and visualizations were made in R version 4.0.4 (www.r-project.org),[97-102] except in Study III, where data management and analyses were performed in SAS version 9.4 (Cary, NC, USA).

RESULTS

Table 5. Characteristics of 1,139 patients identified with first-time hospital-recorded nephrotic syndrome in the Danish National Patient Registry, and of 5,268 patients with first-time recorded nephrotic proteinuria and hypoalbuminemia identified in Danish laboratory databases during 2004-2018.

	Patients with hospital-recorded nephrotic syndrome	Patients with nephrotic proteinuria and hypoalbuminemia
Overall, n (%)	1,139 (100)	5,268 (100)
Male sex, n (%)	682 (60)	3,355 (64)
Age in years, median [IQR]	60 [45, 73]	63 [50, 72]
Hospital-recorded kidney disease (prior 10 years), n (%)		
Glomerulonephritis (excl. NS)	109 (10)	538 (10)
Renal tubulointerstitial diseases	41 (4)	391 (7)
Acute kidney injury and or chronic kidney disease	166 (15)	1,930 (37)
Cystic kidney disease	<5	139 (3)
Hypertension with nephropathy	14 (1)	155 (3)
Diabetic nephropathy	55 (5)	750 (14)
Hospital-recorded comorbidity (prior 10 years), n (%)		
Diabetes	206 (18)	2,032 (39)
Chronic liver disease	29 (3)	163 (3)
Chronic pulmonary disease	114 (10)	580 (11)
Connective tissue disease	67 (6)	402 (8)
Congestive heart failure	72 (6)	458 (9)
Thromboembolic disease	192 (17)	1,328 (25)
Non-hematological cancer (excl. non-melanoma skin cancer)	89 (8)	485 (9)
Hematological cancer	42 (4)	149 (3)
Filled prescriptions (prior 365 days), n (%)		
Antidiabetics, n (%)	203 (18)	2,024 (38)
Anticoagulants, n (%)	370 (32)	2,289 (43)
Thiazides/diuretics, n (%)	364 (32)	1,483 (28)
Beta blockers, n (%)	321 (28)	2,121 (40)
Calcium channel blockers, n (%)	346 (30)	2,527 (48)
ACE inhibitors, n (%)	369 (32)	1,957 (37)
Angiotensin-II receptor antagonists, n (%)	261 (23)	1,736 (33)
Other antihypertensives, n (%)	29 (3)	462 (9)
Statins, n (%)	412 (36)	2,486 (47)
Glucocorticoids	146 (13)	593 (11)
Immunosuppressants	22 (2)	105 (2)
Kidney transplant recipient prior to index date, n (%)	16 (1)	337 (6)
Kidney biopsy recorded +/-6 months from index date, n (%)	696 (61)	1,771 (34)
Any eGFR test before index date, n (%)	1,087 (95)	5,208 (99)
Days since most recent eGFR test, median [IQR]	-7 [-20, -2]	-15 [-54, -3]
Most recent eGFR ml/min/1.73 m^2, median [IQR] [a]	61 [34, 87]	35 [17, 65]

Abbreviations: NS, nephrotic syndrome; IQR, inter quartile range; ACE inhibitors, angiotensin-converting-enzyme inhibitors; eGFR, estimated glomerular filtration rate

[a]eGFR computed from plasma creatinine tests using the CKD-EPI formula

Patient characteristics, and timing and completeness of NS diagnoses (Study I)

In two potentially overlapping cohorts during 2004-2018, we identified 1,139 patients with first-time hospital-recorded NS and 5,268 patients with nephrotic proteinuria and hypoalbuminemia (Table 5), and 760 patients were included in both cohorts (Suppl. Figure 3, Appendix I). Patients identified with nephrotic proteinuria and hypoalbuminemia more often had prior acute kidney injury or chronic kidney disease (37% vs. 15%), diabetes (39% vs. 18%), and thromboembolic disease (25% vs. 17%). Additionally, they had lower eGFR than those with hospital-recorded NS (median [interquartile range] of recent eGFR of 35 ml/min [17-65] vs. 61 ml/min [34-87]). Eighty-seven % of patients had received diagnoses reflecting nephropathy 1 year after presentation with nephrotic proteinuria and hypoalbuminemia, whereas only 18% had received a diagnosis code specific to NS (Figure 5). Initiation of hospital contact with NS correctly reflected the first event of nephrotic proteinuria in the majority of patients (Figure 6). A larger part of patients identified with hospital-recorded NS had kidney biopsies than those identified with nephrotic proteinuria and hypoalbuminemia (Table 1). Minimal change disease, membranous nephropathy, and mesangioproliferative glomerulopathy were common specific findings in both cohorts (Table 3, Appendix I).

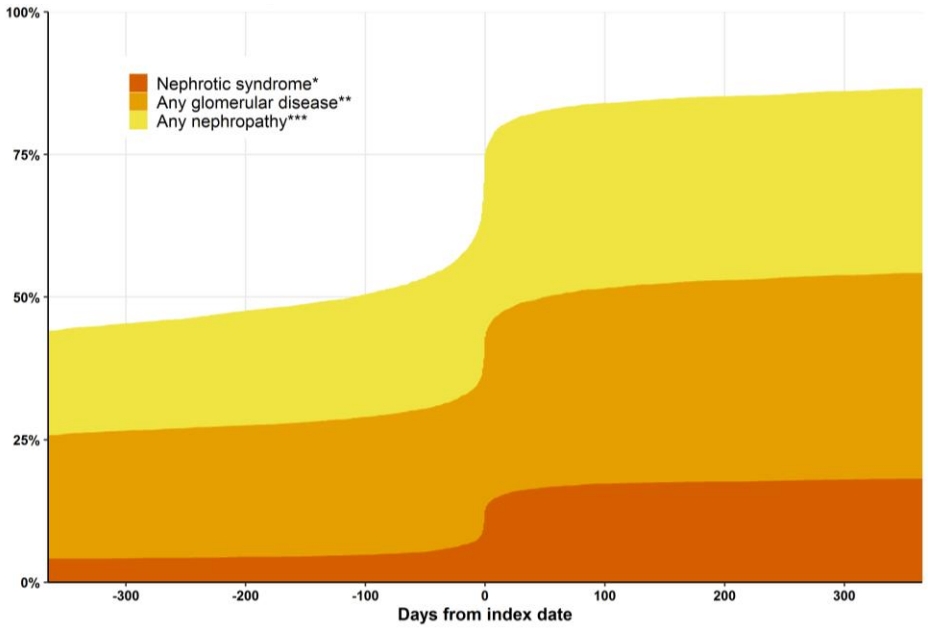

Figure 5. Cumulative proportions of patients with kidney diseases recorded in the Danish National Patient Registry from 1 year before to 1 year after the index date (day 0) among 5,268 adults with first-time recorded nephrotic proteinuria and hypoalbuminemia during 2004-2018.

* Nephrotic syndrome

** Glomerulonephritis (incl. nephrotic syndrome) or diabetic nephropathy

*** Glomerulonephritis (incl. nephrotic syndrome), diabetic nephropathy, systemic lupus erythematosus, Sicca syndrome [Sjögren], glomerular diseases, renal tubulointerstitial diseases, acute kidney failure and chronic kidney disease, disorder of kidney and ureter (unspecified), amyloidosis, and hypertension with nephropathy

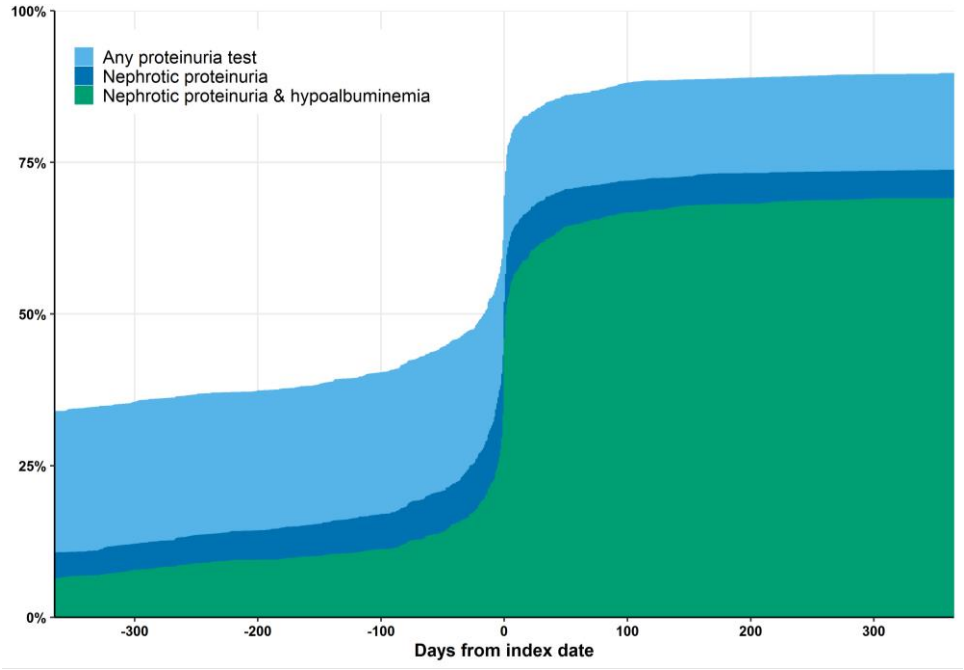

Figure 6. Cumulative proportions of patients with any recorded proteinuria test, nephrotic proteinuria, or nephrotic proteinuria and hypoalbuminemia from 1 year before to 1 year after the index date (day 0) among 1,139 patients with first-time hospital-recorded nephrotic syndrome in areas with complete laboratory coverage.

Comorbidity and glomerular pathology in NS over time and across age groups (Study II)

From 1979 through 2018, we identified 5,446 patients with first-time hospital-recorded NS (Table 1, Appendix II). We observed an increasing prevalence of prior kidney disease, comorbidities, and kidney biopsies in patients with NS over time (Table 1, Appendix II). However, from Figure 7A, it appears that the proportion of patients with NS who had kidney biopsies were quite stable from 1985 and onwards. The most prevalent histopathological findings in patients with NS overall were membranous nephropathy (20%) and minimal changes disease (19%) (Figure 1, Appendix II). The distribution of different histopathological findings among those with a biopsy was rather stable over time, though with small increases in the proportions with focal segmental glomerular sclerosis and diabetic nephropathy (Figure 1, Appendix II). Minimal change disease and "other proliferative glomerulonephritis" were more common among younger adults, whereas membranous nephropathy, focal segmental glomerulosclerosis, and "other glomerulonephritis" were more common in older adults (Suppl. Figure 3, Appendix II). Of note, the biopsy rate was considerably lower in patients above 80 years compared to other adults (Figure 7B)

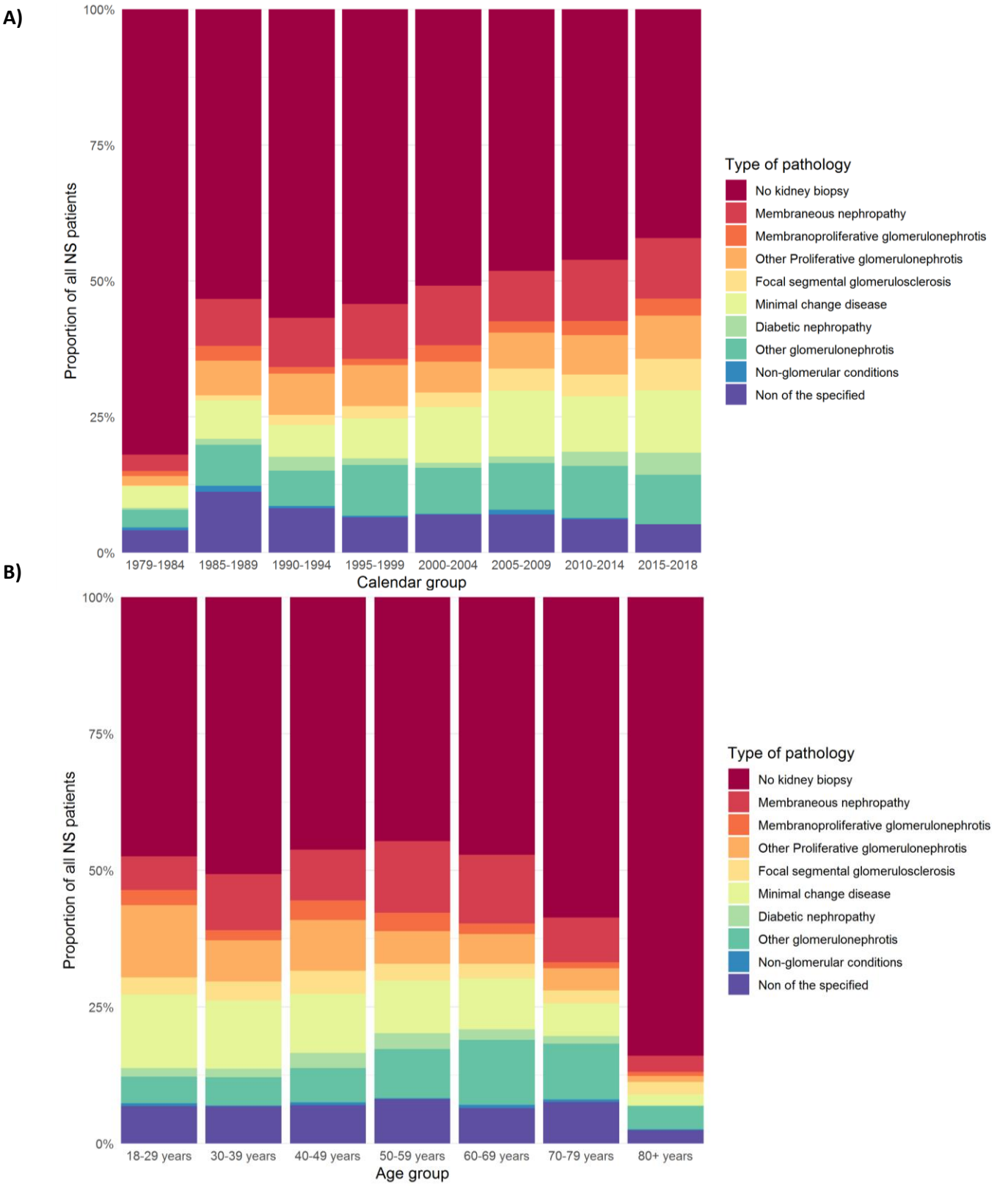

Figure 7. Proportion of patients with hospital-recorded nephrotic syndrome (NS) with a kidney biopsy +/- 180 days from index date and their specific histopathological findings across calendar periods (A) and age groups (B).

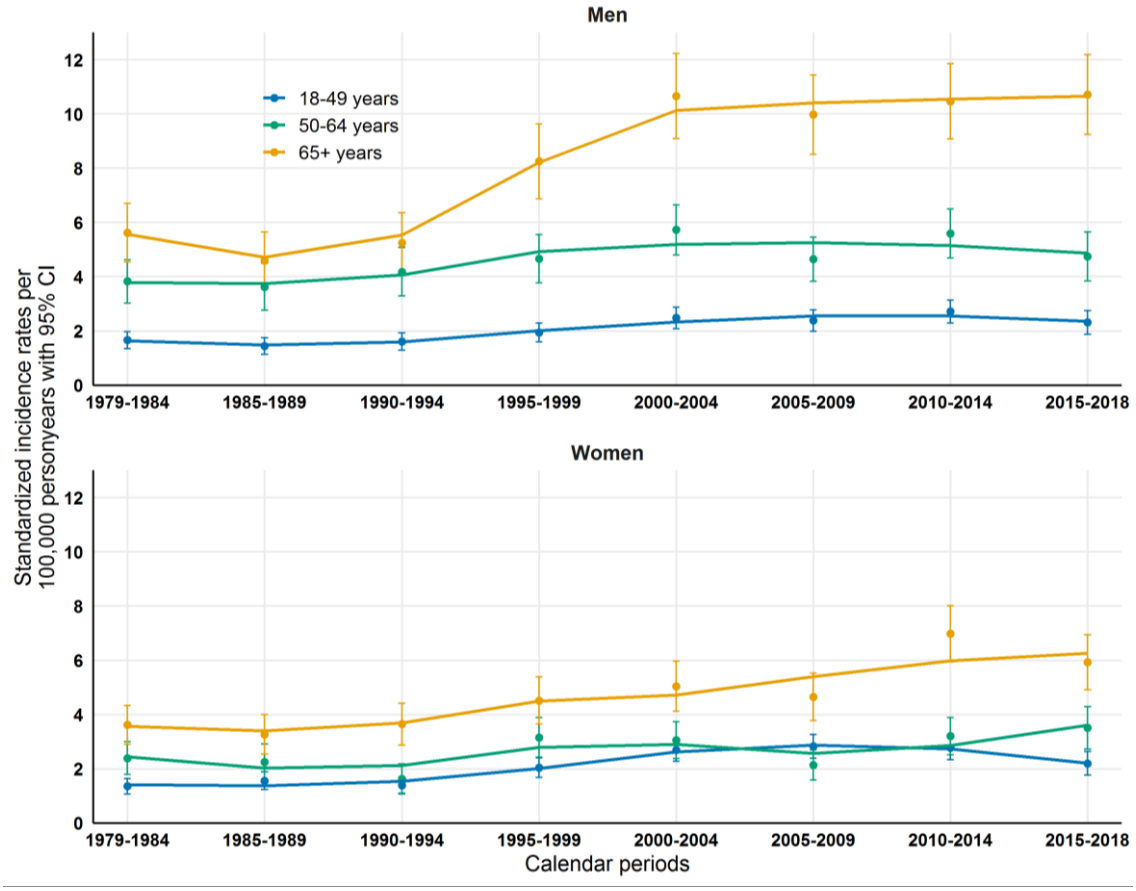

Figure 8. Standardized incidence rates of hospital-recorded nephrotic syndrome in adult men and women by calendar period from 1979 through 2018 stratified by age group (fitted curve smoothed with loess function).

Incidence of NS (Study II)

The incidence of hospital-recorded NS increased from 2.33 per 100,000 person-years (95% CI: 2.18-2.47) in 1979-1989 to 4.22 per 100,000 person-years (95% CI: 4.02-4.42) in 2010-2018 (Table 2, Appendix II). Older age groups experienced the largest increase in NS incidence (Figure 8). Over the study period, the standardized incidence increased gradually in women aged 65+ years, whereas the incidence of NS in men aged 65+ years increased especially from 1990 to 2004 (Figure 8). In general, the incidence of NS was similar in men compared to women below 50 years of age, whereas it was twice as high in men compared to women above 50 years (Figure 9).

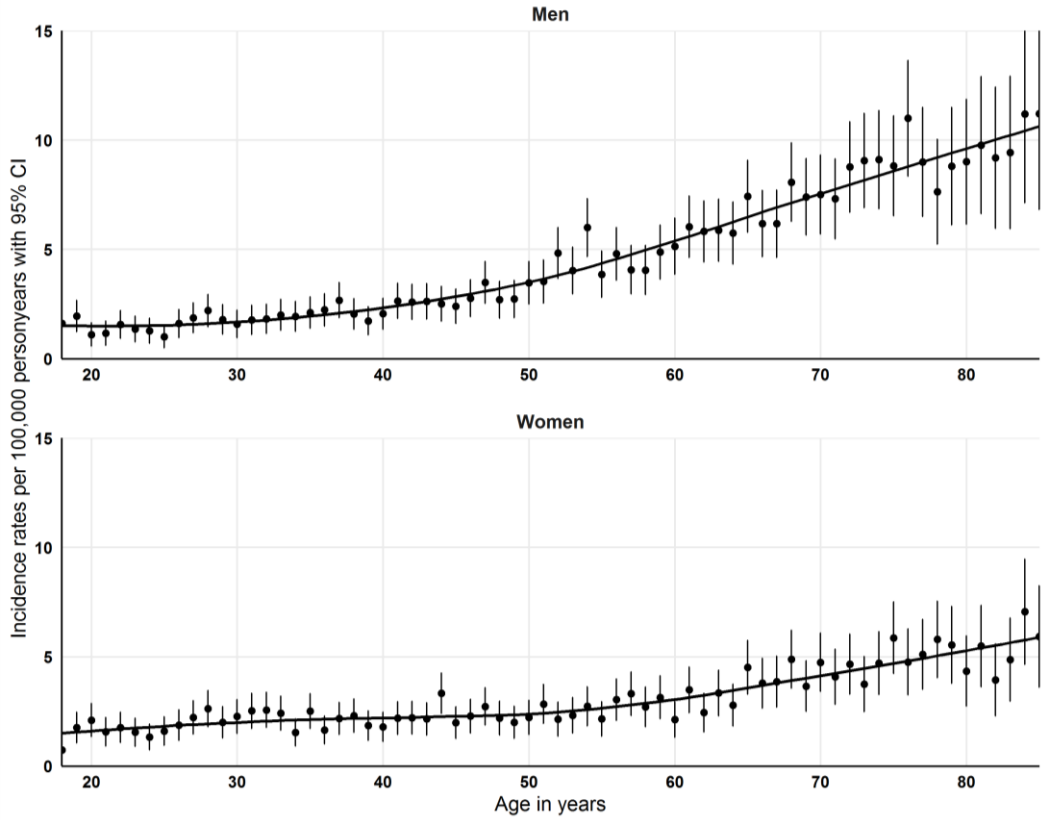

Figure 9. Incidence rates of hospital-recorded nephrotic syndrome in men and women over ages from 18 to 85 years in Denmark during 1979-2018 (fitted curve smoothed with loess function).

Mortality of NS over time and across age groups (Study II)

Over the past four decades, the mortality of NS decreased markedly with the overall 1-year mortality going from 24% (95% CI: 22-27%) in 1979-1989, to 12% (95% CI: 11-14%) in 2010-2018 (Table 3, Appendix II). The mortality differed substantially across age groups, with the 1-year mortality being lowest in patients aged 18-29 years (1% [95% CI: 0-2]) and highest in patients aged 80+ years (43% [95% CI: 38-47]) (Table 3, Appendix II). When accounting for differences in age and sex of patients with NS over time, the standardized 1-year mortality rate in 2010-2018 was one third of that in 1979-1989 (Figure 10). To furthermore account for changing comorbidity load in patients with NS over the study period, we computed HRs of death adjusted for age, sex, and comorbidities prior to NS and found an HR of death of 0.25 (95% CI: 0.21-0.31) in patients with NS in 2010-2018 compared to those in 1979-1989 (Table 3, Appendix II). The magnitude of the HRs of death attenuated with extended follow-up periods (Table 3, Appendix). Finally, the most commonly recorded cause of death in patients with NS was cardiovascular diseases, accounting for 24% of deaths (Suppl. Table 6, Appendix II). Among patients with NS and a kidney

biopsy, the 1-year mortality was highest in those with “other glomerulonephritis” (22%) and diabetic nephropathy (14%), and lowest in those with “other proliferative glomerulonephritis” (5%), focal segmental glomerulosclerosis (5%), minimal change disease (5%), and membranous nephropathy (6%) (Suppl. Table 5, Appendix I).

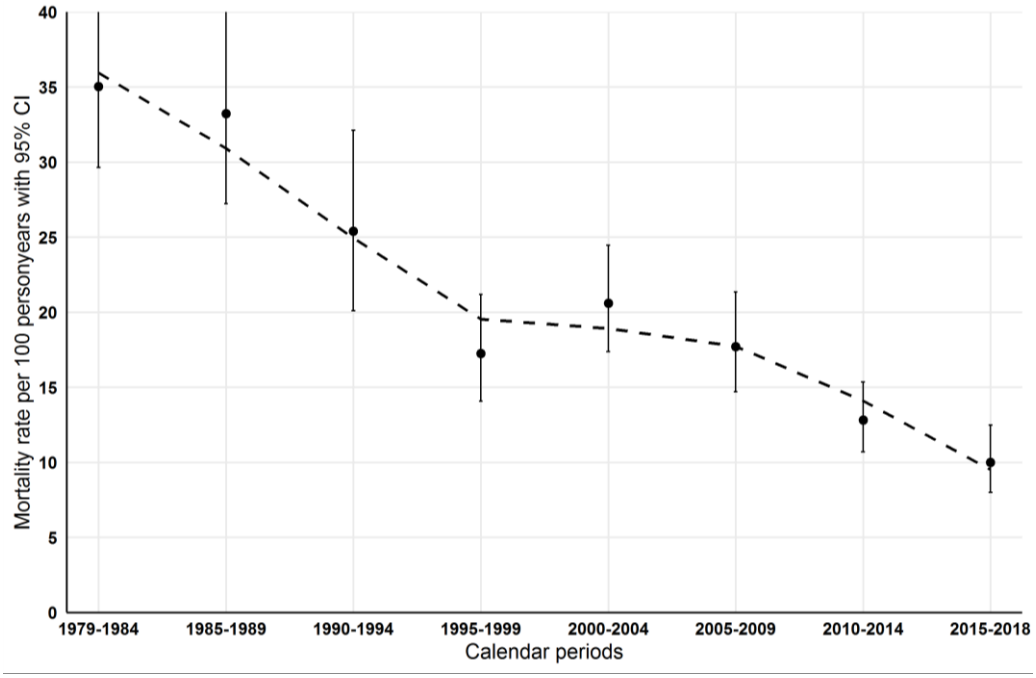

Figure 10. Standardized 1-year mortality rate per 100 person-years among 5,446 patients with first-time hospital-recorded nephrotic syndrome in Denmark from 1979 through 2018 (fitted curve smoothed with loess function). Direct standardization with internal weights by sex and 10-year age group.

Risk of thromboembolic events and bleeding in NS (Study III)

We included 3,967 patients with first-time NS during 1995-2018, and matched these to 39,670 persons from the general population (Suppl. Figure 1, Appendix III). NS patient and general population comparisons were well balanced on age and sex, but the NS cohort had higher prevalence of prior kidney disease, comorbidity, and use of medication. The risk of thromboembolic events in patients with NS was 6.8% (95% CI: 6.0-7.6) during 1 year of follow-up and 20.0% (95% CI: 18.6-21.4) during 10 years of follow-up when considering death a competing risk (Table 6). The first thromboembolic event was more commonly an ischemic stroke or myocardial infarction than any type of VTE (Table 6).

Table 6. One- and 10-year risk of thromboembolic and bleeding events (%) overall and by type among patients with nephrotic syndrome.

	0-1 year		0-10 years	
	No. events	Risk, % (95% Cl)	No. events	Risk, % (95% Cl)
Any thromboembolism	266	6.8 (6.0 - 7.6)	676	20.0 (18.6 - 21.4)
Type of thromboembolism				
Myocardial infarction	58	1.5 (1.1 - 1.9)	175	5.2 (4.5 - 6.0)
Ischemic stroke	90	2.3 (1.9 - 2.8)	244	7.3 (6.4 - 8.2)
Other arterial thromboembolism	14	0.4 (0.2 - 0.6)	29	0.9 (0.6 - 1.2)
Pulmonary embolism	52	1.3 (1.0 - 1.7)	97	2.8 (2.2 - 3.3)
Deep vein thrombosis	34	0.9 (0.6 - 1.2)	80	2.3 (1.9 - 2.9)
Other venous thromboembolism	18	0.5 (0.3 - 0.7)	51	1.5 (1.1 - 2.0)
Any bleeding	203	5.2 (4.5 - 5.9)	570	17.0 (15.7 - 18.3)
Type of bleeding				
Cerebral bleeding	19	0.5 (0.3 - 0.7)	54	1.6 (1.2 - 2.1)
Respiratory tract bleeding	40	1.0 (0.7 - 1.4)	114	3.5 (2.9 - 4.1)
Gastrointestinal bleeding	80	2.0 (1.6 - 2.5)	243	7.4 (6.5 - 8.3)
Urinary tract bleeding	64	1.6 (1.3 - 2.1)	159	4.6 (4.0 - 5.4)

Abbreviations: CI, confidence interval;

We observed a considerably higher risk of thromboembolism and bleeding in patients with NS than in the matched general population during up to 10 years of follow-up (Figure 11). When adjusted for potential confounders, the 1-year hazard rate of thromboembolism was 4-fold higher in patients with NS than general population comparisons (HR_{adj} of 3.99 [95% CI: 3.44-4.62]) (Table 7).

The 1-year risk of hospital requiring bleeding events was 5.2% (95% CI: 4.5-5.9), and the 10-year risk was 17.0% (95% CI: 15.7-18.3) in patients with NS (Table 6). The first bleeding event was most commonly recorded as being gastrointestinal. The adjusted 1-year hazard rate of bleeding was 4-fold higher in patients with NS than general population comparisons (HR_{adj} of 4.02 [95% CI: 3.40-4.75]) (Table 7). The magnitude of the HRs of either outcome in patients with NS was greatest during short-term follow-up and attenuated with increased follow-up time (Table 7).

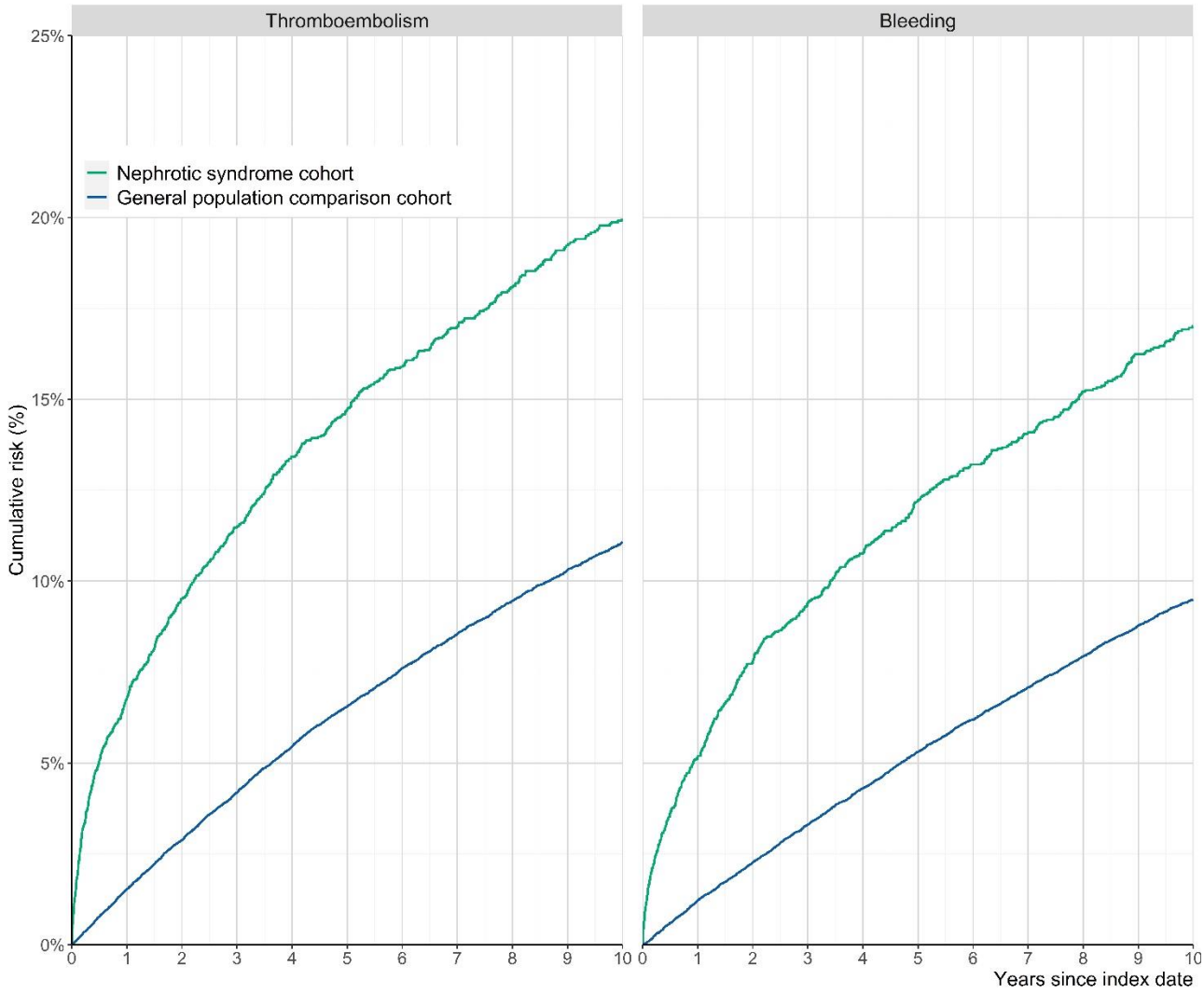

Figure 11. Cumulative risk of any thromboembolism (A) and any bleeding (B) diagnosed in hospital in patients with nephrotic syndrome and persons from the general population (matched on age and sex) during 10 years of follow-up.

In patients with a kidney biopsy, the risk of thromboembolism and bleeding was lowest in patient with minimal change disease and highest in patients with membranoproliferative glomerulonephritis or "other histopathology" (Figure 2, Appendix III). The patients in highest risk when stratified by type of histopathology included older patients with higher prevalence of diabetes, cancer, recent surgery, anticoagulant use, and lower baseline eGFR (Suppl. Table 1, Appendix III).

Table 7. Hazard ratios of thromboembolic and bleeding events during up to 10 years after nephrotic syndrome compared with the general population.

	Hazard ratio (95% confidence interval)	
	Nephrotic syndrome vs. general population cohort	
	Crude*	Adjusted**
Thromboembolic events		
0-30 days	15.09 (10.38–21.94)	11.81 (8.09–17.26)
0-1 year	4.90 (4.24–5.66)	3.99 (3.44–4.62)
0-5 years	2.88 (2.63–3.17)	2.58 (2.35–2.84)
0-10 years	2.54 (2.34–2.76)	2.41 (2.21–2.62)
Bleeding events		
0-30 days	18.02 (11.52–28.20)	14.24 (9.05–22.41)
0-1 year	4.67 (3.96–5.50)	4.02 (3.40–4.75)
0-5 years	2.93 (2.64–3.24)	2.70 (2.43–3.00)
0-10 years	2.51 (2.30–2.75)	2.43 (2.21–2.66)

*Cohorts matched on age and sex, not adjusted.

**Adjusted for age, sex, calendar year, diabetes, prior thromboembolic events, prior bleeding events, cancer, amyloidosis, systemic lupus erythematosus, history of recent trauma/fracture, recent surgery, and use of medication at index date (incl. any anticoagulant drugs, statins, beta blockers, calcium channel blockers, thiazides/loop diuretics, ACE inhibitors, angiotensin-II receptor antagonists, Non-steroidal anti-inflammatory drugs, other antihypertensives, proton pump inhibitors, antidiabetics, glucocorticoids, and antidepressants).

DISCUSSION

Main findings

In Study I, our findings suggest that recording of NS in hospital records is incomplete, as only 18% of patients with nephrotic proteinuria and hypoalbuminemia had a recorded hospital diagnosis code specific of NS 1 year after presentation, whereas 87% had diagnoses reflecting nephropathy. Patients with nephrotic proteinuria and hypoalbuminemia had higher comorbidity burden (e.g. diabetes, thromboembolic disease, and low eGFR), and they less often had kidney biopsies compared to those with hospital-recorded NS. While the hospital diagnoses codes may be incomplete, we found that they correctly identified patients at the time of debut with nephrotic proteinuria and hypoalbuminemia.

In Study II, we found that the incidence of hospital-recorded NS increased during 1979-2018 in Denmark, especially due to increased incidence in men above 65 years. Overall, the incidence of NS increased considerably with age. We found that around half of patients with hospital-recorded NS had a kidney biopsy, and very few of patients above 80 years had a biopsy. The mortality of NS decreased considerably during the study period, especially in elderly patients, while younger age groups had low mortality over the entire study period. The most common cause of death in patients with NS was cardiovascular diseases.

These potentially severe complications of NS were examined in Study III, revealing that patients with NS had a 1-year risk of thromboembolism of 6.8% and of bleeding of 5.2%. The 10-year risk of thromboembolism was 20% and of bleeding was 17%. Interestingly, the risk was highest of ischemic stroke, myocardial infarction, and gastrointestinal bleeding with 10-year risks of 7.3%, 5.2%, and 7.4%, respectively. After adjusting for confounding, the rates of thromboembolism and bleeding were 4-fold higher in patients with NS than in general population comparisons during the first year of follow-up, with attenuating associations with longer follow-up time. Of note, among NS patients with kidney biopsies the risks of thromboembolism and bleeding were highest in those with “membranoproliferative glomerulonephritis” and “other histopathology” and lowest in those with minimal change disease.

Our findings in the context of the existing literature

Identification of patients with NS

No previous study has examined the completeness of the diagnosis code specific of NS or the characteristics of patients with nephrotic proteinuria and hypoalbuminemia. Therefore, I compare the characteristics of patients in our cohorts to those previously reported for patients with recorded NS.

In Study I, our two cohorts included more men than women with great variation in age, which is comparable to previous NS cohorts.[21-25, 27, 103] Similar to other NS cohorts, use of antihypertensives, diuretics, and statins was common in our cohorts, [22, 23, 25, 103] but thromboembolic disease prior to and use of anticoagulants at index date were more common compared to that in Japanese and Dutch NS patients.[23, 24, 103] These differences may well be due to differences in exclusion criteria and settings. In our study, 61% of patients with hospital-diagnosed NS had kidney biopsies, somewhat higher than reported in Japanese patients (biopsy in 46%), yet that study only included biopsies during initial hospitalization.[26] In comparison, only 34% of patients with nephrotic proteinuria and hypoalbuminemia in our study had kidney biopsies, but the distribution of histopathological findings in this cohort did not differ substantially from that in hospital-recorded NS. The age and sex distribution, proteinuria levels, and use of ACE inhibitors in our patients with nephrotic proteinuria and hypoalbuminemia were remarkably similar to those in American patients with diabetic kidney disease and NS.[104] And even though the prevalence of diabetes and antidiabetic drug use were high in both our cohorts, diabetic nephropathy was rarely recorded in kidney biopsies. This suggests that patients with diabetic nephropathy rarely receive the diagnosis code specific to NS, and that kidney biopsy frequency is especially low in patients with diabetes.[27, 54] Finally, the cohort of patients with hospital-recorded NS included very few patients with prior kidney transplantations.

Incidence of NS

Previous studies reporting incidence of NS in adults were based on patients with kidney biopsies indicated by NS, and they reported varying annual incidences from 0.6 to 4.2 per 100,000 persons-years,[27-36] likely reflecting varying biopsy rates across countries.[54] Many of these studies did not restrict to first-time NS patients,[28-30, 33-35] and inclusion of patients with prevalent NS could lead to overestimation of the incidence. Contrary, they may underestimate the incidence of NS significantly, as not all patients with NS have kidney

biopsies (shown in Study I). This can explain the much lower estimated incidence in some previous studies compared to our findings.[27, 28, 32, 33, 35, 36]

Similar to our findings of increasing incidence by age, a French study reported higher incidence of NS in persons aged 65+ years (7.1 per 100,000 person-years) than in those aged 15-64 years (4.2 per 100,000 person-years) and an Italian study reported incidences increasing from 0.31 per 100,000 person-years in those aged 15-24 years to 3.47 per 100,000 person-years in those aged 65-74 years.[30, 32] However, the French and Italian studies included only patients with kidney biopsies, and we showed that these are rarely performed in elderly patients and those with diabetes, thus they may underestimate the incidence in general but especially in elderly patients.

No recent study has examined changes in NS incidence over time, but a French study reported slightly increased incidences of NS from 1976-1980 (3.6 per 100,000 person-years) to 1986-1990 (4.0 per 100,000 person-years).[31] Finally, the increasing prevalence of focal segmental glomerulosclerosis has been described previously,[105] but we lacked data on this among patients with NS outside the USA.

Mortality of NS

Similarly to studies of incidence, previous studies on mortality were based on patients with NS and kidney biopsies.[25, 27, 37-42, 63] Only few of these studies have reported overall mortality among patients with NS without restricting to patients with specific glomerulopathies.[25, 27, 43] A study from Japan reported a 6% mortality in patients with primary NS during 5 years of follow-up, which is considerably lower than our estimates.[25] More similar to our findings, two Scottish studies reported a 5-year mortality of 19% in patients with primary NS and a 3-year mortality of 21% in patients with primary or secondary NS.[27, 43] Studies restricted to NS patients with specific glomerulopathies reported risk of death within 5 years of 6-25% in membranous nephropathy,[25, 38-40, 42, 63] of 4-12% in minimal change disease,[25, 37, 39, 63] and of 3-22% in focal segmental glomerulosclerosis.[25, 39, 63] Large variation in previously reported mortality and differences compared to our findings may reflect true differences between Asian, American, and European populations with NS, or differences in loss to follow-up, exclusion criteria, study periods and settings, or imprecision due to small cohort sizes.[25, 27, 37-42, 63] Importantly, we showed that biopsies are less frequently performed in NS patients with comorbidity and of high age. As previous studies were restricted to patients with biopsies,

inclusion of more elderly and patients with comorbidity (e.g. diabetes) may explain the higher mortality in our study.

Risk of thromboembolism and bleeding after NS

Most previous studies of thromboembolism in NS focused on VTEs, and these have been considered more common than ATEs in NS,[7] however previous studies have reported increased risk of both VTE, myocardial infarction, and ischemic stroke in NS patients.[44, 46, 51] In line with the combined 5-year risk of PE, VTE and other VTEs in our study, study from Scotland estimated a 3-year risk of VTE of 6.8% in 206 patients with primary or secondary NS and recorded in a renal biopsy registry.[27] A study of 7,037 American military veterans with NS, reported a considerably lower risk of VTE (10-year risk of 2.25%), which may be due to difference in settings, inclusion of prevalent NS patients, loss to follow-up, and that they did not consider competing risk of death.[50] Oppositely, a high risk was reported in Japan, were 3% of 7,473 patients had hospital-diagnosed VTE during initial hospitalization with NS. This short-term risk of VTE is higher than the 30-day risk in our study, maybe due to difference in ethnicity or difference in coding practices of NS and VTE events.[26] Also, during 10 years of follow-up of 289 patients with NS in the Netherlands, the average annual risk of VTE was 1.0% and of ATE was 1.5%.[23] These risks are higher than the combined risk of VTEs or ATE, respectively, in our study, maybe because we censored patients at the first thromboembolic event of any kind (arterial or venous). Similar to the previous Dutch study, we found higher risk of ATE (especially myocardial infarction and ischemic stroke) than of VTEs in patients with hospital-recorded NS. The mechanism behind and potential prevention of these complications should be addressed in future studies. Interestingly, previous studies reported highest risk of thromboembolism in patients with membranous nephrotpahty,[66] yet in our study the long-term risk was highest in patients with membranoproliferative glomerulonephritis or “other histopathology”. In three Chinese studies that screened all NS patients for thromboembolism, between 10% and 36% of patients had radiologically verified VTE, and this higher prevalence of VTE likely is due to limited detection of asymptomatic or mild VTEs in our study.[45, 47, 48]

No previous study, had the primary aim of examining the absolute and relative risk of bleeding in NS. A Danish study examining the effect of anticoagulation in 79 consecutive patients with NS described major bleeding events in 2.5% of patients after medical record review.[52] A Scottish study examining risk of VTE in 206 patients with NS and kidney biopsies reported that 3.4% of patient with NS had a major bleeding event

during in median 3 years follow-up.[43] The risk of bleeding in NS patients in our study was considerably higher, likely because we examined the long-term risk of all hospital-requiring bleeding events. The short-term risk of urinary tract bleeding in patients with NS may reflect complications to kidney biopsies, and the reasons for and consequences of bleeding events in NS needs to be elucidated.

Methodological considerations

Large population-based registries as the ones used in the current studies enable studies of occurrence and prognosis that would be unfeasible in clinical settings.[90] The registries provide nationwide data collected over several decades, and the (in principle) equal access to healthcare in tax-funded hospitals minimizes the selection of study participants. Furthermore, the data collected in routine clinical practice are collected prospectively, and such recording of the exposure before knowing the outcome reduces the risk of bias due to misclassification of the exposure (e.g. recall bias).[83] Despite these advantages, research based on secondary data has common limitations e.g. due to lack of control over which variables to measure, and when and how to measure them, and lack of clinical detail of severity of the disease under consideration.[90]

The internal validity addresses if the results are trustworthy within the given setting of a study, whereas the external validity concerns if the results are applicable outside the given setting of a study. The external validity in epidemiological literature is commonly addressed using the terms generalizability and transportability.[90] The internal validity can be impaired due to random error or systematic error. The random error describes the statistical concepts of uncertainty or imprecision of estimates, and this can be improved by increasing the sample size. Systematic errors (bias) lead to systematic over or underestimation of associations. They are commonly categorized as selection bias, misclassification (information bias), or confounding. Selection and information bias are inherent in the design of studies and cannot be corrected in the analytical phase, while confounding can be corrected in either the design or the analytical phase of a study.[90] Below, these concepts are briefly described and discussed in relation to the current Studies I-III.

Generalizability and transportability

We find it unlikely that NS in Danish patients is fundamentally different from NS in other settings, and we consider our studies to have high internal validity. Therefore, we deem our findings generalizable to other patients with NS.[90] However, the transportability of our findings to other settings needs to be addressed. First, the findings of incomplete recordings of NS in hospital-registries in Study I may be specific for the

Danish setting, as coding practices likely differ from one healthcare system to another. Yet, it is unlikely that nephrologists apply completely different diagnosis codes to their patients, and the transportability may be fair at least to settings with a healthcare system comparable to the Danish one. In countries with hospital data recordings based on insurance claims, the completeness of NS diagnoses may differ substantially. Also, in Studies II and III, the transportability of our findings will depend on the prevalence of risk factors for NS, death, thromboembolism, and bleeding in other populations.

As described above, most previous studies of incidence and prognosis of patients with NS were based on patients with NS and kidney biopsies. In Study II, we saw that especially elderly NS patients and those with diabetes less frequently had kidney biopsies, and these patients may have different prognoses than younger patients and those without diabetes. Thus, previous estimates of the risk of NS only considered risk in a selected sample of NS patients, and therefore their findings may not be generalizable to all patients with NS. We did not restrict to patients with biopsies in any of the studies, and in Studies II and III, we included all patients with hospital-recorded NS in Denmark, so our findings may be more generalizable to a broader range of patients with NS. Study I, however, revealed that NS may be underreported especially among patients with diabetes, and these patients without the diagnosis code specific of NS may have a different risk of both mortality, thrombosis, and bleeding. Thus, the estimates of prognosis in our Studies II and III may not apply to all patients with NS, but mainly the ones with hospital-recorded NS.

Random error

Random error, or uncertainty, addresses how likely it is that estimates/associations are due to chance alone. Many of the previous studies reporting incidence of NS,[27-36] mortality of NS,[37-42, 63] or risk of thrombosis in NS[43, 45, 48, 49] lacked measures of uncertainty. As NS is a rare disorder, most studies of the disorder are limited by small sample sizes, making the point estimates less precise. The nationwide registries allowed us to identify relatively large cohorts of NS patients, and we addressed the uncertainty of both incidence and outcome estimates in our studies by calculating corresponding 95% CIs.

Selection of NS patients

Selection bias occur when the association between the exposure and the outcome is different in participants and non-participants who fulfilled the inclusion criteria. We had no selection of potential participants (i.e. those with hospital-diagnosed NS) based on their willingness or ability to participate in the

study. The period-specific HR has a built-in selection bias if computed by subsequent time-periods (i.e. with changing starting time of follow-up), due to selection of healthy survivors.[106] Therefore, we started the follow-up at the index date in every period when estimating HRs by time period.[106] We observed attenuating HRs of death, thromboembolism, and bleeding by extending follow-up periods, and these reflect the average HRs during the respective periods.[90, 106] Of note, the HR of death cannot be interpreted as a mortality rate ratio or relative risk when the absolute risk of the outcome in the reference group is high, which was the case in Study II during 5 years of follow-up and Study III during 10 years of follow-up.[107]

Misclassification of NS

Previous studies of NS likely underestimated the incidence of NS, as only about half of patients with NS diagnoses have a kidney biopsy, as described in Study I. However, we also found that recording of NS in hospital registries may be incomplete. This is a concern in Study II, where we examined the incidence of NS based on hospital records, as we potentially overlooked patients with NS without a formal diagnosis. Also, we cannot rule out that the amount of NS patients who received codes reflecting the underlying disease instead of NS specifically have changed over time. This potentially made us under or overestimate the changes in NS incidence over time. Furthermore, data from outpatient and emergency rooms were only available since 1995, and increased data availability may have contributed to the observed increase in NS incidence. However, we saw no abrupt changes in incidence from 1994-1995, and as continuously more patients were diagnosed as outpatients from 1995-2018, it is unlikely that lack of these before 1995 would alone explain the increase in NS over time. When interpreting the findings, one should bear in mind that we examined the incidence of hospital-recorded NS and its changes over time. Correspondingly, in theory the comparison population in Study III could contain patients with NS who had not received the diagnosis code specific to NS. Yet, as we sampled comparisons among millions of Danish residents and NS being a rare disorder, the effect of such contamination would be minimal.

We cannot rule out that recording of the specific NS diagnosis code depended on the risk of complications, e.g. that patients with NS and high risk of thrombosis were more likely to receive the NS diagnosis code than patients with NS and a lower risk of thrombosis. Such misclassification could lead to an overestimation of the association between thromboses and NS. Contrary, if the diagnosis code specific to NS is related to a referral to specialized nephrologist care resulting in improved clinical performance and better adherence,

these patients may have a better prognosis. However, we do not expect NS to be noticeably differentially misclassified in Studies II or III, as a recording of NS (exposure) is rather unlikely to depend on the future events of mortality, thrombosis, or bleeding (outcomes), especially when examining patients' long-term prognosis.

The criteria of NS includes rather arbitrary cutoff values of proteinuria,[4] and different reviews and guidelines have used different cutoff values of hypoalbuminemia.[4, 7-9] These cutoff levels may be used to guide clinicians in addition to the clinical presentation when diagnosing NS, but future studies should examine if alternative cutoffs could more precisely detect patients with NS. One previous study developed algorithms to detect glomerular disease in children based on diagnosis and procedure codes,[108] and it may be relevant for future studies to develop algorithms based on e.g. hospital and laboratory data for identification of adults with NS.

Misclassification of outcomes

Previous studies of mortality of NS may be limited by loss to follow-up, i.e. incomplete information about the outcomes during the study period.[90] We obtained information on death from the Danish Civil Registration System, which is highly valid and provides virtually complete information on death in Danish residents.[80] Thus, loss to follow-up and misclassification of death are unlikely in Study II.

In Study III, however, we cannot rule out that VTE is more completely recorded in patients with NS than in the general population, especially shortly after presentation with NS, as VTEs are well-known complications to NS. However, previous studies screening patients by ultrasound and CT reported much higher prevalence of VTE at debut with NS than the 30-day risk we observed.[45, 47, 48] Furthermore, we find it unlikely that recording of myocardial infarction, ischemic stroke, and bleeding events would depend on a previous diagnosis of NS, as these are not commonly mentioned as complications from NS.[6] Thus, surveillance bias alone is unlikely to explain the observed associations in Study III. Contrary, non-differential misclassification of the outcome due to general underreporting of bleeding events in both cohorts would lead to bias towards the null.[90]

Confounding

Confounding is a concept used in research of causality describing a confusion of causes.[90] Historically, confounding factor (confounder) has been defined by its association with both the exposure and outcome of interest, leading to a misinterpreted causal association between the exposure and outcome. For example, when studying if NS increases the risk of thromboembolism, cancer could confound the observed association as it is a risk factor for both NS and thromboembolism. In causal epidemiology, one wants to isolate the effect of an exposure on the outcome, by removing the effect of confounding. Confounding can be avoided in the design phase by randomization, restriction, matching, or in the analytical phase of a study by stratification, standardization, adjustment, or G-methods.[109] In Study II, we standardized incidence and mortality estimates to a standard population to remove the effect of changing age and sex over time. To further account for changes in comorbidity on the mortality, we computed HRs of death adjusted for potential confounders recorded before NS. In Study III, we matched NS patients to comparisons to account for confounding by age and sex, and we further adjusted for a wide range of factors that potentially could confound the association between NS and thromboembolism and bleeding. We selected covariates to adjust the HR in Studies II and III using a "disjunctive cause criterion" including factors recorded pre-exposure that were expected to be related to the exposure, or the outcomes, or both.[90] But even adjusted estimates can be confounded, by residual confounding (not fully captured in factors used for adjustment), unmeasured confounding, or unknown confounding. As an example, we cannot rule out that residual confounding due to hypertension (included by use of antihypertensive drugs) may contribute to the observed association between NS and thromboembolism in Study III. Additionally, we did not have data on smoking, yet we adjusted for smoking-related disorders (e.g. chronic pulmonary disease) probably yielding residual confounding. Finally, we lacked data on ethnicity and BMI, and these factors may give unmeasured confounding as it is related to both NS and risk of thromboembolism. To examine if the observed adjusted 1-year HR of thromboembolism of 3.99 could be explained by confounding, we calculated an E-value based on the point estimate, CIs, and the notion that the outcome was rare (<15% absolute risk).[110] The E-value was 7.44 meaning that: 1) an unmeasured, unknown, or residual confounder (or set of confounders) had to be 7 times more prevalent in the NS patients than in the general population, and 2) the confounder itself should increase the risk of thromboembolism 7-fold to explain the observed association. Such a highly

prevalent and strongly associated confounder is very unlikely, given that we already adjusted for a wide range of known confounders.

Additional considerations

In Study I, we found that recording of NS in hospital may be incomplete, yet, we used only data on hospital diagnoses to identify patients with NS in Studies II and III for two reasons. Firstly, the patients with nephrotic proteinuria and hypoalbuminemia do not necessarily have NS, and we believed that basic epidemiological data on NS were needed.[4] Secondly, laboratory data were only available during the latter part of the 40-year study period.

In analyses restricted to patients with biopsies in Studies II and III, we considered how to avoid immortal time bias and avoid conditioning on the future at start of follow-up. We could either start follow-up at NS index date with delayed entry in different histopathology groups, or start follow-up on the biopsy date, or at a landmark after assessment of biopsies. Few patients had a biopsy before NS index date so very few patients would be "at risk" had we started follow-up at the NS index date. Therefore, each event would affect the estimated cumulative risk substantially more at early than at later time points where more patients had a kidney biopsy (i.e. more patients at risk). If the risk of outcome was high within the first days/weeks after NS, then this phenomenon would affect the estimated cumulative risks considerably. NS is usually not considered an acute deadly disease, so we did not expect a massively increased short-term risk of death in NS and therefore, in Study II, we chose to start the analyses at date of biopsy or NS index date, whichever came latest. Starting follow-up at the biopsy date enabled us to capture more events, yet at the expense of following patients from different time points in the disease course. As we expected a high risk of especially thromboembolism right after the NS diagnosis, we chose to start the analyses at the landmark day 180 in Study III. Starting at a landmark (e.g. day 180) eased the comparison of risk between groups as all patients were followed from a set date after the NS presentation.

A discussion of future aspects

We addressed some important knowledge gaps in the evidence of NS. While we provide some answers, our findings generated multiple questions that should be clarified.

The relevance and consequences of the somewhat arbitrary cutoffs for proteinuria and varying cutoffs for hypoalbuminemia used when defining NS need to be scrutinized.

The reasons for and consequences of the incomplete recording of NS in discharge diagnoses from hospitals need to be elucidated. Future studies may compare the follow-up in care and prognoses in patients with and without diagnosis to clarify if the prognoses differ in these.

In Study I, our findings suggested that NS may by underreported in hospital records. In future studies, we will aim to examine the incidence and prognosis of patients with nephrotic proteinuria and hypoalbuminemia. Specifically, the prognostic value of proteinuria and p-albumin levels on the risk of thromboembolic complications needs to be clarified. In addition, we find it very relevant to examine the incidence and prognosis in patients with varying degrees of proteinuria, and in these we plan to examine the prognostic value of proteinuria and p-albumin level.

As the prevalence of risk factors for NS, such as diabetes and cancer, is expected to increase with an increasingly ageing global population, the incidence of NS needs to be examined in the decades to come.

Given that the existing evidence of NS is based in large on patients with biopsies, there are huge knowledge gaps concerning patients with secondary NS. The occurrence and prognosis of secondary NS may be better addressed if using also hospital diagnoses and laboratory findings to identify NS patients. To differentiate between patients with and without NS among those with biochemical features of NS, more refined algorithms based on different data sources could be developed.

In addition to the complications we examined, the risk of infections, acute kidney injury, chronic kidney disease, and bone disorders remains largely unclarified in adult patients with NS.

I believe that the Nordic population-based health registries can provide invaluable evidence of the long-term prognosis in NS patients. It is my hope that the findings in our current and future studies combined with detailed clinical data from prospective studies like NEPTUNE and CureGN will close some of the wide gaps in the evidence of NS.

CONCLUSION

- Adults with hospital-recorded NS comprises only one fifth of adults with biochemical features of NS, suggesting that NS may be incompletely recorded. Patients with hospital-recorded NS are substantially different to those identified with biochemical features of NS. Studies based on the hospital diagnosis specific to NS may especially overlook patients with NS and diabetes. This is crucial for the interpretation and design of studies of NS, and it supports the use of both laboratory records and hospital records in studies of NS.
- The incidence of hospital-recorded NS has increased in Denmark over the past 40 years, and this could not alone be explained by aging of the population. The increase in incidence was largest in men above 65 years of age. The histopathological findings in patients with NS were rather stable, yet, with slightly more patients with focal segmental glomerulosclerosis and diabetic nephropathy in recent periods. The mortality of NS decreased considerably over the past 40 years, especially in older age groups. More complete recording, and higher awareness and diagnostic activity of NS, and improvement in data availability may have contributed to the observed changes over time. Projections suggest that risk factors for NS will be more common in the future, which calls for updated analyses on the epidemiology of NS in the years to come.
- The risk of thromboembolism and bleeding events in patients with hospital-recorded NS is high, and during the first year of follow-up, the risk is 4-fold that in the general population. Stratified by subtype of the first event, the absolute risk was highest of ischemic stroke, myocardial infarction, and gastrointestinal bleeding in NS patients. We observed the highest risk of thromboembolism in patients with low eGFR, systemic lupus erythematosus, and diabetes. Among those with a kidney biopsy, the risk of thromboembolism and bleeding was highest in those with membranoproliferative glomerulonephritis and “other histopathology”, and lowest in those with minimal change disease. These findings, especially those of high risk of ATE and bleeding in NS patients, need further exploration.

ENGLISH SUMMARY

Nephrotic syndrome (NS) is a rare renal condition characterized by severe proteinuria and hypoalbuminemia in patients with primary glomerular diseases (primary NS) or systemic conditions (secondary NS). Previous observational studies of NS included mainly patients with kidney biopsy indicated by NS, and they may only include a selected subset of all patients with NS. Also, data on incidence and mortality of nephrotic syndrome in adults are in general limited. Finally, venous thromboembolic events are common and potentially severe complications of nephrotic syndrome, but the long-term risk of venous or arterial thromboembolism and bleeding after NS remains unclarified.

In cohort studies, we used Danish population-based registries to address knowledge-gaps in the epidemiology of NS. We examined 1) which patients are recorded with NS in hospital, by comparing patients with laboratory-recorded nephrotic proteinuria and hypoalbuminemia to patients with hospital-recorded NS from 2004 to 2018, 2) trends in incidence, histopathology, and mortality of hospital-recorded NS from 1979 to 2018, and 3) the risk of hospital-diagnosed thromboembolic or bleeding events in patients with hospital-recorded NS from 1995 to 2018.

In Study I, we found that only 18% of patients with recorded nephrotic proteinuria and hypoalbuminemia had recorded hospital diagnoses compatible with NS within 1 year, while 87% had diagnoses reflecting any kind of nephropathy. When compared to patients with hospital-recorded NS, patients with nephrotic proteinuria and hypoalbuminemia comprise a larger cohort with higher comorbidity burden. This is essential knowledge when designing and interpreting registry-based studies of risk and prognosis of NS.

In Study II, we found that the incidence of hospital-recorded NS in adults increased from 2.33 per 100,000 person-years (95% confidence interval [CI]: 2.18-2.47) in 1979-1980 to 4.22 per 100,000 person-years (95% CI: 4.02-4.42) in 2010-2018, and the distribution of histopathological findings was rather stable. The 1-year mortality of NS decreased from 25% (95% CI: 22-28) in 1979-1989 to 12% (95% CI: 11-14) in 2010-2018. These changes may in part be explained by more complete recording of NS and increasing data availability.

In Study III, we found that patients with NS have high absolute risk of thromboembolic (10-year risk of 20.0% [95% CI: 18.6-21.4]) and bleeding events (10-year risk of 17.0% [95% CI: 15.7-18.3]). The risk of both outcomes was manifold higher compared to that in matched comparisons from the general population

even after adjusting for differences in prevalence of potential confounders at index date. We provided accurate long-term risk data of thromboembolism and bleeding in NS in a large cohort with minimal loss to follow-up. The mechanism and consequences of the high risk of arterial thromboembolism and hospital-requiring bleeding events need to be clarified.

DANSK RESUMÉ

Nefrotisk syndrom (NS) er en sjælden tilstand hos patienter med primær glomerulonefritis (primær NS) eller systemisk sygdom (sekundær NS), med stort tab af protein til urinen, albuminmangel i blodet og perifære ødemer. Den eksisterende viden om NS stammer hovedsageligt fra studier baseret på data fra biopsi-registre, og de inkluderede muligvis kun en særlig del af patienterne med NS. Derudover er der generelt meget sparsom viden om forekomst og dødelighed af NS hos voksne. Slutteligt har tidligere studier af risikoen for blodpropper hos patienter med NS fokuseret på venøse blodpropper, hvorimod risikoen for arterielle blodpropper og blødninger hos patienterne er ukendt.

Vi anvendte danske registerdata til at undersøge forekomsten og prognosen af NS i tre kohortestudier. Vi undersøgte: 1) Hvilke patienter der bliver kodet med NS efter hospitalsbesøg, ved at sammenligne alle patienter med laboratoriefund som kunne indikere NS (nefrotisk proteinuri og hypoalbuminæmi) med patienter med hospitalskodet NS i årene 2004-2018. 2) Tendenser i forekomsten af NS, og biopsifund og dødelighed hos patienter med NS i årene 1979-2018. 3) Risikoen for blodpropper og hospitalsdiagnosticerede blødninger hos patienter med hospitalsdiagnosticeret NS i årene 1995-2018.

Studie I viste, at kun 18 % af patienter med laboratoriefund som kunne indikerer NS modtog hospitalskoden specifik for NS, men 87 % modtog hospitalskoder der indikerede nyresygdomme forenelige med NS. Patienter med laboratoriefund som kunne indikere NS havde dårligere nyrefunktion og mere komorbiditet end patienter med hospitalskodet NS. Dette kan indikere, at NS hospitalskoden primært benyttes til udvalgte patienter med NS, hvilket er vigtigt ved opsætning og fortolkning af studier af NS.

Studie II viste, at forekomsten af NS steg i løbet af de seneste fire årtier. Nye tilfælde af NS hos voksne steg fra 2,33 per 100.000 personår (95 % confidence interval [CI]: 2,18-2,47) i 1979-1980 til 4,22 per 100.000 personår (95 % CI: 4.02-4.42) i 2010-2018. Fordelingen af de forskellige typer af biopsifund var nogenlunde stabil igennem studieperioden. Derimod faldt den gennemsnitlige 1-års dødelighed fra 25 % (95 % CI: 22-28) i 1979-1989 til 12 % (95 % CI: 11-14) i 2010-2018. Disse ændringer i forekomst og dødelighed af NS kan muligvis delvist forklares af øget registrering af NS samt øget tilgængelighed af data.

Studie III viste, at patienter med NS har en høj risiko for blodpropper (10-års-risiko på 20,0 % [95 % CI: 18,6-21,4]) og blødning (10-års-risiko på 17,0 % [95 % CI: 15,7-18,3]). Risikoen for blodpropper og blødninger var

mange gange højere hos patienter med NS sammenlignet med personer af samme køn og alder fra bagrundbefolkningen (også efter justering for anden sygdom). Vi viste, at patienter med NS har høj langtidsrisiko for blodpropper og blødninger, og særligt risikoen for arterielle blodpropper samt blødninger er tidligere ubeskrevet. Årsagerne til disse og konsekvenser heraf bør undersøges nærmere.

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APPENDICES WITH MANUSCRIPTS

Appendix I Paper I

Appendix II Paper II

Appendix III Paper III

The papers have been removed from the file due to copyright issues