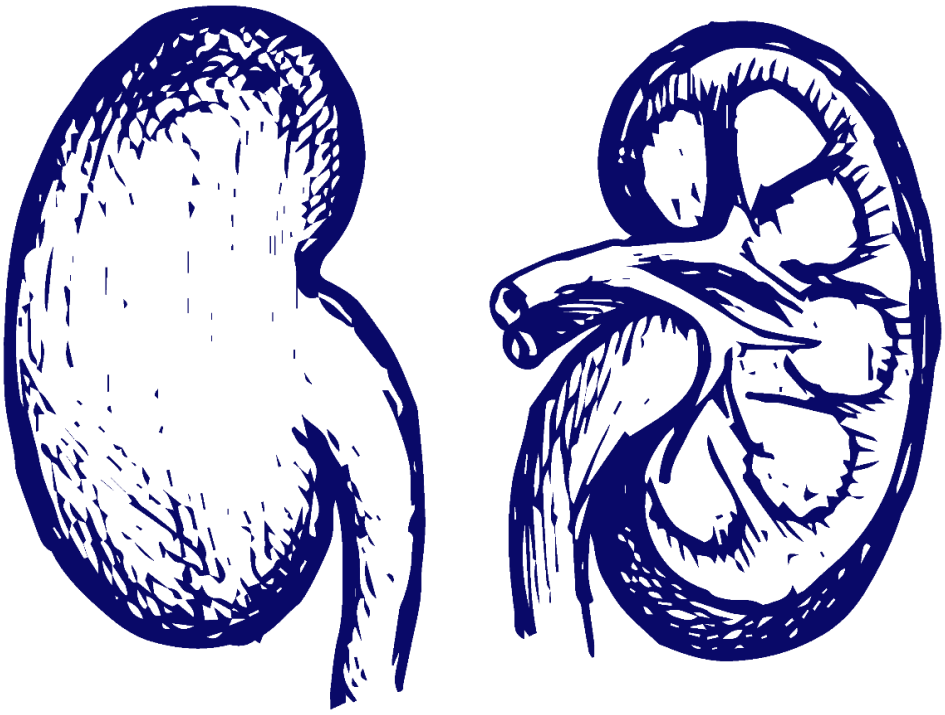
AARHUS UNIVERSITY
HEALTH

Epidemiology of acute pyelonephritis

Occurrence, microbiology, and prognosis

PhD thesis

Lise Skovgaard Svingel



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List of papers

The thesis is based on the following four original studies, which will be referred to in the following text by their Roman numerals (I-IV). The four thesis papers are provided in the appendices.

I. **Svingel LS**, Christiansen CF, Birn H, Søgaard KK, Nørgaard M.
Temporal changes in hospitalization rate for acute pyelonephritis: a 19-year population-based Danish cohort study [SUBMITTED]

II. **Svingel LS**, Nørgaard M, Christiansen CF, Birn H, Nielsen HL, Søgaard KK.
Microbial etiology of severe acute pyelonephritis in a 19-year population-based cohort study [IN PREPARATION]

III. **Svingel LS**, Christiansen CF, Heide-Jørgensen U, Søgaard KK, Birn H, Nørgaard M**.**
Risk of chronic kidney disease in patients hospitalized for acute pyelonephritis: a matched population-based cohort study [IN PREPARATION]

IV. **Svingel LS**, Christiansen CF, Jensen SK, Heide-Jørgensen U, Søgaard KK, Birn H, Nørgaard M.
The risk of chronic kidney disease after acute kidney injury in relation to acute pyelonephritis [IN PREPARATION]

Abbreviations

APN:	acute pyelonephritis
ATC:	Anatomical Therapeutic Chemical classification
CAKUT:	congenital anomalies of the kidney and urinary tract
CCI:	Charlson Comorbidity Index
CI:	confidence interval
CFU:	colony forming units
CKD-EPI:	Chronic Kidney Disease Epidemiology Collaboration
CRS:	Civil Registration System
DAG	directed acyclic graph
DNPR:	Danish National Patient Registry
eGFR:	estimated glomerular filtration rate
HR:	hazard ratio
ICD-8:	International Classification of Diseases, 8th Revision
ICD-10:	International Classification of Diseases, 10th Revision
IQR:	interquartile range
LABKA:	Clinical Laboratory Information System (LABKA) Research Database
pCr:	plasma creatinine
PY:	person year
RFR:	renal functional reserve
RLRR:	Register of Laboratory Results for Research
spp.:	species
UTI:	urinary tract infection

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1. Introduction

1

This PhD thesis came to be during the biggest pandemic of modern times, the Covid-19 pandemic. Whereas this pandemic was by many experienced as a bolt from the blue, this thesis deals, in essence, with an infectious disease that is also affecting all populations across all continents, yet in a more slowly progressing manner. Still, for affected individuals it may have serious consequences.

The primary focus for this thesis is acute pyelonephritis (APN), one of the most severe forms of the most common community-acquired infection – urinary tract infection (UTI).[1] Possibly due to the high frequency of UTIs at the global and community level,[2,3] mild cases have in the antibiotic era often been regarded as fairly banal. Thus, the rising prevalence of pathogens causing UTIs[4] has come on like a twilight, not noticeable from one moment to the next. Therefore, we have to make an effort to register its progression as it is best observed over a longer period of time.

For this purpose, epidemiologic investigations are key in producing research with the potential to guide clinical care towards the goal of predicting the course of disease and improving its prognosis.[5,6] We therefore find it highly relevant to take stock of the extent and the aftermath of APN. One these grounds, this thesis deals with the occurrence of, the microbiology associated with, and the prognosis of APN in Denmark over the past two decades.

2. Background

2.1. Acute pyelonephritis

The story of pyelonephritis is in many ways long and winding. Although the clinical entity was described already in the earliest days of modern medicine, it did not gain much attention until the 1950s.[7] Throughout time, uncertainty has revolved around the consequences of pyelonephritis, in part due to controversy regarding the terminology and diagnostic definition.[8,9] The urinary tract is composed by the urethra, bladder, ureters, and kidneys, and any part of this system can become site of a UTI (Figure 1).[2] In our current understanding, APN is defined as an acute infection of the upper urinary tract, kidney pelvis, and kidney parenchyma.[10] Most often, APN develops as a dissemination of a lower UTI when bacteria, uropathogens, ascend from the bladder to the kidney.[11] From the kidney, the infection might spread to the bloodstream, causing bacteremia.[2] Thus, the distinction between lower and upper UTI is often not clear due to overlapping clinical signs.[12] Moreover, the presentation of pyelonephritis wary widely between individuals and can include signs and symptoms of both bladder inflammation, *e.g.*, urinary frequency, urgency, and dysuria; kidney involvement presenting as flank pain or tenderness; and systemic inflammation evident by, *e.g.*, fever, malaise, and sepsis.[2,10,12]

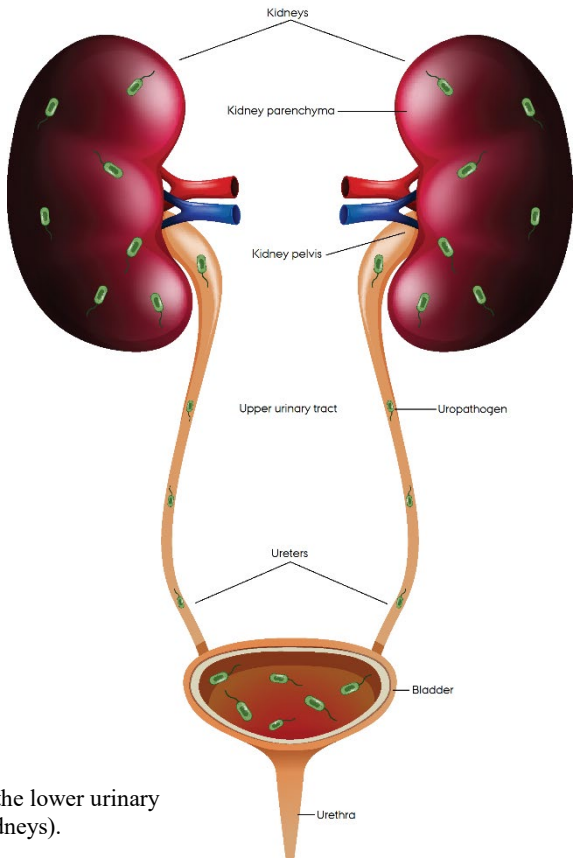

Figure 1. Diagram of the urinary tract with uropathogens present in both the lower urinary tract (*i.e.*, urethra and bladder) and upper urinary tract (*i.e.*, ureters and kidneys).

2.1.1. Occurrence

With an estimated 150 million annual infections worldwide,[2,3] UTIs are among the most common bacterial infections in both childhood and adulthood, and a major societal and health care burden.[13] More than half of all women will experience one or more UTIs in their lifetime.[14] Although only approximately 1% of uncomplicated UTIs is estimated to progress to APN within 30 days,[15] APN is a relatively common disease with an estimated annual incidence of approximately 1 per 1000 persons.[16] The incidence varies by demographic characteristics, and specific subpopulations are at increased risk.[13] Hence, the risk is reportedly highest in infants, young women, and older persons.[17] Female individuals are, with the exception of infants and elderly men, substantially more prone to APN than are male, chiefly due to differences in anatomy, including a shorter urethra in females.[18] Besides sex and age, known risk factors for APN include factors that dispose to bacterial invasion, including diabetes, manipulation of the urinary tract, foreign bodies (including urinary catheters), and sexual activity; and factors that impede urinary flow, including pregnancy, anatomic abnormalities, urolithiasis, and an enlarged prostate.[12,19-21] Other proposed risk factors include genetic predisposition, high bacterial load, and pathogen virulence attributes.[2,22] Hence, the occurrence of APN is determined by an interplay between host and pathogen factors.[2,23]

2.1.2. Diagnosis and microbiology

Clinical suspicion of pyelonephritis is based on medical history and physical examination, and may be supported by rapid screening tests such as urine dipstick and microscopic urinalysis.[20,24] Furthermore, in patients with suspected pyelonephritis, urine should be collected for culture to confirm the clinical diagnosis and to guide antibiotic therapy.[10,25] Urine specimens should be obtained prior to initiation of antibiotic therapy in order to maximize the likelihood of pathogen identification and successful antimicrobial susceptibility testing. A positive urine culture, identifying one or more uropathogens in a concentration considered significant ($\geq 10^3$ colony forming units [CFU]/ml for primary uropathogens, including *Escherichia coli*, in a mid-stream urine specimen),[26] is regarded as the cardinal confirmatory test of a UTI. Additional tests, including blood culture and imaging of the urinary tract, may assist in establishing the diagnosis in severe or ambiguous cases, *e.g.*, in patients having received antibiotic therapy prior to urine collection.[10,24]

The microbial etiology of APN is more diverse than that of lower UTI due to both host and pathogen factors.[23,27] Thus, certain patient characteristics, underlying conditions, and medical procedures that predispose to APN may promote infection by opportunistic pathogens that rarely cause infection in otherwise healthy persons. Still, the main microbial cause of all UTIs, including APN, is Gram-negative bacteria, primarily *E. coli*, a bacteria commonly found in the gastro-intestinal flora. Depending on the population and setting, *E. coli* is estimated to cause 45-92% of pyelonephritis cases.[10,17,24,28,29] Other common causes of APN are *Enterobacteriaceae* including *Klebsiella* species (spp.), *Proteus* spp., and *Enterobacter* spp., and also

Pseudomonas aeruginosa and Gram-positive bacteria such as *Staphylococcus saphrophyticus*, *Staphylococcus aureus*, and *Enterococcus* spp.[17,19,27,30-32]

2.1.3. Treatment

The treatment of APN involves source control, *e.g.*, removal of urinary catheter or drainage, antibiotic therapy as well as symptomatic treatment, including analgesics and antipyretics. While the majority of patients with APN can be treated with oral antibiotics in primary care or in an outpatient setting, the most severe cases (*i.e.*, in children, pregnant women, immunocompromised, and fragile elderly persons) require initial intravenous antibiotic therapy and supportive care under hospitalization.[10,17,33,34] Thus, according to national recommendations, all children with suspected APN should be referred for hospital treatment.[29,35,36] The proportion of patients with APN being hospitalized is estimated to be lower than 20% in young, non-pregnant women and as high as 70% in pregnant women and patients with diabetes.[10,17,32]

Selection of empirical antibiotic therapy is guided by assessment of patient-specific conditions and local data on pathogen spectrum and antibiotic resistance patterns. Once the microbial etiology is established, the antibiotic regimen should be targeted appropriately.[20,27,37,38] The recommended treatment duration depends on patient and pathogen characteristics and is guided by national guidelines.[25,36,37]

2.1.4. Prognosis

The clinical spectrum of APN ranges widely from relatively mild infections to life-threatening disease. In the most severely ill patients, the infection can lead to life-threatening and/or long-term complications, including sepsis, acute kidney injury (AKI), chronic kidney disease (CKD), and possibly death.[10,39-44] Bacteremia is estimated to occur in approximately 21-23% of patients with APN,[45,46] and UTIs are considered one of the leading causes (9%) of all sepsis episodes in Europe.[47] The overall mortality in patients hospitalized for APN has been estimated to be 12% in men and 15% in women.[48] Both the short- and long-term prognoses of APN are greatly affected by patient characteristics, including age and underlying conditions, and the quality of treatment. Although results are somewhat conflicting in different populations,[49,50] much research point to the importance of prompt and efficient antibiotic therapy for symptom remission and reduction of length of hospital stay, rate of complications, and mortality.[51-54] In the long term, especially in children and in cases where effective antibiotic treatment is delayed,[55-59] APN has been suggested to potentially lead to scarring of the kidney parenchyma,[41,55,60-63] with subsequent hypertension, impairment of kidney function, and ultimately CKD.[57,64-67] Yet, while pyelonephritis, in the early years of modern nephrology, was perceived as the leading cause of kidney failure, the present-day understanding is more complex and multifactorial.[7,68]

2.2. Kidney function and disease

The human kidneys possess regulatory, excretory, and endocrine functions of imperative importance for sustaining life and health. The kidneys filter blood by glomerular filtration followed by tubular reabsorption and secretion leading to the production of urine that is essential for the regulation of acid-base, fluid, and electrolyte balances; and for the excretion of metabolic and exogenic waste products, including medications (Figure 2). Moreover, they secrete or metabolize important factors, such as erythropoietin, vitamin D, renin, insulin, and prostaglandins.[69] The kidneys are, thus, vital for a wide range of body and organ functions, and loss of kidney function is associated with dysfunctions of multiple tissues and organs.[70]

2.2.1. Laboratory assessment

Kidney function is most commonly assessed by the glomerular filtration rate (GFR), defined as the volume of blood plasma filtered by the kidney glomeruli per unit of time.[69] In clinical practice, GFR is mostly estimated as the plasma clearance of an endogenously produced metabolite, which is excreted mainly by glomerular filtration, such as the muscle waste product creatinine, and normalized to body surface (1.73 m^2). Since the production rate of creatinine is constant under normal conditions with stable muscle mass, *i.e.*, steady state, changes in plasma creatinine (pCr) are inversely related to and applicable as a measure of changes in GFR. Hence, pCr together with information on sex and age (to account for inter-individual differences in, *e.g.*, muscle mass) are included in widely used formula for calculating the estimated GFR (eGFR) in clinical and research practices. Yet, in addition to sex and age, the pCr can be affected by many factors including muscle injury, diet, physical activity, and extra-renal clearance. Thus, eGFR should be considered a rough measure of the GFR.[71] Importantly, estimation of GFR does not capture the renal functional reserve capacity that can institute compensatory hyperfiltration in functioning nephrons, thus maintaining GFR within normal range until more than 50% of nephrons are lost.[69,72]

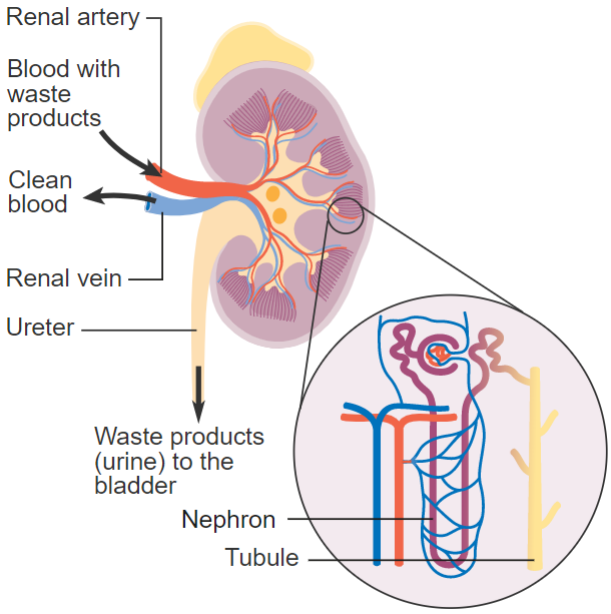

Figure 2. Diagram of the kidney function.

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2.2.2. Changes in kidney function

The kidneys develop progressively throughout fetal life, and GFR matures rapidly after birth to reach adult values, when normalized to body surface, by the age of one year.[73] Following, GFR remains largely constant until the age of 40 years, and hereafter the average GFR decreases by approximately 1 ml/min/year as part of

the aging process.[74] However, substantial diversity exists between individuals regarding the structure of the kidneys, the number of nephrons, and the functional characteristics. Both physiological and pathological factors can affected kidney function. These include pregnancy, changes in blood pressure, obstruction from, *e.g.*, urolithiasis, and edema of the kidney parenchyma.[71] Moreover, processes involving kidney inflammation can lead to tubule-interstitial fibrosis and altered glomerular filtration.[70]

2.2.3. Acute kidney injury

Acute kidney injury is an acute (within hours to days) reduction in GFR that is potentially reversible. It is a heterogeneous syndrome including not only kidney damage per se, but also functional impairment relative to physiologic demands such as aging, *i.e.*, a demand beyond the renal functional reserve capacity.[71,75] Thus, the term AKI covers the entire spectrum of acute changes in kidney function from minor derangements in markers of kidney function to conditions requiring renal replacement therapy.[75] Owing to the many functions of the kidneys, sustained AKI is associated with impairment of a vast range of body functions. These include retention of metabolic waste products and fluid; alterations in acid-base balance, electrolyte disturbances, and hormonal dysregulation; and potentially multisystem organ failure.[75,76] The diagnosis is typically made by laboratory testing documenting accumulation of metabolic waste products (often creatinine) or observation of a decreased urine output.[77] In hospitalized patients, AKI is estimated to occur in one out of three children and one out of five adults, with mortality rates ranging from 14% in children to 24% in adults.[78] Many disorders, including infections and in particular sepsis, may confer AKI, and its manifestation is often multifactorial.[69,79] In Denmark, the 30-day risk of AKI in adults hospitalized for acute APN was recently reported to be 16-47%, depending on pre-admission kidney function.[44] The risk of AKI is increased in patients with pre-existing, reduced GFR. Furthermore, AKI is considered a risk factor for CKD development and progression.[80-84] Thus, AKI may result in loss of functioning nephrons and long-term adverse outcomes including CKD, even in patients with apparent full recovery of kidney function.[71,85,86]

2.2.4. Chronic kidney disease

Chronic kidney disease refers to a number of heterogeneous disorders, in which kidney damage is considered irreversible and has implications for the health of an individual.[70] The current Kidney Disease Improving Global Outcomes (KDIGO) consensus definition covers all disorders with abnormalities of kidney structure or function that is present for more than three months and has health implications.[87] Health implications include both increased mortality and morbidity, including anemia, bone disease, cardiovascular disease, and cancer.[88] While CKD, in its early stage, is often asymptomatic, the condition is often progressive with a continuous decline in kidney function until the final stage of kidney failure.[69,89] In kidney failure, glomerular filtration is severely reduced and often insufficient to maintain essential body functions, necessitating renal replacement therapy in form of dialysis or donor kidney transplantation to prolong life.

With a global prevalence of approximately 10%, CKD is an important health problem.[80,88,90,91] The global burden of CKD is enlarging due to increased longevity, population growth, and changing risk factors.[92]

Well-described risk factors for CKD, in addition to AKI, include sex (women are at higher risk of early stages of CKD, and men are at higher risk of progression to advanced stages), older age, obesity, smoking, and various acute and chronic medical conditions, the most prominent being hypertension and diabetes.[69,80,81]

2.3. Conceptualizing the relationship between acute pyelonephritis and kidney disease

Despite many known risk factors for CKD, the exact etiology in individual patients is often not clear.[87] A "multi-hit" model has been proposed for the pathogenesis of most cases of CKD, excluding only the minority situations characterized by rapid and complete destruction of kidney structure and functions by a well-established, single pathophysiological mechanism.[93,94] In this understanding, several "hits", *i.e.*, the compilation of several risk factors, form the basis for CKD (Figure 3). As such, the possible first "hit", *e.g.*, a congenitally reduced number of nephrons, heralds a susceptibility to kidney injury.[92,95] The second and any subsequent "hits" represents acquired kidney injury, increases the risk of manifest and progressive kidney disease.[93]

In extension hereof and in an epidemiologic framework, CKD may be conceptualized by the co-called sufficient-component cause model.[96] This implies that CKD development may be perceived as the manifestation of a sufficient cause, which comprises a multitude of component causes, *i.e.*, events or conditions associated with disease development. Each single component cause and, thus, the overall causal mechanism may vary between individuals.

Understanding each of the elements adding to the combined mechanisms for CKD development is of crucial importance for both primary and secondary prevention, including therapeutic interventions to minimize progressive kidney disease.

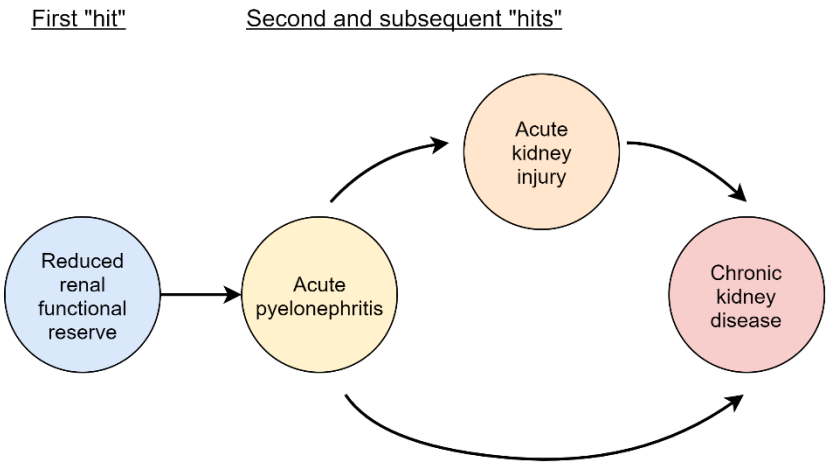

Figure 3. Diagram of a "multi-hit" model conceptualizing the pathogenesis from acute pyelonephritis to chronic kidney disease.

2.4. Summary of existing literature and evidence gaps

A review of the literature on the epidemiology of APN was performed to gain an overview of the existing knowledge base and potential evidence gaps. Specifically, we searched for studies addressing: the incidence/hospitalization rate of APN and the associated patient characteristics (Study I); the microbial etiology, length of stay, and mortality associated with APN (Study II); the risk of CKD following APN (Study III); and the risk of CKD following APN with AKI (Study IV).

The literature search was performed in MEDLINE (PubMed®) for each study separately. Searches were performed using the search builder with Medical Subject Headings (MeSH) and the Boolean operators AND/OR. The searches were restricted to studies in humans, and was most recently performed on December 29, 2022. Additionally, the reference lists of the relevant papers were screened.

The following summarizes the relevant literature with a focus on studies applying population-based designs.

2.4.1. Occurrence of acute pyelonephritis (Study I)

Only few population-based studies from North America, Asia, and Europe have described the incidence of APN over time and in all age groups.[17,33,97]

A study by Nicolle et al conducted in Canada during 1989-1992 was among the first to describe APN incidence in a population-based setting.[97] They included both codes for pyelonephritis and unspecified UTI and reported mean hospitalization rates of 10.86 (± 0.51) per 10,000 population in the female population and 3.32 (± 0.27) per 10,000 in the male population. A widely cited population-based study by Czaja et al, including 3,236 patients with inpatient and outpatient APN, reported an annual incidence rate of 15-17 cases per 10,000 in the female population and 3-4 cases per 10,000 in the male population in the United States in the period 1997-2001.[17]

Two large studies from South Korea, covering the periods 1997-1999 and 2010-2014 have estimated the APN incidence based on insurance claims data.[33,98] Ki et al investigated a total of 496,289 claims for APN in all ages and reported annual incidence rates (inpatient and outpatient) of 59.0 per 10,000 in the female population and 12.6 per 10,000 in the male population.[33] The more recent study by Kim et al was restricted to the population aged ≥15 years and included 845,656 claims from 2010-2014.[98] They described increasing APN incidences (inpatient and outpatient) with annual incidence rates per 10,000 persons increasing from 65.1 to 79.3 in women and from 5.8 to 8.0 in men.

In the European setting, a previous Danish population-based cohort study focused on APN in renal transplant recipient during 1990-2009 and included a matched cohort of general population individuals for comparison.[34] This comparison cohort comprised 49,226 individuals, and the incidence of first-time APN per 1,000 person years (PY) in this cohort was 0.26 (95% confidence interval [CI]: 0.21–0.31) compared with

18.5 (95% CI: 16.4-20.9) in renal transplant recipients. In the comparison cohort, the incidence increased from 0.25 (95% CI: 0.18–0.35) during 2000–2004 to 0.35 (95% CI: 0.28–0.44) during 2005–2009.

Incidence rates have consistently been reported higher in women than in men,[17,33,34,97-99] and in studies considering all age group, with peaks in infants, young women, and individuals aged ≥55.[17,33,97,99,100] In children, Copp et al examined trends in hospitalization rate per 100,000 persons aged <18 years in California, United States, and found the rate to increase from 17 in 1985 to 31 in 2005.[101] The increase in this population was primarily brought on by a nine fold increase observed in children aged <1 year, from 28 per 100,000 persons in 1985 to 238 per 100,000 persons in 2005. In addition, another American study by Sood et al examined trends in emergency department visits and hospitalizations associated with pediatric UTI during 2006-2011.[102] They described an increase in the incidence of female emergency department visits from 709 visits per 100,000 in 2006 to 844 visits per 100,000 in 2011, while the male incidence remained unchanged.

Additionally, the incidence and risk of APN has been described in many selected populations, including children, young women, and individuals of older age and with pre-disposing conditions, such as diabetes and pregnancy.[12,19,32,103] However, these studies were often restricted to small samples, single-center settings, or a cross-sectional design.

Owing to, *e.g.*, differences in health care systems, the results from previous population-based studies may not be applicable to a Northern European setting. Moreover, the incidence of and hospitalization rate for APN is likely to change over time owing to other time-varying factors. Firstly, the proportion of the population living with risk factors for APN such as advanced age, chronic diseases, and foreign bodies in the urinary tract may be enlarging due to changes in demography, lifestyle, and advances in treatments.[10,17] Secondly, advances in diagnostic technologies, including ultrasound-guided bladder puncture for retrieving urine samples, may promote diagnostic accuracy.[104] Thirdly, changes in hospitalization rate may result from implementation of modern treatment regimens favoring early discharge after initial care in the emergency department and avoidance of regular inpatient admission.[10,20,29,32]

Thus, up-to-date epidemiological data describing the hospitalization rate for APN in all age groups are warranted.

2.4.2. Microbiology, length of stay, and mortality associated with acute pyelonephritis (Study II)

The microbial etiology of UTIs, including APN, has been described in a large body of literature, displaying substantial differences in the pathogen distribution between geographical locations, clinical settings, patient populations, and applied metologies.[10,23,105]

Still, across studies, *E. coli* is recognized as the primary causative pathogen in APN (often ascertained as complicated/hospitalized UTI) with a reported prevalence of 45%-88%. Few studies examining the bacterial spectrum of APN have considered all ages.[99,105,106] Tandogdu et al conducted a point prevalence study in 70 countries in Europe, Asia, South America, and Africa during 2003-2013 and identified 1,606 patients with pyelonephritis. In these, *E. coli* accounted for 45%.[105] Using data from Dutch laboratory information systems, Koningstein et al identified 27,922 isolates from 23,357 patients hospitalized for complicated UTI in 2012 and found *E. coli* in 47.2%.[106] Also considering hospitalized UTI, Laupland et al identified *E. coli* as the causative pathogen in 70% of 40,618 episodes in 30,851 Canadian residents from 2004-2005.[99]

Moreover, a range of studies restricted to adolescents and adults, and conducted in Europe[43,48] and Asia,[107-109] during 1997-2013 reported *E. coli* as the primary causative pathogen in 46%-88% of cases. One study by Efstathiou et al including 225 patients hospitalized for APN in Greece, described *E. coli* as more prevalent in women compared with men (60% versus 53%) and in patients aged ≤65 years compared with patients aged >65 years (59% versus 53%).[48]

Of non-*E. coli* spp., *Klebsiella* spp. were observed in 6-14%,[43,99,105-107,109] *Proteus* spp. in 7%,[43,48,106] *Pseudomonas aeruginosa* in 5-16%,[48,106,107,109] and *Enterococcus* spp. in 6-15%.[48,99,106] Efstathiou et al described a higher prevalence of *Klebsiella* spp. in men than in women (7.8% versus 2.4%) and in patients aged >65 years than in younger patients (6.5% versus 3.4%).[48]

In children, Ghiro et al described the bacterial spectrum of 1,333 patients aged 0–17 years hospitalized for APN during 1994-1998 in Italy, and found *E. coli* in 90%, followed by *P. mirabilis* in 4% and *K. oxytoca* in 2%.[110]

The length of stay for patients hospitalized for APN has been described in few studies.[48,50,107,109] In a multinational, multi-center cohort study, conducted in 20 countries in Europe and the Middle East during 2013-2014, Eliakim-Raz et al described a median length of stay of 6 days (IQR: 4-10) in 198 adults hospitalized for APN.[50] Tal et al reported a mean duration of hospitalization of 26.6 days (±18.9) in 191 patients aged ≥75 year in Israel,[109] and Chung et al reported a mean length of stay of 12 days (range 3-46) in 68 adult patients hospitalized for APN in Hong Kong during 2007-2012.[107] Efstathiou et al observed prolonged hospitalizations, defined as hospitalizations with duration ≥10 days, in 27.5% of patients hospitalized for APN.[48] Moreover, they found that patients with diabetes, and patients >65 years with long-term catheterization were especially prone to such prolonged hospitalizations.

Mortality in APN has previously been described in patient with complicated UTI, including pyelonephritis.[43,50,100,107,108] The overall mortality observed in relation to APN hospitalization has been in the range from 1-7%[32,43,107,108] up to as high as 33% in geriatric patients.[109] The 30-day mortality has been reported in a range from 2% in APN[50] to 31% in patients with catheter-associated UTI, including APN.[49] A

few of these studies reported mortality by uropathogen. Thus, Eliakim-Raz et al described a higher 30-day mortality in patients with complicated UTI caused by *K. pneumoniae* (10%) compared with *E. coli* (6%),[50] while Tal et al described a more modest difference in in-hospital mortality between *Klebsiella* spp. APN and *E. coli* APN (31% versus 28%) in geriatric patients.[109]

Thus, population-based data on the APN-associated microbial etiology and clinical outcomes, such as length of stay and 30-day mortality, that are generalizable to a Northern European setting are scarce.

Denmark has an effective national surveillance program, the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP), for systematic and continuous monitoring of antimicrobial drug consumption and antimicrobial resistance in humans, animals, and food.[111] Included in this program are, *e.g.*, data on isolates of *E. coli* from urine and blood samples from humans. Reports based on these data have demonstrated both a 37% increase in the number of *E. coli* isolated from urine samples from hospitalized patients from 2012 to 2021, and an increasing number of invasive *E. coli* cases from 70.3 to 102.4 cases per 100,000 inhabitants over the past decade.[4] Still, as prior research has raised attention towards pathogen diversity between different types of UTI (cystitis, pyelonephritis, and urosepsis), it may prove necessary to assess the bacterial spectrum for each condition specifically in order to achieve the most precise knowledge base for proper treatment strategies.[32,112]

Thus, it is highly relevant to generate up-to-date epidemiological data elucidating changes in the prevalence of uropathogens causing APN and on associated measures of short-term prognosis, including length of stay and 30-day mortality.

2.4.3. Risk of chronic kidney disease after acute pyelonephritis (Study III)

Prior studies of long-term sequelae of APN, sometimes referred to as febrile UTI, in children and adults, have produced diverging results. Thus, long-term sequelae, including CKD, have been suggested from observational studies; however, most of these were small, including less than 400 individuals from selected populations, and applied study designs prone to recall bias.[64,113,114] After decades of concern revolving primarily around vesicoureteral reflux and risk of kidney scarring in relation to pyelonephritis,[56,64] a systematic review from 2011 concluded that children without structural kidney abnormalities were not at significant risk of developing CKD after UTIs.[114] Yet, an increasing body of literature, including recent studies, have suggested that APN, especially in children and in cases with delayed initiation of effective antibiotic treatment,[55-59] may lead to kidney scarring,[41,55,60-62] with subsequent hypertension, impairment of kidney function, and ultimately CKD.[57,64-66] A recent study by Calderon-Margalit et al investigated the risk of treated kidney failure (dialysis or kidney transplant) in 7,231 individuals with a history of pyelonephritis and found an adjusted HR of 4.03 (95% CI: 3.16-5.14).[66]

However, to advance this field of research, there is a need for large-scale, population based studies investigating the risk of CKD following hospitalized APN in patient of all ages and across a range of comorbidities in order to identify populations at increased risk.

2.4.4. Risk of chronic kidney disease after acute pyelonephritis with acute kidney injury (Study IV)

A previous Danish study by Graversen et al investigated the risk of AKI by pre-admission kidney function in adult patients with APN during 2000-2017.[44] They described 30-day risks of AKI in the range 16-47%, depending on pre-admission eGFR. In addition, in a single-center study from South Korea by Jeon et al, AKI was reported in as much as 61.2% of adult patients hospitalized with APN.[115]

Furthermore, progression from AKI in APN to CKD has been described in patients with pre-existing kidney disease and obstructive uropathy.[115,116] Thus, Jeon et al observed a higher proportion of subsequent CKD in patients with pre-existing kidney disease and AKI compared with patients without AKI (10.3% versus 2.7%). In line with this, another South Korean study by Lee et al, including patients with urolithiasis with or without APN and AKI, observed the highest risk of CKD in patients with concomitant APN and AKI compared with patients without one or both of these conditions.[116]

Thus, the prior knowledge from population-based studies on the importance of AKI for subsequent development of CKD in patients with APN is limited, yet necessary to improve our understanding of the prognosis following APN.

3. Aims

3

Study I

- To examine temporal changes in hospitalization rate for APN.
- To characterize the demographic characteristics associated with hospitalization for APN.

Study II

- To examine the utilization of microbiological diagnostics in hospital-diagnosed APN including the proportion of hospital-diagnosed APN confirmed by positive urine culture.
- To investigate temporal changes in the microbial etiology associated with hospital-diagnosed APN.
- To examine length of stay and 30-day mortality by isolated uropathogen.

Study III

- To examine the absolute and relative risk of *de novo* CKD after first-time hospitalization for APN compared with the general population without APN.
- To examine patient characteristics associated with *de novo* CKD after first-time hospitalization for APN.

Study IV

- To examine the risk of *de novo* CKD after first-time hospitalization for APN in patients with or without concomitant AKI.
- To assess the risk of *de novo* CKD after first-time hospitalization for APN in patients recovering from concomitant AKI.

4. Methods

The following describes the methods used in Study I-IV. Table 1 provides an overview of the applied study designs, data sources, and analyses for each study.

4.1. Setting

Studies I-IV were all conducted in a Danish hospital setting. Denmark has a welfare state model offering its residents free, public health care and partial reimbursement of prescribed medications in the tax-funded healthcare system.[117] Public hospitals provide the majority of hospital services in Denmark, including acute and specialized care and laboratory services.

Following a reform of the regional administrative government in 2007, Denmark is currently divided in five administrative Regions, governing primary and secondary health care services (Figure 4).[117] From 2000 to 2018, the Danish population grew from 5.34 million to 5.78 million, with a decrease in the proportion of children younger than 15 years (from 17.5% to 15.7%) and an increase in the proportion of persons aged 75 years or older (6.8% to 7.7%).[118] In the geographical area now administered by the North Denmark Region (Study II), the population increased from 0.49 million to 0.59 million during this period.

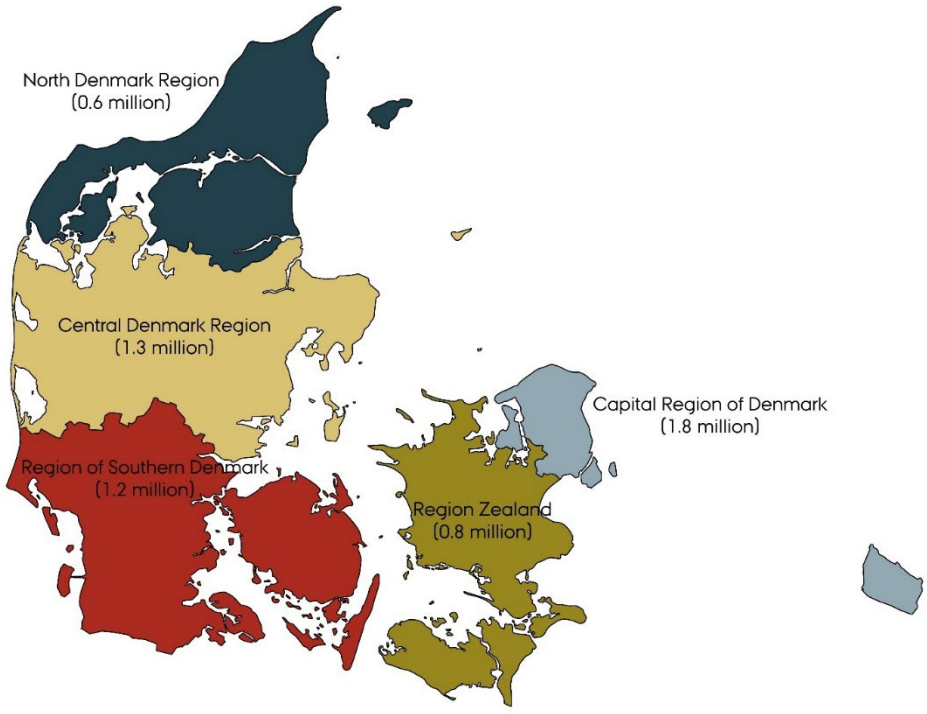

Figure 4. Map of Denmark with the five administrative Regions and 2018-populations in parentheses.

4.2. Data sources

Denmark has a long history for record keeping, and all contacts with any health care sector (primary, secondary, and tertiary) generates prospectively collected data in the medical databases.[117] Although the administrative and health databases were not originally established for research purposes, they provide unique sources for detailed individual-level data. These data can be unambiguously linked using a unique 10-digit personal identification number (Central Personal Register [CPR] number).[119] While some databases are regional,[120,121] most of the medical databases used have national coverage. Study I-IV were all register-based, linking data from the following data sources:

The Danish Civil Registration System (CRS)[119] (Study I-IV)

This registry, established in 1968, has been updated daily since 1989. The CRS assigns a unique CPR number to all residents upon birth or immigration, and enables virtually complete follow-up of all individuals.
From this registry, we utilized information on age, sex, residency, and emigration and vital status.

The Danish National Patient Registry (DNPR)[122] (Study I-IV)

The DNPR was established in 1977 and gained complete nationwide coverage in 1978. It contains information on virtually all somatic hospital inpatient admissions (used interchangeably with the term "hospitalizations" henceforth) since 1977 and on psychiatric hospitalizations, outpatient visits, and emergency department visits (this term will be used also to cover medical admission unit) since 1995. Data include dates of admission and discharge, procedures, surgeries, and discharge diagnoses (one primary and up to twenty secondary diagnoses for each contact). Discharge diagnoses are recorded according to International Classification of Diseases (ICD), 8th revision (ICD-8) until 1994 and 10th revision (ICD-10) thereafter.
We used data from the DNPR to obtain information on exposure, outcome, and descriptive and potentially confounding variables.

The Danish National Prescription Registry[123] (Study II-IV)

Since 1995, this registry has held complete data from the electronic dispensing systems of community pharmacies on all redeemed prescription medications. The records include date of dispensing and type of medication (according to Anatomical Therapeutic Chemical [ATC] classification codes).

We retrieved information on prescriptions for treatment of diabetes and hypertension, and UTI-specific antibiotics for descriptive purposes and/or assessment of potential confounders.

StatBank Denmark (Statistics Denmark)[118] (Study I)

This publicly available online tool and database, holding Danish population statistics, was used to obtain data on the Danish population numbers according to calendar year, sex, age, and region of residence.

wwLab/ADBakt (Autonik AB, Nyköping, Sweden) (Study II)

This laboratory information system records microbiologic test results for all urine and blood cultures performed at the Department of Clinical Microbiology, Aalborg University Hospital, Denmark. Microbiological diagnostic services for general practitioners and hospitals in North Denmark was provided by the Department of Clinical Microbiology, Aalborg University Hospital throughout the study period from the year 2000 through 2018.[124]

We used this system to retrieve urine and blood cultures for outcome assessment. Urine culture was performed in accordance with the European Urinalysis Guidelines,[125] while blood culture was performed by use of automated blood culture systems.[126] Conventional biochemical diagnostic methods, supplemented with MALDI-TOF (Bruker, Bremen, Germany), were used for bacterial identification.[124] Antimicrobial susceptibility testing was performed in accordance with The Swedish Reference Group of Antibiotics[127] until 2010, and the European Committee of Antimicrobial Susceptibility Testing (EUCAST) guidelines hereafter.[128]

The Clinical Laboratory Information System (LABKA) Research Database (Aarhus University, Denmark)[120,121,129] (Study III-IV)

Beginning in the 1990s, laboratory information on biochemical tests required at general practitioners and hospitals in the Central Denmark Region and the North Denmark Region has been collected in this database. The geographical coverage is considered complete since November 2004 in the North Denmark Region and since November 2009 in the Central Denmark Region.[121]

The Register of Laboratory Results for Research (RLRR)[120,121,129] (Study III-IV)

The RLRR is a national register collecting laboratory data on biochemical tests required in relation to daily clinical care at general practitioners and hospitals. Data collection started at different time points in the Danish Regions (from October 2013 in the North Denmark Region and Region Zealand); thus, its coverage is temporally varying but considered complete from October 2015.

The data in the LABKA Research Database and RLRR include information on type of analysis (according to the International System of Nomenclature for Properties and Units [NPU] and national or local analysis numbers), time and date of sampling, reporting unit, and result of tests analyzed at hospital-based laboratories. From these two registers, we obtained data on creatinine measurements for assessment of AKI and CKD.

4.3. Study designs

Studies I-IV were all population-based cohort studies covering the years 2000-2018. Studies I, III, and IV were based on the Danish entire population, while Study II was conducted in the North Denmark Region and also used a cross-sectional design. In Study III, we included a matched comparison group. The study designs are graphically depicted in Figure 5-8.

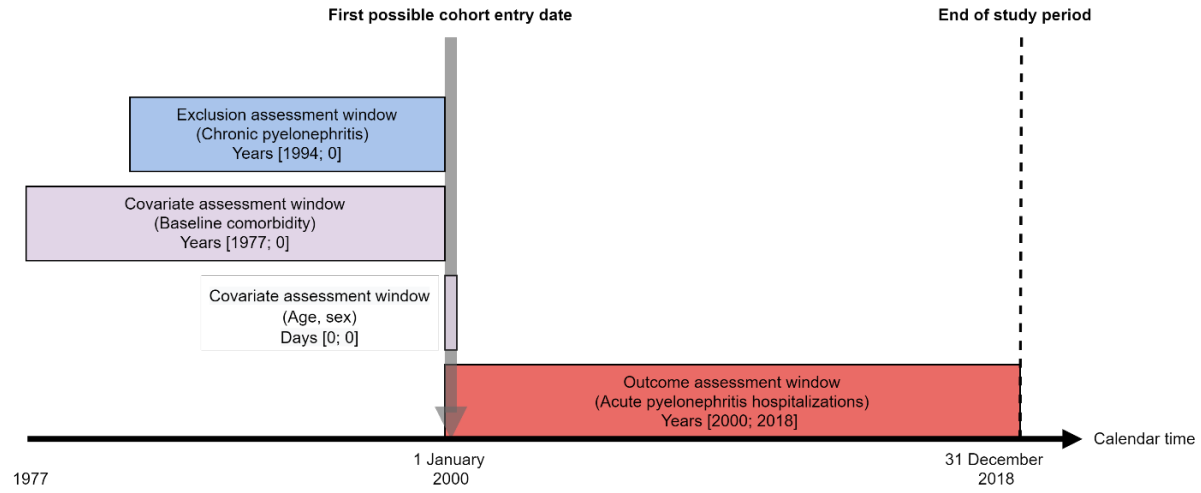

Figure 5. Design of Study I.
Note: The definition of an acute pyelonephritis hospitalization included all hospital inpatient APN diagnoses registered less than 30 days apart (with index date defined by the first admission date).

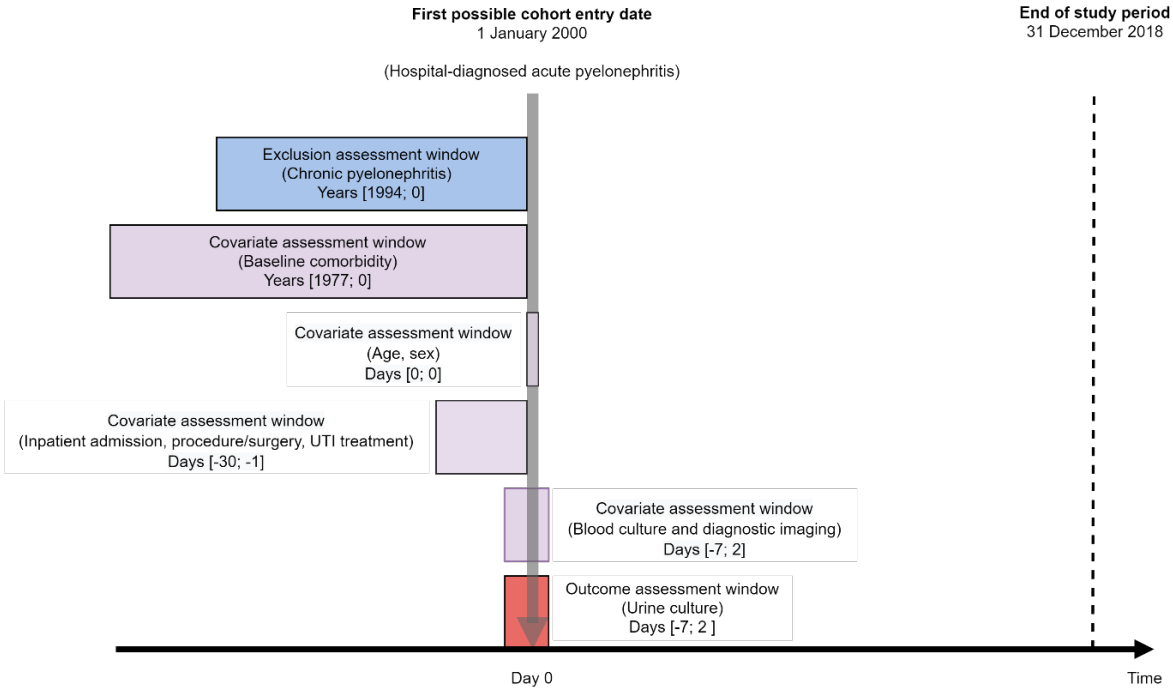

Figure 6. Design of Study II.
Note: The definition of an episode of hospital-diagnosed acute pyelonephritis included all hospital inpatient and emergency department APN diagnoses registered less than 30 days apart (with index date defined by the first admission date.

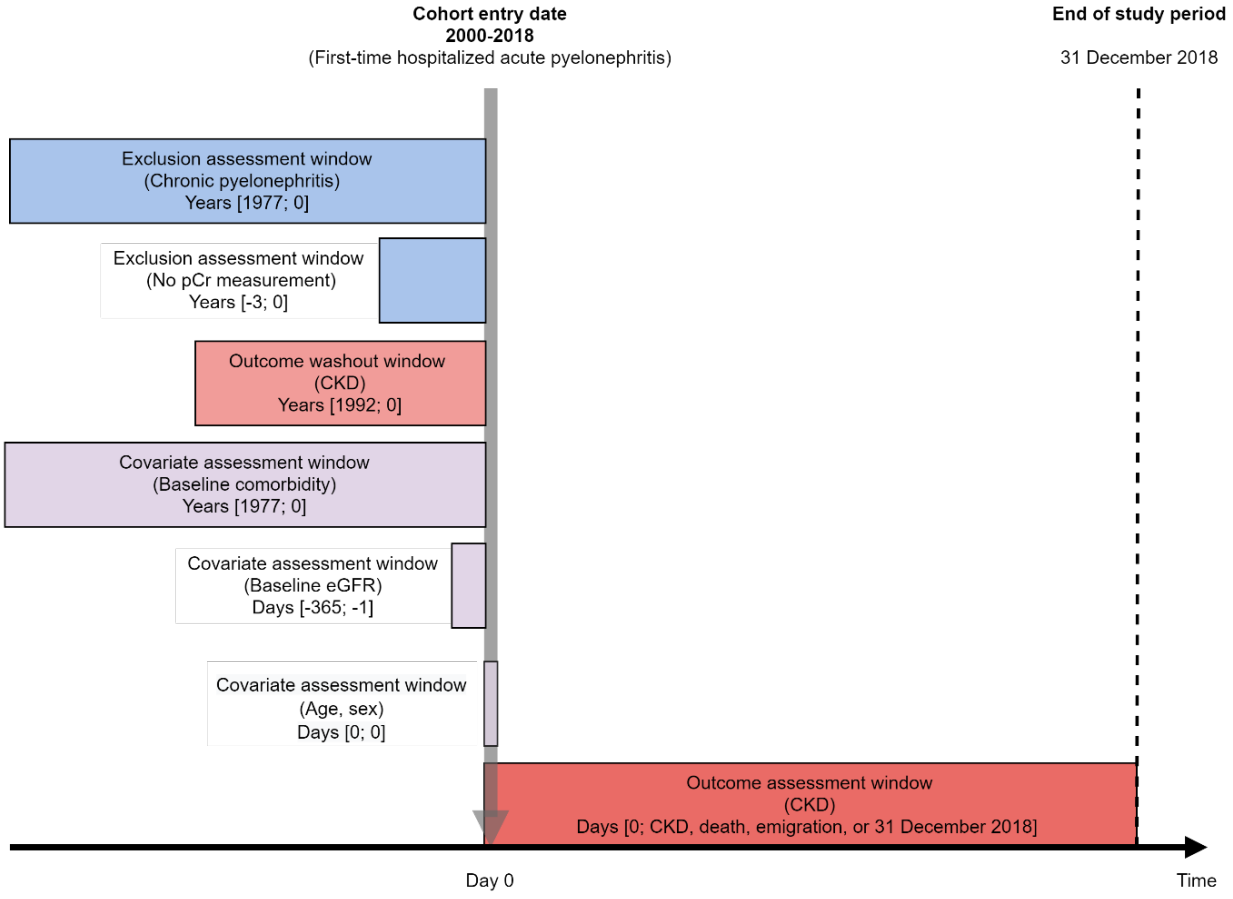

Figure 7. Design of Study III.
Note: Index date for individuals in the general population comparison cohort was the date of admission with first-time hospitalization for acute pyelonephritis in their matched patient.

Abbreviations: CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; pCr: plasma creatinine

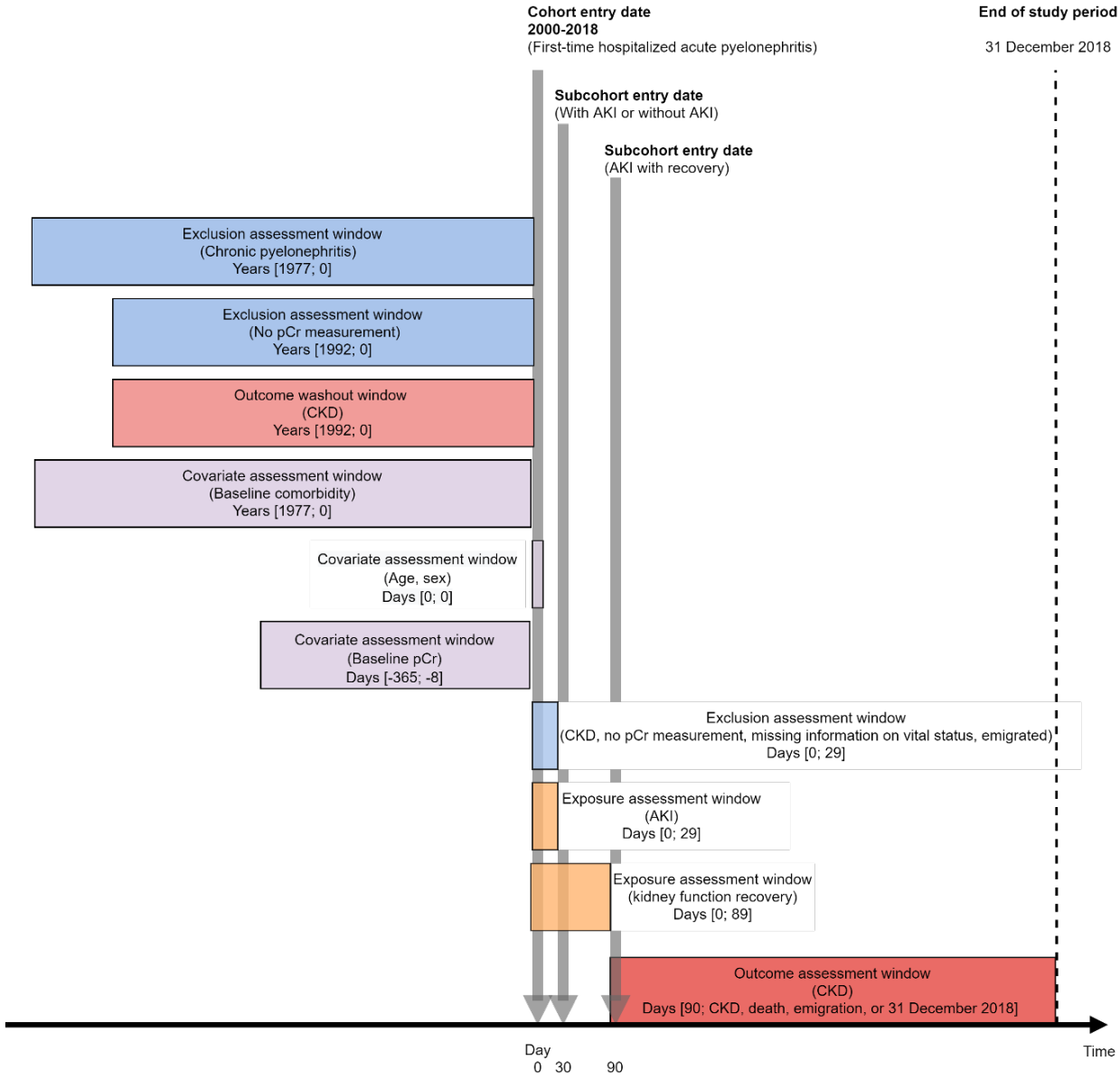

Figure 8. Design of Study IV.

Abbreviations: CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; pCr: plasma creatinine; AKI: acute kidney injury

4.4. Study populations

A chart of the study populations is presented in Figure 9, which also summarize diagnosis codes used for identification of APN and criteria applied for exclusion. To focus on associations related to acute episodes of pyelonephritis, patients with any diagnosis indicating chronic pyelonephritis were excluded from all of the following cohorts. Thus, all of the patient cohorts were derived from an identified population of 57,164

patients with 66,939 hospital-diagnosed (inpatient and emergency department) episodes of APN, to which varying additional criteria were applied to serve the aim of each study.

In Study I, we constructed a nationwide cohort of patients of all ages with one or more hospitalizations for APN during 2000-2018 (Figure 1, Appendix I).

The study population of Study II comprised all patients residing in and diagnosed with APN at a hospital (inpatient and emergency department) in the North Denmark Region during 2000-2018 (Figure S1, Appendix II).

In Study III-IV, the patient cohorts were subsets of the nationwide cohort from Study I. They comprised patients aged two years or older with a first-time hospitalization for APN. Patients younger than two years were not included as the outcome definition for laboratory-based CKD did not apply in this age group.[130] Moreover, patients with pre-existing CKD were excluded.

Furthermore, in Study III, we excluded patients without any prior (within three years) creatinine measurement recorded in the laboratory databases to ensure that we only included patients who were covered by the laboratory system so that we could catch a potential laboratory-based CKD outcome. The comparison cohort was constructed by randomly matching each patient in the APN cohort with up to 10 individuals from the general population without APN, chronic pyelonephritis, and CKD before the matching date (Figure 2, Appendix III). Patients and comparison individuals were matched by age, sex, calendar year, and municipality; municipality was included as a matching variable as a proxy for laboratory database coverage. Matching date (date of admission with APN in patients) was used as index date for comparison individuals.

In Study IV, we applied several restrictions in order to ensure that the AKI exposure could be ascertained, and the population was at risk of the CKD outcome. Thus, we excluded from the APN cohort patients who, within the 30-day AKI assessment window, fulfilled the criteria for CKD, had no creatinine measurement, died or emigrated, or reached the end of study period (Figure S2, Appendix IV). The APN cohort was divided in two cohorts depending on the occurrence of concomitant AKI. Lastly, a subcohort of patients who recovered from AKI within 90 days was constructed.

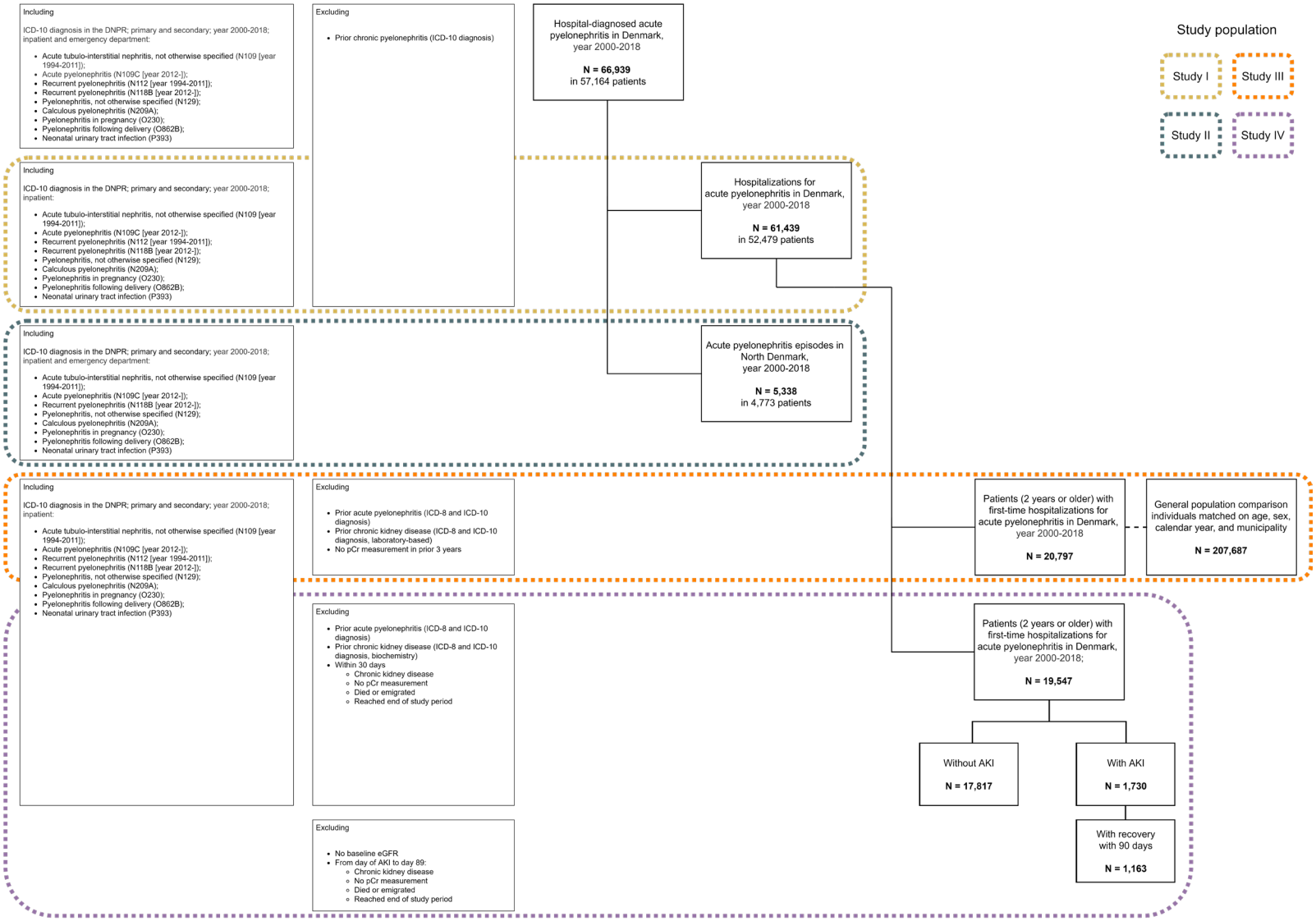

Figure 9. Chart of study population in Study I-IV

Table 1: Summary of materials and methods.

	Study I	Study II	Study III	Study IV
Objectives	To examine a) temporal changes in hospitalization rate for APN; and b) characterize the demographic characteristics associated with hospitalization for APN.	To examine a) the utilization of microbiological diagnostics in hospital-diagnosed APN including the proportion of hospital-diagnosed APN confirmed by positive urine culture; b) temporal changes in the microbial etiology associated with hospital-diagnosed APN; and c) length of stay and 30-day mortality by isolated uropathogen.	To examine a) the absolute and relative risk of *de novo* CKD after first-time hospitalization for APN compared with the general population without APN; and b) patient characteristics associated with *de novo* CKD after first-time hospitalization for APN.	To examine a) the risk of *de novo* CKD after first-time hospitalization for APN in patients with or without concomitant AKI; and b) the risk of *de novo* CKD after first-time hospitalization for APN in patients recovering from concomitant AKI.
Setting	Danish hospitals, Jan 2000-Dec 2018.	Hospitals in the North Denmark Region, Jan 2000-Dec 2018.	Danish hospitals, Jan 2000-Dec 2018.	Danish hospitals, Jan 2000-Dec 2018.
Design	Nationwide cohort study.	Population-based cross-sectional study and cohort study.	Nationwide matched cohort study.	Nationwide cohort study.
Data sources	CRS, DNPR, StatBank Denmark.	CRS, DNPR, The Danish National Prescription Registry, wwLab/ADBakt.	CRS, DNPR, The Danish National Prescription Registry, LABKA, RLRR.	CRS, DNPR, The Danish National Prescription Registry, LABKA, RLRR.
Study population	N = 52,479 patients with N = 61,439 hospitalizations with APN.	N = 4,773 patients with N = 5,338 hospitalizations with APN and N = 2,849 isolated uropathogens.	APN patient cohort (≥2 years): N = 20,797 and matched comparison cohort: N = 207,687.	N = 19,547 patients (≥2 years) with APN, divided according to AKI occurrence: cohort without AKI (N = 17,817) and cohort with AKI (N = 1,730); and a subcohort with AKI and recovery (N = 1,163).
Matching	-	-	Age, sex, calendar year, municipality.	-
Exposure	Calendar year from 2000-2018.	Hospital-diagnosed APN. Uropathogen (*E. coli* and non-*E. coli*).	First-time hospitalization for APN.	AKI within 30 days after first-time hospitalization for APN. Biochemically confirmed recovery from AKI.
Outcome	Hospitalization for APN.	Microbiological test results with microbial etiologies. Length of stay and 30-day mortality.	*De novo* CKD with reduced kidney function.	*De novo* CKD with reduced kidney function.
Covariates	Sex, age, and municipality, CCI score, diabetes mellitus, kidney disease, CAKUT. UTI and sepsis.	Sex, age, and municipality, hypertension, diabetes mellitus, and uropathies (including CAKUT, acquired obstructive and reflux uropathy, urolithiasis, cancer of the kidney or urinary tract, other kidney disease, other urinary tract disease), imaging of the kidneys and urinary tract, recent inpatient admissions,	Sex, age, and municipality, hypertension, diabetes mellitus, uropathies (including CAKUT, acquired obstructive and reflux uropathy, urolithiasis, cancer of the kidney or urinary tract, other kidney or urinary tract tumor, other kidney disease, and other urinary tract disease), baseline eGFR, and the	Sex, age, calendar period, municipality, hypertension, diabetes mellitus, cardiovascular disease, and uropathies (including CAKUT, acquired obstructive and reflux uropathy, urolithiasis, cancer of the kidney or urinary tract, other kidney or urinary tract tumor, other kidney disease, and other urinary tract disease), AKI stage,

		urogenital or kidney procedure/surgery and UTI-specific antibiotic treatment.	number of eGFR measurements in first year of follow-up.	baseline eGFR, time since most recent eGFR, and the number of eGFR measurements during the first 30 days of follow-up.
Statistical analysis	Descriptive statistics; crude and age- and sex-standardized annual hospitalization rates for APN.	Descriptive statistics including proportion of APN episodes accompanied by urine culture and positive urine culture; tabulation of microbial etiologies with stratification by calendar periods, patient characteristics, and vital status.	Descriptive statistics; Cumulative incidence (using the Aalen-Johansen estimator) of hospitalization for APN, treating death as a competing risk; Cox models to calculate unadjusted and adjusted (for sex, age, calendar time, diabetes mellitus, and uropathy) HRs of CKD. Forest plots. Stratifying by sex, age group, calendar period, hypertension, diabetes mellitus, and uropathy.	Descriptive statistics; Cumulative incidence (using the Aalen-Johansen estimator) of hospitalization for APN with or without AKI, treating death as a competing risk. Stratifying by sex, age group, calendar period, hypertension, diabetes mellitus, and uropathy.
Subgroup and sensitivity analyses	Age- and sex-standardized annual hospitalization rates of selected UTI and sepsis in the same source population.	Included episodes with a urine culture performed a) within 14 days before and 4 days after index date, and b) within 30 days before and 4 days after index date.	a) Including APN diagnoses from the emergency department; b) defining the exclusion criteria of pre-existing CKD by a single outpatient eGFR <60 ml/min/1.73 m^2 prior to index; and c) defining the laboratory-based CKD outcome by severely decreased eGFR <30 ml/min/1.73 m^2.	Cumulative incidence (using the Aalen-Johansen estimator) of hospitalization for APN with recovery from AKI, treating death as a competing risk.

Abbreviations: AKI: acute kidney injury; APN: acute pyelonephritis; CAKUT: congenital anomalies of the kidney and urinary tract; CCI: Charlson Comorbidity Index; CKD: chronic kidney disease; CRS: Civil Registration System; DNPR: Danish National Patient Registry; *E. coli*: *Escherichia coli*; HR: hazard ratio; LABKA: the Clinical Laboratory Information System (LABKA) Research Database; RLRR: 7the Register of Laboratory Results for Research.

4.4. Exposures

4.4.1. Calendar period (Study I)

In Study I, the exposure was calendar year from 2000 to 2018.

4.4.2. Acute pyelonephritis (Studies II and III)

In Studies II and III, APN was identified based on a composite of ICD-10 codes, including both primary and secondary discharge diagnoses, in the DNPR during 2000-2018 (Figure 9).[122] Diagnoses of APN registered less than 30 days apart were assumed to belong to the same episode with index date defined as the first admission date.

In Study II, diagnoses from both inpatient admissions and emergency department visits were included in order to capture the microbial range associated with all hospital-diagnosed APN (Table S1, Appendix II). We allowed each patient to contribute more than one episode and included all their consecutive APN episodes.

In Study III, the exposure was defined as first-time hospitalization for APN (Table S1, Appendix III).

4.4.3. Acute kidney injury subsequent to acute pyelonephritis (Study IV)

In Study IV, the exposure was AKI subsequent to (defined as occurring within the first 30 days after) first-time hospitalization for APN recorded in the DNPR (Table S1, Appendix IV).[122] We identified AKI in the laboratory databases according to the KDIGO guideline.[75,120,121,129] Hence, AKI was defined by a) an absolute increase in plasma pCr of ≥26.5 μmol/l within 48 hours and/or; b) a relative increase in pCr to ≥1.5 times the lowest level within the prior 7 days; and/or c) an increase in pCr to ≥1.5 times preadmission pCr.[75] Preadmission pCr was defined as the median outpatient pCr value recorded within 8-365 days preceding the admission date.[131]

In a subgroup analysis, the exposure was AKI with recovery, defined as having at least one (outpatient or inpatient) pCr measurement yielding an eGFR at or above baseline eGFR level from AKI date to day 89 after admission with APN (Figure 8).

4.5. Outcomes

4.5.1. Acute pyelonephritis (Study I)

In the primary analysis in Study I, we identified hospitalizations for APN based on a composite of ICD-10 codes, including both primary and secondary discharge diagnoses, in the DNPR during 2000-2018 (Table S1, Appendix I). Diagnoses registered less than 30 days apart were considered as belonging to the same hospitalization, and each hospitalization was attributed to the calendar year of the first recorded admission date. In a sensitivity analysis, we included also diagnoses from emergency department visits.

4.5.2. Microbiological tests, length of stay, and mortality (Study II)

In Study II (using a cross-sectional design), we examined microbiological test results in form of microbial etiologies of hospital-diagnosed APN confirmed by urine culture. Moreover, we evaluated temporal changes from 2000-2018 in etiology and use of microbiological diagnostic testing, *i.e.*, urine and blood culture, in the diagnostic workup in patients diagnosed with APN in hospital.

The criteria applied for defining a positive urine culture were adapted from the European Urinalysis Guidelines and are summarized in Table 2.[125] When these criteria for positive urine culture were not met, the urine culture was classified as negative.

Finally, we estimated (using a cohort study design) clinical outcomes including length of hospital stay, defined as duration in days from first admission date until last discharge date for diagnoses comprising an episode, and 30-day mortality, stratified by *E. coli* and non- *E. coli* APN.

4.5.3. Chronic kidney disease (Studies III and IV)

We identified CKD by applying both a hospital diagnosis-based algorithm using DNPR diagnosis codes implying chronic conditions with reduced kidney function and a laboratory-based algorithm using data from RLRR and LABKA (Tables S1 and S2, Appendices III and IV).[120-122,129] The laboratory-based definition was applied in order to increase sensitivity[132] and defined CKD based on decreased GFR in accordance with the KDIGO criteria.[87] Thus, CKD was defined by two eGFR measurements <60 ml/min/1.73 m^2 estimated from outpatient pCr measurements at least 90 days apart. Since we were estimating GFR in both children, adolescents, and adults, we calculated eGFR using the full age spectrum CKD-EPI40 equation (Chronic Kidney Disease Epidemiology Collaboration [CKD –EPI] formula[133] with age and creatinine values adjusted to 40 years for patients ≥2 years and <40 years).[134] This formula was selected to achieve reliable estimates of eGFR in children in the lack of information on height (needed for applying the Chronic Kidney Disease in Children [CKiD] equations[135]) and to improve accuracy of eGFR in young adults.[136] The CKD-EPI40-formula has been externally validated in a Canadian cohort of adolescents and young adults with type 1 diabetes mellitus and found to outperform the CKD-EPI equation[133] by showing improved bias (5.3 ml/min/1.73 m^2 [95%CI: 1.2-9.3] versus 26.4 ml/min/1.73 m^2 [95%CI: 22.4-30.2]) and accuracy similar to that of the CKiD equation (15.1 ml/min/1.73 m^2 [95% CI: 12.3-17.5]).[135,137]

Table 2. Categorization of uropathogens and classification of the result of urine culture

Species	**Species category (group)[a]**	**Concentration (CFU/mL)[b]**	**Additional requirements**	**Urine culture classified as positive[c]**
Escherichia coli	Primary uropathogen (group I)	$\geq 10^3$	One or two species isolates[d] and accompanying antimicrobial susceptibility testing	Yes
Salmonella spp.				
Staphylococcus saprophyticus				
Klebsiella spp.	Secondary uropathogen (group II)	$\geq 10^3$	One or two species isolates[d] and accompanying antimicrobial susceptibility testing	Yes
Citrobacter spp.				
Enterobacter spp.				
Proteus spp.				
Serratia spp.				
Morganella morganii				
Providencia spp.				
Raoultella spp.				
Pseudomonas aeruginosa				
Haemophilus spp.				
Staphylococcus aureus				

Enterococcus spp.[e]				
Aerococcus spp.				
Haemolytic streptococci (except group B[e])				
Pseudomonas spp. (except *P. aeruginosa*)	Tertiary uropathogen (group III)	$\geq 10^3$	One species isolated and accompanying antimicrobial susceptibility testing	Yes
Acinetobacter spp.				
Stenotrophomonas maltophilia				
Candida spp.				
Coagulase-negative staphylococci (except *S. saprophyticus*)				
Group B streptococci[f]				
Non-haemolytic streptococci	Normal flora (group IV)	Any	Same species was identified in blood culture and accompanying antimicrobial susceptibility testing	Yes
Other normal urogenital flora				

[a] As defined by European Urinalysis Guidelines.[26]
[b] In urine collected from mid-stream urine, indwelling catheter, suprapubic aspiration, cystoscopy, or single urethral catheterization.
[c] Urine containing any concentration of group I-IV species was considered positive if the same species was identified in blood culture.
[d] Urine was considered contaminated when more than two species were present.
[e] *Enterococcus* spp. was categorized as positive only when it was not isolated from a urine specimen also containing a group I or another group II uropathogen (or if same species was identified in blood culture).
[f] Group B streptococci were classified as group II uropathogen in female patients aged 15-40 years (considered fertile age).

Abbreviations: CFU: colony forming units; spp: species.

4.6. Covariates

For each study participant, we obtained information on demographic variables, including sex, age, and municipality, from the CRS,[119] used for characterization (Studies I-IV), standardization (Study I), and stratification (Study I-IV), and adjustment (Study III). In Study I-IV, we also retrieved data on baseline comorbidities, defined by diagnoses in the DNPR and/or prescriptions in The Danish National Prescription Registry.[122,123] The baseline comorbidities we found relevant to include in each study based on our knowledge from the existing literature are described in Table S1, Appendix I-IV. These comorbidities included hypertension, diabetes mellitus, cardiovascular disease, and uropathies, including congenital anomalies of the kidney and urinary tract (CAKUT), acquired obstructive and reflux uropathy, urolithiasis, cancer of the kidney or urinary tract, other kidney or urinary tract tumor, other kidney disease, and other urinary tract disease.

In Study I, baseline comorbidity status was furthermore assessed by the Charlson Comorbidity Index (CCI) score, based on the complete hospital discharge history.[138] The CCI includes 19 major disease categories, yet in our study we did not include kidney disease when we computed the CCI score (Table S1, Appendix I). We categorized CCI score as 0 versus ≥1 in order to make a binary distinction between previously healthy individuals versus individuals with any pre-exciting comorbidity considered clinically relevant. Furthermore, we recorded selected UTI and sepsis diagnoses in the source population during the study period in order to assess concomitant changes in hospitalization rates for these diseases (Figure S2, Appendix I). Descriptive variables in Study II additionally included radiological and nuclear medicine imaging of the kidneys and urinary tract (performed within 7 days before and 2 days after index date); recent (within 30 days before index) inpatient admission and urogenital or kidney procedure/surgery recorded in DNPR,[122] and UTI-specific antibiotic treatment recorded in the Danish National Prescription Registry[123] (Table S1, Appendix II).

Studies III and IV furthermore included information derived from records of pCr measurements in RLRR and LABKA, including baseline eGFR (calculated from outpatient pCr measurements using the CKD-EPI40 equation[134]), time since most recent eGFR measurement, and the number of eGFR measurements during follow-up.

In Study III, potential confounders were identified from existing knowledge and by construction of a directed acyclic graph (DAG) (Figure 10),[139] and included sex, age, calendar year, diabetes mellitus, and uropathy.

In Study IV, AKI stages were defined in accordance with the KDIGO guideline.[75] Stage 1 AKI was defined by a) a relative increase in pCr of 1.5–1.9 times the lowest level within the prior 7 days or preadmission pCr, or b) an absolute increase in pCr of ≥26.5 μmol/l. Stage 2 AKI was defined by a relative increase in pCr of

2.0–2.9 times the lowest level within the prior 7 days or preadmission pCr. Finally, stage 3 was defined by a) a relative increase in pCr of ≥3.0 times the lowest level within the prior 7 days or preadmission pCr, or b) pCr ≥353.6 μmol/l. Preadmission pCr was defined as the median outpatient pCr value recorded within 8-365 days preceding the admission date.

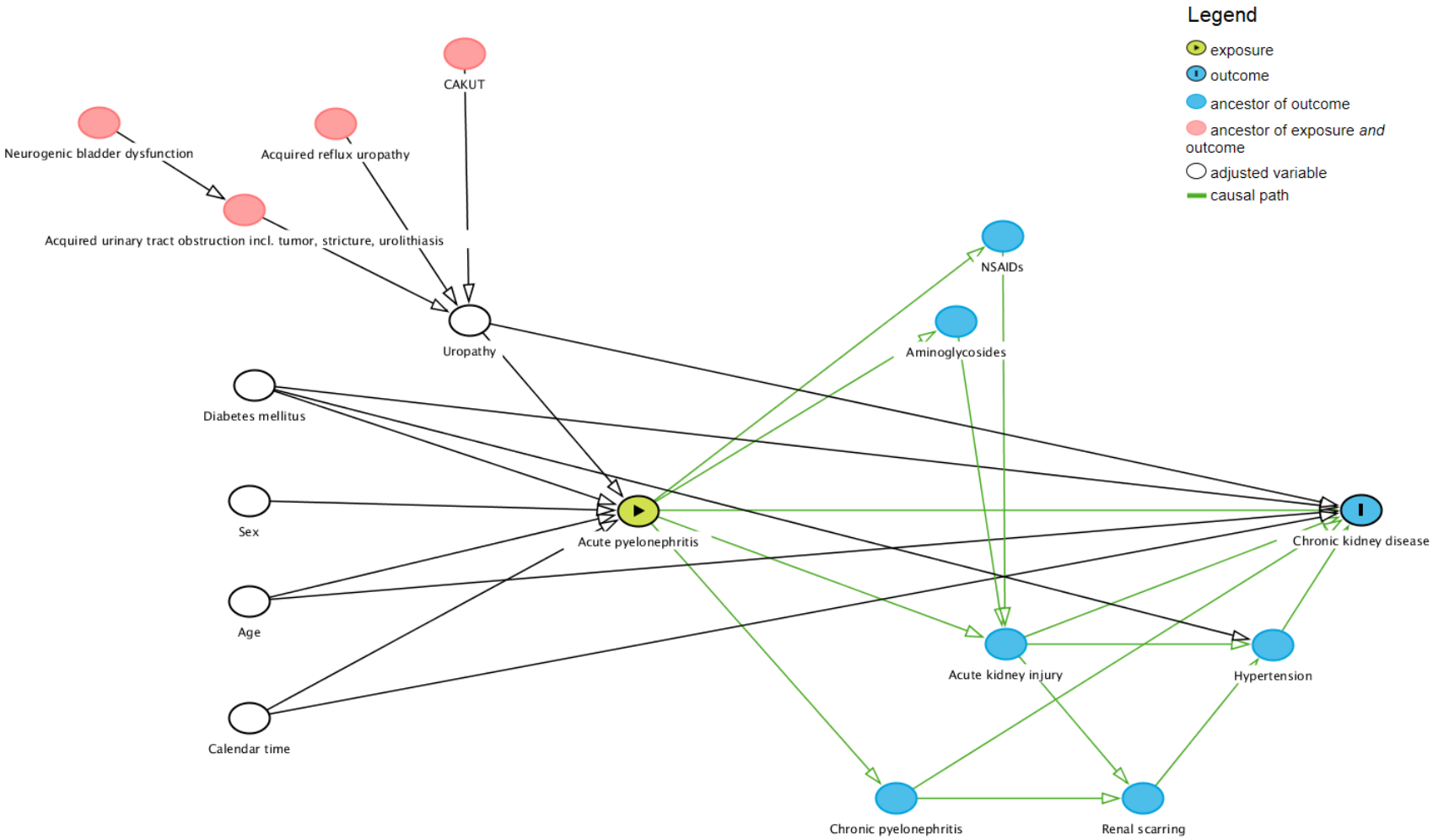

Figure 10. Directed acyclic graph (DAG) for exploration and identification of confounders when assessing the association between acute pyelonephritis on chronic kidney disease.

Note: Based on the above graph, the proposed minimal sufficient adjustment for estimating the effect of acute pyelonephritis on chronic kidney disease include: sex, age, calendar time, diabetes mellitus, and uropathy. The DAG was created with DAGitty.[139]

Abbreviations: CAKUT: congenital anomalies of the kidney and urinary tract; NSAIDs: non-steroidal anti-inflammatory drugs

4.7. Statistical analyses

A summary of the statistical analyses conducted in each study is described below and in Table 1; further details are included in Appendix I-IV.

All statistical analyses were conducted on the remote servers of the Danish Health Data Authority. Data management, including matching, was performed in SAS version 9.4 (Cary, NC, USA), while additional data management, analyses, and visualizations were performed in R version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria).[140]

4.7.1. Descriptive statistics of participants and microbiology (Study I-IV)

Descriptive statistics were described by frequencies and proportions for categorical variables, and by medians with interquartile range (IQR) for continuous variables. We characterized the cohorts according to the descriptive variables described above, overall, and across strata of exposure categories.

In the primary analysis in Study II, we estimated the proportion of APN episodes accompanied by urine culture (performed within 7 days before and 2 days after index date) and positive urine culture (Table 1, Appendix II) as a measure of microbiologically confirmed diagnosis. The study population was sub-grouped in episodes with a) performed urine culture; b) positive urine culture; c) positive urine culture with the same species identified in blood culture; d) negative urine culture; and e) no performed urine culture. Microbial etiologies were tabulated and stratified by calendar periods, patient characteristics, and vital status. In order to assess the sensitivity of the time restriction applied to define urine cultures related to the APN episode, we performed two sensitivity analyses applying extended time windows. For this, we included episodes with a urine culture performed 1) within 14 days before and 4 days after index date, and 2) within 30 days before and 4 days after index date, respectively. In these sensitivity analyses, blood cultures and imaging of the kidneys and urinary tract performed within 7 days before and 4 days after index date were considered related to the APN episode.

In Study IV, we characterized the study population (overall and with or without AKI) at the time of admission with APN. Additionally, we characterized APN patients (who did not meet the criteria summarized in Figure 9) and comparison individuals who were missing prior pCr and baseline eGFR to elucidate potential demographic differences between these groups and those with such records in the laboratory databases.

4.7.2. Incidence rate of hospitalization for acute pyelonephritis (Study I)

In the primary analysis in Study I, we estimated annual APN hospitalization rates per 10,000 PY with 95% CIs, using the Danish population of 1 January of each respective year as denominator.[141] Since the demographics have changed in the source population over the study period (as outlined under “Setting”),

we computed crude rates and rates standardized to the sex and age distribution of the population in year 2000 to allow for comparison of rates over time. Rates were presented graphically and stratified by sex, age groups, age in 0-2 year-olds, diagnosis code, and region of residence. In a sensitivity analyses, we included APN diagnoses from the emergency department for the purpose of capturing the entire severity spectrum of hospital-based APN diagnoses. Finally, we similarly estimated standardized hospitalization rates of selected UTI and sepsis diagnoses per 10,000 PY with 95% CIs in the same source population to identify systematic changes in coding practice that could potentially affect the use of APN diagnoses.

4.7.3. Cumulative incidence of chronic kidney disease (Study III and IV)

In Studies III and IV, we estimated cumulative incidence of CKD as a measure of the absolute CKD risk, using the Aalen-Johansen estimator, accounting for the competing risk of death.[142]

In Study III, we followed patients with first-time hospitalization for APN and their comparison individuals from the index date (date of hospital admission in patients) until *de novo* CKD, death, emigration, or end of follow-up (31 December, 2018), whichever came first (Figure 7). Absolute CKD risks were assessed for the first year and for the subsequent years (year >1-15). In this study, we had to stop follow-up after 15 years due to small risk sets in some strata (total of 798 in the patient cohort and 9120 in the comparison cohort) beyond this time point. We plotted the risk curves with 95% CIs, and results were stratified by age group, sex, calendar period, hypertension, diabetes, and uropathy to assess variations in CKD risk. Moreover, we assessed and plotted the cumulative mortality with 95% CI to illustrate differences in survival time between the cohorts. In this study, we undertook several supplemental analyses, applying different inclusion, exclusion, and outcome criteria to evaluate the robustness of our findings. First, we included APN diagnoses from the emergency department in order to capture the entire spectrum of hospital-diagnosed APN. Next, we defined the exclusion criteria of pre-existing CKD by a single outpatient eGFR <60 ml/min per 1.73 m^2 before index to reduce the probability of including study participants with prevalent CKD. Finally, we defined the laboratory-based CKD outcome by severely decreased eGFR <30 ml/min per 1.73 m^2 to increase specificity of the outcome and to evaluate the influence of potential surveillance bias on the association between APN and CKD.

In Study IV, we evaluated whether patients with first-time hospitalization for APN experienced AKI within the first 30 days after admission; thereby categorizing patients with APN into those with AKI and those without concomitant AKI. Study participants who had at least one pCr measurement and had not been diagnosed with CKD, died, emigrated, or reached end of study during the first 30 days after admission with APN were included and could contribute risk time. Thus, to avoid immortal time bias, we set a landmark at 30 days after admission with APN and followed the two cohorts from this time until *de novo* CKD, death, emigration, or end of follow-up (31 December, 2018), whichever came first (Figure

8).[143] We estimated the absolute CKD risk and risk difference between patients with and without AKI in the first year and the subsequent years (year >1-10); stopping follow-up after year 10 due to a small risk set (2502 patients without AKI and 123 with AKI) beyond this time point.[142] Risk curves were plotted, and results were stratified by age group, sex, hypertension, diabetes, uropathy, calendar period, and AKI stage. In a subgroup analysis, we followed only patients with biochemically confirmed recovery from AKI and who had not been diagnosed with CKD, died, emigrated, or reached end of study during 90 days after admission with APN. They were followed for 1 year from a landmark at 90 days after admission with APN and from this time until *de novo* CKD, death, emigration, or end of follow-up (31 December, 2018), whichever came first.

4.7.4. Cox models (Study III)

To compare the risk of CKD in patients with first-time hospitalization for APN to that in the general population (without APN), we used Cox proportional hazard regression analysis to calculate unadjusted and adjusted (for sex, age, calendar time, diabetes, and uropathy) hazard ratios (HRs) of CKD. The proportional hazards assumption was examined by Schoenfeld residual plots and was initially found to be violated.[144] Therefore, we split follow-up at 1 year, and HRs were presented as period-specific HRs for the first and subsequent years (year >1-15). Both unadjusted and adjusted results were stratified by sex, age group (if number of events allowed), calendar period, hypertension, diabetes, and uropathy. The HRs were tabulated and presented in forest plots.

4.8. Ethical considerations

The studies were approved by the Danish Data Protection Agency through institutional registration (record number 2015–57-0002, Aarhus University record number 2016–051-000001/812). According to Danish legislation, registry-based studies do not require approval from an ethics committee or informed patient consent. However, microbiological data is classified as part of the medical records, and access was approved by the Danish Patient Safety Authority (journal number 31-1521-215/31-1522-60), and the Central Denmark Region (journal number 1-45-70-5-21/1-45-70-5-21).

To comply with the Danish Health Data Authority's rules for data protection, any cell counts below or equal to five (and related cells) were masked with "≤".

5. Results

5

The main findings from Study I-IV are presented below. For a more detailed description, please see the full manuscripts in Appendix I-IV.

5.1. Hospitalization rate for acute pyelonephritis (Study I)

The final study population of Study I comprised 52,479 patients with 61,439 hospitalizations with APN from 2000-2018 (Figure 1, Appendix 1). The median age was 33 years (IQR: 9-64), and the majority of hospitalizations occurred in female patients (73.1%). Overall, the majority of patients did not have any pre-existing comorbidities; 63.5% had a CCI score of 0. Over time, the prevalence of pre-existing comorbidity increased slightly, yet of note, we did not observe increasing prevalence of CAKUT in the age group 0-2 years (Table 2, Appendix I). Further details on baseline characteristics of the study population can be found in Appendix I.

Over the study period, we observed increasing annual crude and sex- and age-standardized hospitalisation rates for APN. Thus, from 2000 to 2018, the standardized rate roughly doubled, increasing from 6.4 (95% CI: 6.1-6.7) to 12.9 (95% CI: 12.5-13.3) per 10,000 PY in the female population and from 2.6 (95% CI: 2.4-2.8) to 4.0 (95% CI: 3.8-4.3) per 10,000 PY in the male population (Figure 11 and Table S3, Appendix I). Rates increased in all age groups (Figure 11B-C) and to the largest extent in the age groups 0-2 years (female and male population), 15-29 year-old (female population), and ≥75 years (female and male population) (Figure 11B-C). Particularly high absolute and relative increases were observed in infants younger than one year (girls: from 7.4 [95% CI: 4.4-10.4] to 63.4 [95% CI: 54.4-72.4] per 10,000 PY, and boys: from 17.1 [95% CI: 12.7-21.5] to 51.2 [95% CI: 43.3-59.1] per 10,000 PY) (Figure 12A-B). In addition to the temporal increase in hospitalization rate for APN, we observed concomitant declines in hospitalization rates for unspecified UTI and sepsis in the most recent years of the study period (Figure S2, Appendix I).

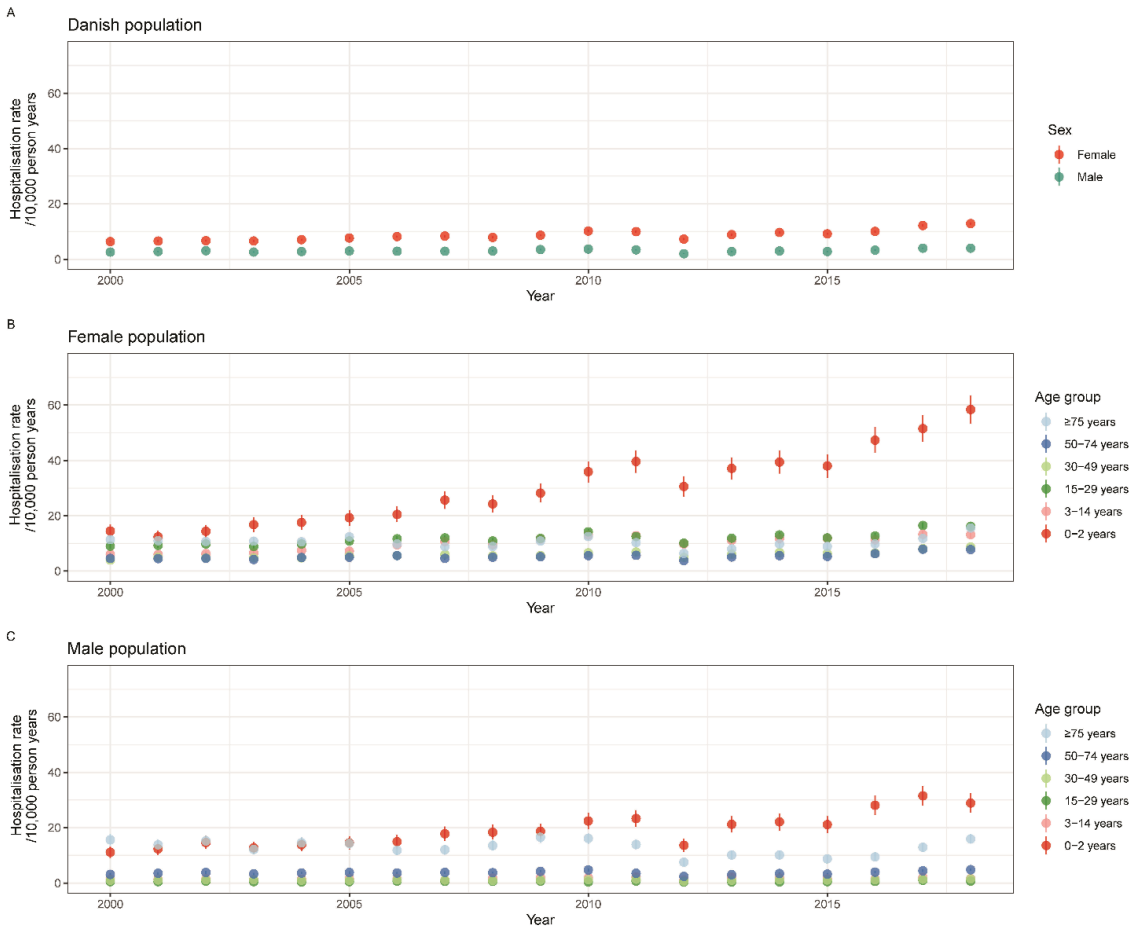

Figure 11. Sex- and age-standardized hospitalization rates for acute pyelonephritis in the Danish female (A and C) and male (A and B) population, stratified by sex (A) and age group (B and C).

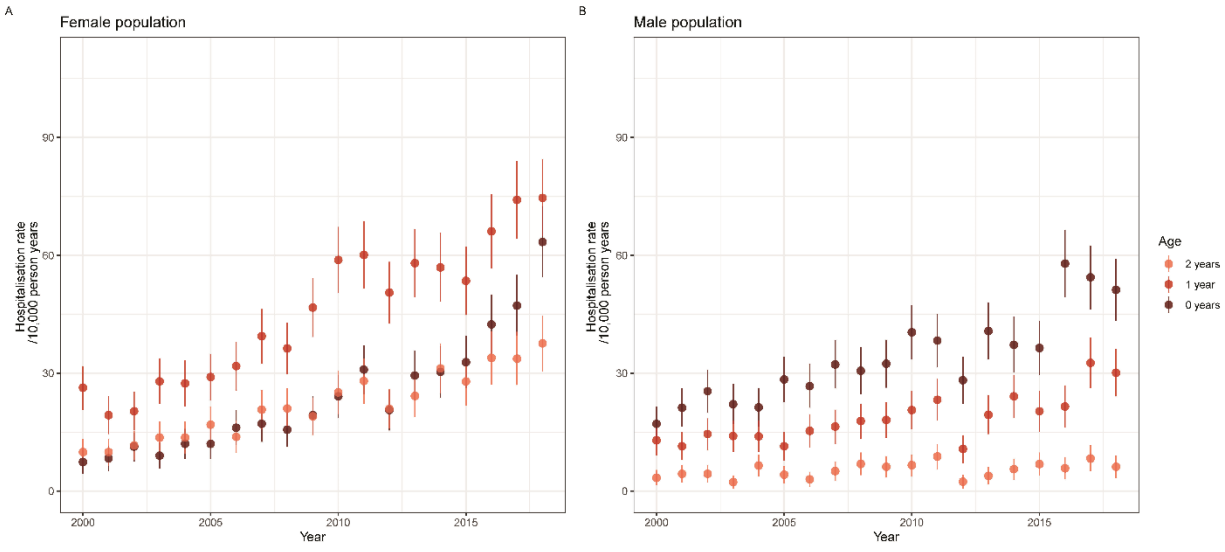

Figure 12. Sex- and age-standardized hospitalization rate for acute pyelonephritis in the Danish female (A) and male (B) population aged 0-2 years, stratified by age in years.

5.2. Microbial etiology associated with acute pyelonephritis (Study II)

Study II included 4,773 patients with 5,338 episodes of APN diagnosed during inpatient admission or emergency department visit at a hospital in the North Denmark Region from 2000-2018. Detailed baseline characteristics of the study population are available in Appendix II. We found that 85.3% of all episodes was accompanied by at least one urine culture performed within seven days before and two days after admission date. Over time, there was an increase in both the proportion accompanied by urine culture (from 75.1% in 2000-2006 to 92.9% in 2013-2018) and in the proportion accompanied by blood culture (from 64.1% in 2000-2006 to 80.7% in 2013-2018) (Table S2, Appendix II). At the same time, the proportion of hospital-diagnosed APN episodes confirmed by a positive urine culture increased from 44.5% in 2000-2006 to 56.7% in 2013-2018. In 2015-2018, as many as 85.8% of children aged 0-2 had their APN confirmed microbiologically (Table S2, Appendix II).

In 2,773 positive urine cultures, a total of 2,849 isolates were identified (Table 3). The main causative pathogen was *E. coli*, accounting for 79.8% of all isolates. During the study period, the frequency of *E. coli* infection increased in all age groups (Figure S2, Appendix II), and the overall proportion of *E. coli* increased from 77.3% in 2000-2006 to 81.9% in 2013-2018. Secondary uropathogens accounted for 18.3% of all isolates, with *Klebsiella pneumoniae* as the most common (4.4% of all isolates). Non-*E. coli* spp., especially *Klebsiella* spp., were relatively more common in men, patients aged 50 years or older, and patients with diabetes or CAKUT. In addition, the 30-day mortality was higher in patients infected with a non-*E. coli* spp. (4%) than in patients infected with *E. coli* (1%).

Defining urine cultures related to the APN episode based on an expanded time-window had minimal effect on the number of positive urine cultures (Table S3, Appendix II).

Table 3. Species isolated from positive urine culture from patients with hospital-diagnosed acute pyelonephritis, overall and by calendar period.

		All isolates	Calendar period[a]		
			2000-2006	2007-2012	2013-2018
	Total, n (%)	2,849 (100.0)	609 (100.0)	917 (100.0)	1,323 (100.0)
Species category	**Species, n (%)**				
Primary (group I)	All	2,305 (80.9)	[b]	[b]	[b]
	Escherichia coli	2,274 (79.8)	471 (77.3)	719 (78.4)	1,084 (81.9)
	Other[c]	20 (0.7)	[b]	[b]	[b]
Secondary (group II)	All	521 (18.3)	127 (20.9)	179 (19.5)	215 (16.3)
	Klebsiella pneumoniae	125 (4.4)	35 (5.7)	44 (4.8)	46 (3.5)
	Klebsiella oxytoca	48 (1.7)	10 (1.6)	19 (2.1)	19 (1.4)
	Klebsiella aerogenes	5 (0.2)	[b]	[b]	[b]
	Klebsiella species, other	9 (0.3)	[b]	[b]	[b]
	Proteus mirabilis	35 (1.2)	13 (2.1)	11 (1.2)	11 (0.8)
	Proteus vulgaris	9 (0.3)	20 (3.2)	24 (2.5)	25 (1.9)
	Enterobacter cloacae	19 (0.7)	34 (5.5)	73 (7.7)	79 (5.9)
	Citrobacter freundii	9 (0.3)	[b]	[b]	[b]
	Citrobacter koseri	5 (0.2)	[b]	[b]	[b]
	Morganella morganii	8 (0.3)	[b]	[b]	[b]
	Serratia marcescens	5 (0.2)	[b]	[b]	[b]
	Enterobacterales, other[d]	17 (0.6)	[b]	[b]	[b]
	Pseudomonas aeruginosa	69 (2.4)	20 (3.3)	24 (2.6)	25 (1.9)
	Staphylococcus aureus	33 (1.2)	11 (1.8)	6 (0.7)	16 (1.2)
	Enterococcus faecalis	7 (0.2)	[b]	[b]	[b]
	Enterococcus species[e]	103 (3.6)	18 (3.0)	37 (4.0)	48 (3.6)
	Aerococcus urinae	10 (0.4)	[b]	[b]	[b]
	Haemolytic streptococci group A/C/G	5 (0.2)	[b]	[b]	[b]

Tertiary (group III) and normal flora (group IV)	All	23 (0.8)	[b]	[b]	[b]
	Coagulase-negative staphylococci	12 (0.4)	[b]	[b]	[b]
	Other	11 (0.4)	[b]	[b]	[b]

[a] The catchment population increased from approximately 494,000 in year 2000 to approximately 590,000 in year 2018.

[b] Not reported in order to comply with the Danish Health Data Authority's rules for data protection.

[c] *Salmonella* species and *Staphylococcus saprophyticus*.

[d] Including *Providencia* species and *Raoultella* species.

[e] Isolated in urine culture not containing a group I or II uropathogen in significant concentration.

5.3. Risk of chronic kidney disease after acute pyelonephritis (Study III)

The study population of Study III consisted of 20,797 patients hospitalized for first-time APN in 2000-2018 and 207,687 matched comparison individuals from the general population without APN, chronic pyelonephritis, and CKD. The median age was 38 years (IQR: 20-61) and the majority of study participants were female (77.1%). Patients hospitalized for APN had higher prevalence of all included comorbidities than comparison individuals (Table 1, Appendix III). The most prevalent comorbidities were hypertension (patients: 12.5%, and comparison individuals: 7.8%), diabetes (patients: 8.5% and comparison individuals: 4.5%), and other urinary tract disease (patients: 8.2% and comparison individuals: 1.5%). In individuals with a registered pCr at baseline, the median baseline eGFR was comparable between patients (88 ml/min/1.73 m^2 [IQR: 74-101]) and comparison individuals (87 ml/min/1.73 m^2 [IQR: 76-99]). Nearly half (45.7%) of study participants were included in the calendar period 2015-2018, and the median follow-up time was 3.3 years (IQR: 1.2-7.3) in patients and 3.9 years (1.6-7.9) in comparison individuals. During follow-up, *de novo* CKD with reduced kidney function was identified in 2,096 (10.1%) patients and in 12,322 (5.9%) comparison individuals, and patients with APN exhibited a shorter time to CKD (median 1.2 years [IQR: 0.4-3.7]) than comparison individuals (median 3.0 years [IQR: 1.2-6.1]).

The absolute risk of CKD was consistently higher in patients with APN than in comparison individuals. At one year after start of follow-up, the CKD risk was 4% (95% CI: 4-5) in the APN cohort and 1% (95% CI: 1-1) in the comparison cohort (Figure 13A and Table S4, Appendix III). During the subsequent years (year >1-15), the CKD risk was 7% (95% CI: 7-8) in patients and 6% (95% CI: 5-6) in comparison individuals. These increased absolute risks of CKD corresponded to unadjusted HRs of 3.63 (95% CI: 3.36-3.91) in the first year following APN admission and 1.35 (95% CI: 1.27-1.43) in the subsequent years (Figure 14). Adjusting for sex, age, calendar time, diabetes, and uropathy only changed relative risks modestly. Thus, the adjusted HRs were 3.86 (95% CI: 3.58-4.16) in the first year and 1.79 (95% CI: 1.69-1.91) in the subsequent years following APN admission (Figure 14). First-time hospitalization for APN was associated with increased risk of CKD in both female and male patients. Thus, in female patients the risk was 3% (95% CI: 3-4) in the first year and 6% (95% CI: 5-6) in the subsequent years, while it was 8% (95% CI: 7-8) in the first year and 13% (95% CI: 12-14) in the subsequent years in male patients. Furthermore, as illustrated in Figure 13, first-time hospitalization for APN was associated with an increased CKD risk in patients compared with individuals without APN across all additional strata (age groups, hypertension, diabetes, uropathy). The absolute CKD risk was, however, very low (≤1%) in all study participants younger than 30 years. In individuals aged 75 years or older and in individuals with hypertension, the difference in risk of CKD in patients and comparison individuals leveled off over the course of follow-up.

Moreover, APN remained associated with an increased CKD risk in patients relative to comparison individuals in all sensitivity analyses (Figure S4-S6, Appendix III).

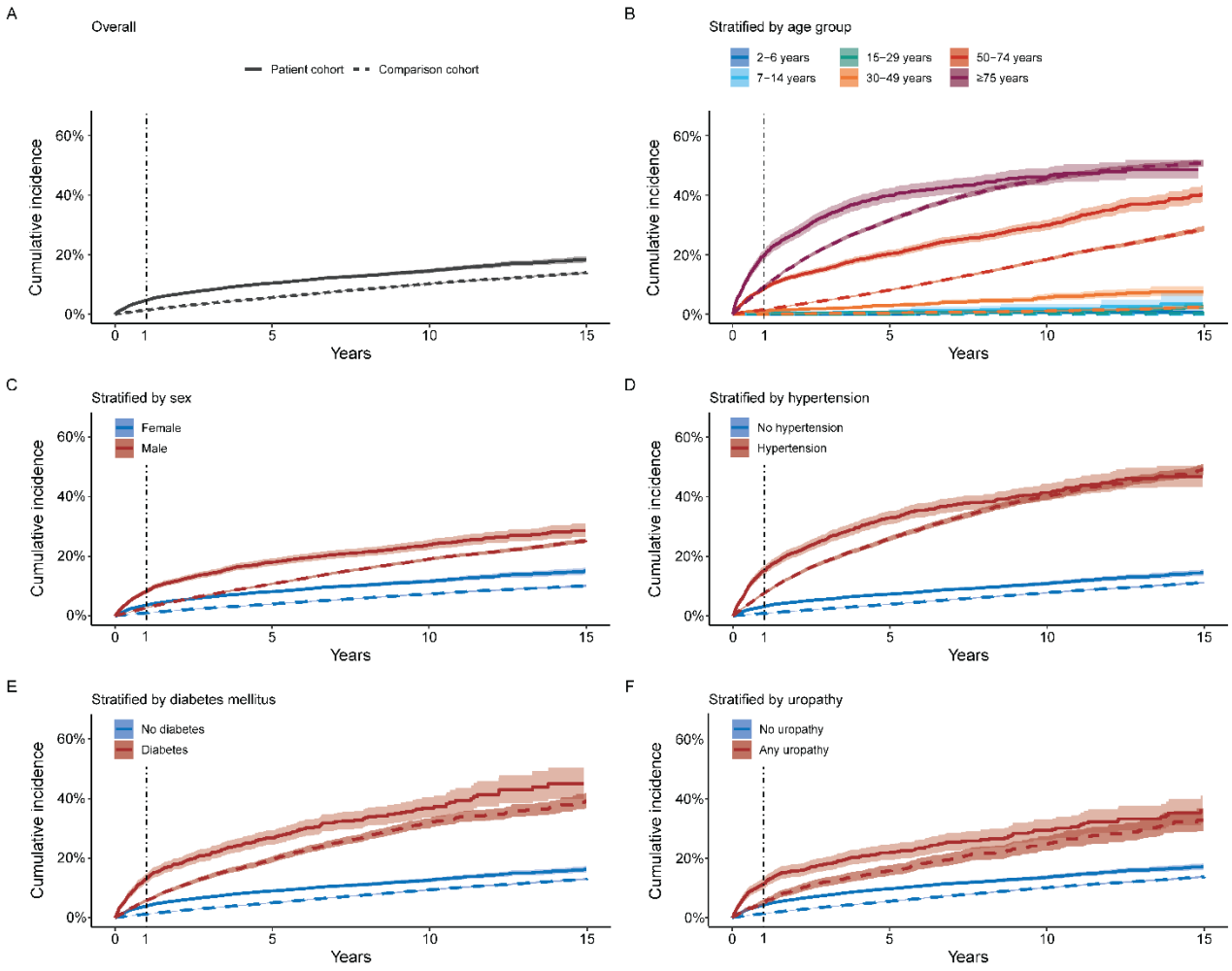

Figure 13. Cumulative incidence of chronic kidney disease (death as competing risk) in patients with acute pyelonephritis and comparison individuals without acute pyelonephritis, stratified by baseline characteristics.

Note: Overall (A), stratified by age group (B), sex (C), hypertension (D), diabetes mellitus (E), and uropathy (F). Patients with acute pyelonephritis are depicted with a solid line; patients without acute pyelonephritis are depicted with a dashed line. Cumulative incidence is shown with 95% confidence interval.

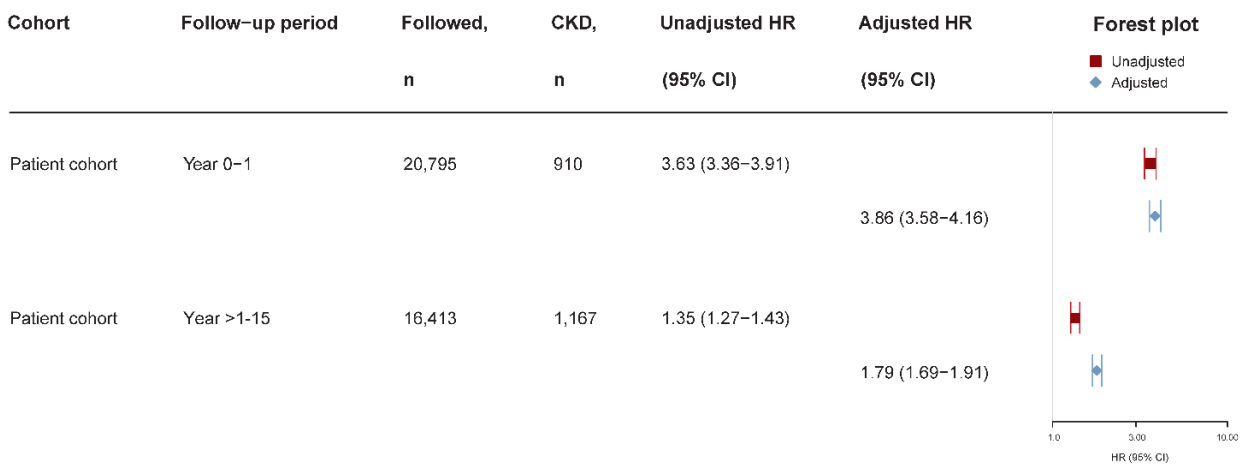

Figure 14. Hazard ratios for chronic kidney disease in patients following first-time hospitalization for acute pyelonephritis.

5.4. Risk of chronic kidney disease after acute pyelonephritis with acute kidney injury (Study VI)

Study IV included a total of 41,243 patients with first-time hospitalization for APN in 2000-2018. Within the first 30 days after admission, 1,892 (4.6%) patients had AKI. Hereof 63.1% had AKI stage 1, 21.0% stage 2, and 15.9% stage 3. Patients with AKI were more likely male (38.7% versus 23.3%), older (median age 64 years [IQR: 48-75] versus 37 years [IQR: 20-63]), and had higher prevalence of comorbidities (hypertension: 31.2% versus 10.2%; other cardiovascular disease: 29.2% versus 15.6%; and diabetes: 19.5% versus 6.9%). than patients without recorded AKI. Detailed baseline characteristics of the cohorts can be found in Appendix IV. From these cohorts, 1,730 patients with AKI concomitant with APN and 17,817 patients without AKI were eligible for follow-up from 30 days after admission. From this time point, the cohort with AKI was followed for a median of 1.5 years (IQR: 0.4-4.1), while the cohort without AKI was followed for a median of 3.4 years (IQR: 1.3-7.4). During follow-up, 514 (29.7%) of patients with AKI developed *de novo* CKD compared with 1,242 (7.0%) of patients without AKI.

We found that the absolute CKD risk following acute pyelonephritis was consistently higher in the patients with concomitant AKI compared with patients without AKI. This association was observed across all characteristics (sex, age groups, hypertension, diabetes, uropathy, calendar periods [first year risk], and AKI stages), and in both the first and subsequent years (year >1-10) of follow-up (Figure 15 and Table 4). Overall, in the first year, the CKD risk was 18% (95% CI: 16-20) in patients with AKI and 3% (95% CI: 2-3) in patients without AKI, resulting in a risk difference of 15% (95% CI: 14-16). In the subsequent years, the CKD risk was 19% (95% CI: 17-22) in patients with AKI and 5% (95% CI: 5-5) in patients without AKI, yielding a risk difference of 14% (95% CI: 13-15). The first-year risk difference decreased from 30% (95% CI: 20-40) in year 2000-2004 to 18% (95% CI: 15-23) in year 2015-2018, and from 34% (95% CI: 16-34) in AKI stage 3 to 13% (195% CI: 1-15) in

AKI stage 1. On note, the absolute CKD risk was low in study participants younger than 30 years (2% [95% CI: 0-4] in patients with AKI and 0% [95% CI in patients without AKI]).

We identified 1,163 patients who, as assessed by eGFR measurements, recovered from AKI subsequent to APN and were eligible for follow-up from 90 days after admission. After one year of follow-up, (from day 90), 208 patients had developed CKD, thus, their risk of CKD was 18% (95% CI: 16-20) and, thus, identical to that of the overall AKI cohort in the first year (from day 30) after admission.

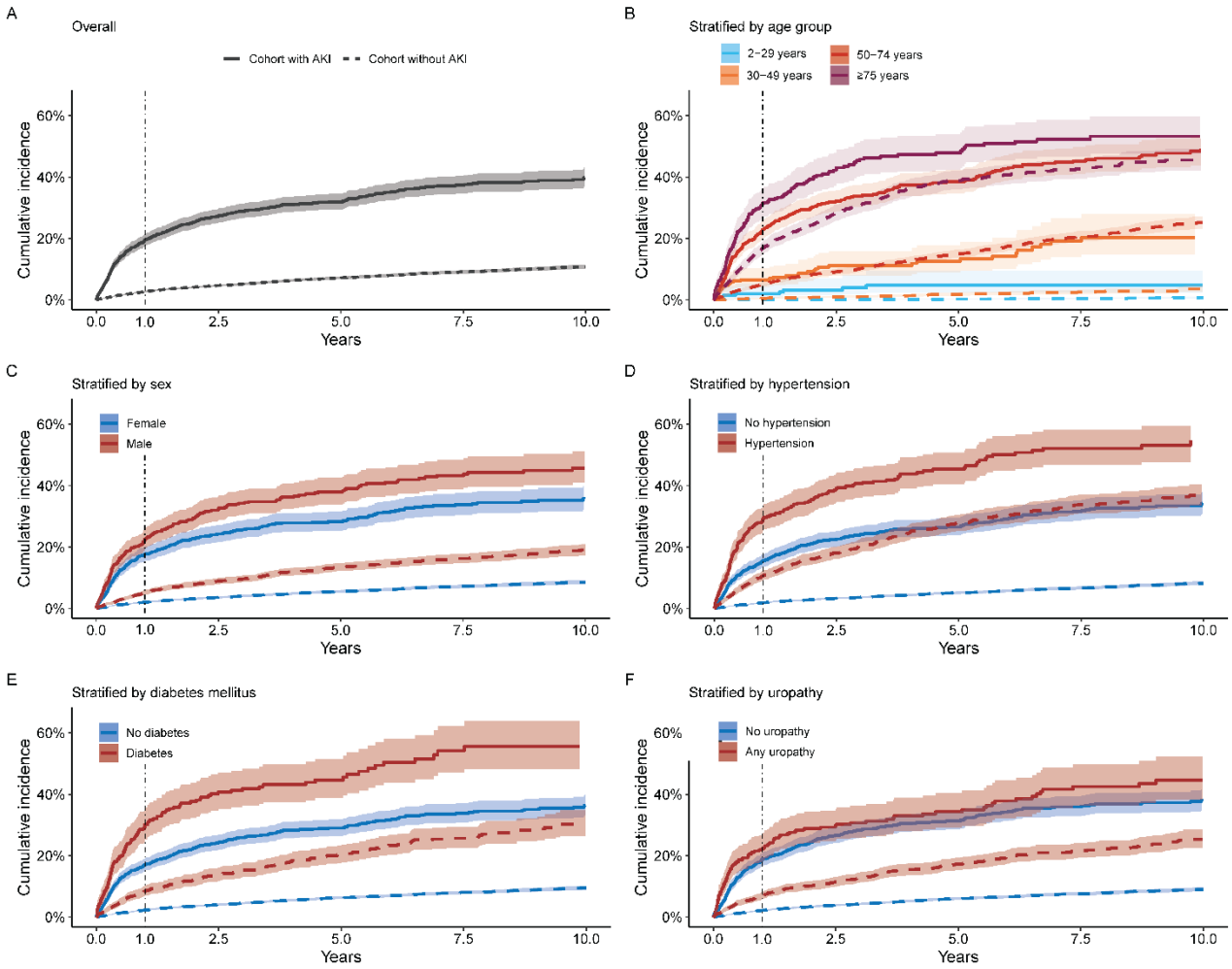

Figure 15. Cumulative incidence of chronic kidney disease (death as competing risk) in patients with and without acute kidney injury following acute pyelonephritis, stratified by baseline characteristics.

Note: overall (A), stratified by age group (B), sex (C), hypertension (D), diabetes mellitus (E), and uropathy (F). Patients with acute kidney injury are depicted with a solid line; patients without acute kidney injury are depicted with a dashed line. Cumulative incidence is shown with 95% confidence interval.

Table 4. Absolute risk of and risk difference for chronic kidney disease (death as competing risk) in patients with and without acute kidney injury following acute pyelonephritis; by follow-up period; overall and stratified by baseline characteristics.

		Cohort with AKI		Cohort without AKI		
	Follow-up period, year	**Events, n**	**Absolute risk, % (95% CI)**	**Events, n**	**Absolute risk, % (95% CI)**	**Risk difference, % (95% CI)**
Overall	0-1	316	18 (16-20)	450	3 (2-3)	15 (14-16)
	>1-10	193	19 (17-22)	728	5 (5-5)	14 (13-15)
Sex						
Female	0-1	178	17 (14-19)	273	2 (2-2)	15 (14-16)
	>1-10	111	17 (14-19)	454	4 (4-4)	13 (12-14)
Male	0-1	138	21 (18-24)	177	5 (4-6)	16 (14-18)
	>1-10	82	24 (20-29)	274	10 (9-11)	14 (12-16)
Age in years						
2-29	0-1	≤5	2 (0-4)	6	0 (0-0)	2 (2-2)
	>1-10	≤5	3 (0-5)	19	0 (0-0)	3 (3-3)
30-49	0-1	18	6 (3-9)	15	0 (0-1)	6 (5-7)
	>1-10	20	9 (5-13)	55	2 (1-2)	7 (6-8)
50-74	0-1	180	21 (18-24)	210	5 (4-5)	16 (15-17)
	>1-10	118	25 (21-29)	422	12 (11-13)	13 (11-15)
≥75	0-1	114	29 (25-34)	219	16 (14-17)	13 (10-16)
	>1-10	49	34 (26- 41)	232	29 (26-32)	5 (1-10)
Hypertension						
No hypertension	0-1	177	15 (13-17)	282	2 (2-2)	13 (12-14)
	>1-10	129	17 (14-19)	503	4 (3-4)	13 (12-14)
Hypertension	0-1	139	27 (23-30)	168	10 (8-11)	17 (15-19)
	>1-10	64	27 (21-33)	225	19 (17-22)	8 (5-11)
Diabetes mellitus						
No diabetes	0-1	225	16 (14-18)	356	2 (2-2)	14 (13-15)
	>1-10	150	18 (15-20)	609	4 (4-5)	14 (13-15)
Diabetes	0-1	91	28 (23-33)	94	8 (6-9)	20 (17-23)
	>1-10	43	26 (19-33)	119	14 (12-16)	12 (8-16)
Uropathy						

No uropathy	0-1	240	18 (15-20)	324	2 (2-2)	16 (15-17)
	>1-10	150	18 (16-21)	550	4 (4-5)	14 (13-15)
Any uropathy	0-1	76	21 (17-25)	126	7 (5-8)	14 (12-16)
	>1-10	43	23 (17-29)	178	13 (11-14)	10 (7-13)

Abbreviations: AKI: acute kidney injury; CI: confidence interval.

6. Discussion

6

6.1. Comparison with existing literature

6.1.1 Occurrence of acute pyelonephritis (Study I)

Our observations of an increasing hospitalization rate for APN in the Danish population extend previous findings of a rising incidence of urinary tract, including APN, and systemic infections caused by uropathogens in the Danish population over the past decade.[44,145,146] The temporally increasing APN incidence is also supported by international findings, including the observations by Ki et al and Kim et al from South Korea during 1997-1999 and 2010-2014, respectively.[33,98] However, compared with our estimates, the incidences reported by Kim et al in the adult female population of South Korea were much higher (inpatient rate: 18.9 per 10,000 persons; and outpatient rate: 54.4 per 10,000 persons).[98] Also, the overall APN incidence rates reported by Czaja et al from the United States during 1997-2001, including all ages but not recurrent episodes, were approximately two-fold greater (inpatient and outpatient: 15-17 per 10,000 persons in the female population and 3-5 per 10,000 in the male population) than our year-2000 estimates.[17] However, the inpatient-only hospitalization rates reported by Czaja et al were lower (3-4 per 10,000 in the female population and 1-2 per 10,000 male population) compared with our estimates. The finding of incidences being highest in infants, young women, and older individuals was similar between studies. Moreover, consistent with our findings, other studies have reported the APN hospitalization rate to have increased over time in infants.[101,102] Thus, a nearly nine-fold increased incidence was reported by Copp et al among infants in California during 1985-2006.[101] The trend was further supported by the study by Sood et al during 2006-2011, showing increasing incidence of female emergency department visits for UTIs, including pyelonephritis, with the highest incidences in infants and adolescents.[102]

6.1.2. Microbiology, length of stay, and mortality associated with acute pyelonephritis (Study II)

Substantial geographical variation in pathogen distributions have been described,[147] and consistent with this, our findings differ from those reported in a recent global review describing a prevalence of *E. coli* in APN of merely 45%.[23,105] However, our findings are in line with observations by Czaja et al and Laupland et al from studies from Canada[17,99] reporting *E. coli* as the causative pathogen in approximately 80% of female APN cases, and several studies describing a lower *E. coli* prevalence in men (53-74%) and older age groups (59-76.1%).[17,48] Moreover, we found that non-*E. coli* spp. especially *Klebsiella* spp. were more frequent in patients with diabetes or CAKUT. Although diabetes is not generally recognized to favor specific bacterial pathogens, a comparable proportions of *Klebsiella* spp. causing UTIs in diabetic patients has previously been described.[148-150] Similarly,

Klebsiella spp. has been reported to cause a relative large proportion of UTIs in children with CAKUT.[151]

The length of stay related to hospitalization for APN reported in the existing literature was mostly longer than our estimates indicate. Thus, in previous accounts, it has range from 6-27 days, depending on the population between populations.[50,107,109] To the best of our knowledge no other study has assessed length of stay by the causative pathogen.

Many previous accounts of mortality in complicated UTI were in the range 7-33%, depending on the patient populations,[43,49,50,107-109] however, 30-day mortalities comparable to those observed in the present study (1-2%) have been described in multinational and nationwide samples.[50,100] Moreover, a higher 30-day mortality has previously been reported in patients with complicated UTIs caused by non-*E. coli* (*Klebsiella* spp.: 10.3-30.8%) that by *E. coli* (5.8-28.1%),[50,109] and male sex, older age, and comorbidities such as diabetes likely contribute to this association.[50,152]

6.1.3. Risk of chronic kidney disease after acute pyelonephritis (Study III)

The relative risk of CKD we observed in young adults following APN hospitalization (adjusted HR 6.28 [95% CI: 3.71-10.63]) was fairly comparable with the adjusted HR for kidney failure of 4.03 (95% CI: 3.16-5.14) reported by Calderon-Margalit et al in adolescents/young adults with a history of pyelonephritis.[66] Calderon-Margalit et al speculated that their observations implied an even greater risk of earlier stages of CKD. However, in the present study, we did not find the absolute risk of CKD to exceed 1% in patients with APN younger than 30 years. When examining the association between APN and CKD by sex, we found a larger absolute difference in CKD risk in male patients than in female patients, while female patients had a higher relative CKD risk. The sex-stratified absolute risk estimates contrasted with reports of the overall sex-specific CKD prevalence being greater in the female population.[153] Yet, the higher CKD prevalence in women likely reflected a longer life expectancy for women compared with men at all ages and eGFR categories, other than in severely decreased eGFR.[90,154] The relatively higher HR in female compared with male APN patients is consistent with prior observations of higher frequency of post-infection kidney scars in female patients.[65,155]

6.1.4. Risk of chronic kidney disease after acute pyelonephritis with acute kidney injury (Study IV)

Only few prior studies have examined outcomes of AKI specifically in APN, and a comparison between studies demonstrates some discrepancies. The South Korean study by Jeon et al reported AKI in as much as 61.2% of adult patients hospitalized with pyelonephritis but did not identify any cases of progression to CKD in patients without pre-existing reduced kidney function.[115] In line with our findings, they did, however, observe a higher proportion of subsequent CKD in patients with pre-existing kidney disease and AKI compared with patients without AKI (10.3% versus 2.7%).

We found that the risk of CKD following APN was higher in patients with AKI and pre-existing comorbidities including acquired obstructive or reflux uropathy, including urolithiasis. In line with this, the study by Lee et al including patients with urolithiasis with or without APN and AKI, observed the highest risk of CKD in patients with concomitant APN and AKI compared with patients without one or both of these conditions.[116]

Our principal finding of an increased risk of CKD in patients with AKI is overall in support of the existing literature on all-all cause AKI and CKD. Thus, prior observational studies have also shown a strong association between AKI and subsequent CKD, also in patients who initially recover renal function following AKI.[156]

6.2. Methodological considerations

Population-based registries, like the medical databases used in Study I-IV, are important data sources for large-scale studies of disease occurrence and prognosis in all population subgroups over vast time spans and with virtually complete follow-up. Thus, such registries enables us to conduct cost-effective observational studies of associations that would often not be feasible, or ethical, to study in an experimental setting.[5] The tax-funded Danish healthcare system with, at least in principle, equal access for all residents to health care and the nationwide or regional data collection minimize the risk of distorted selection of study participants. Data collection is prospective and conducted routinely as part of daily clinical health care, thereby reducing the risk of systematic deviation, *i.e.*, bias, of results due to misclassification of the exposure, *e.g.*, by recall bias.[117]

Importantly though, when basing studies on secondary data, the researcher does not have influence on which variables are measured, how these variables are measured, and with what level of detail the variables are recorded.[5] Thus, if registry data cannot be supplemented with, *e.g.*, information from medical records, accuracy might be limited due to a lack of clinical information that is usually obtained by medical history and physical examination, such as presence and severity of signs and symptoms of disease.

6.2.1. Study design

The aims of the present epidemiologic studies were, in essence, to estimate disease occurrence by the measures of prevalence (Study II), incidence rate (Study I), and risk (Study II-IV), and to compare these measures across different characteristics.[96] For these purposes, we found the cross-sectional study design (Study II) and the cohort study design (Study I-IV) suited.

The cross-sectional design was applied in Study II for estimating the prevalence of acquired and positive urine cultures and microbial etiologies in patients with hospital-diagnosed APN. Since diagnostic urine cultures are often acquired already prior to hospital admission (or prior to admission with APN as discharge diagnosis), a longitudinal study with the premise of temporality, *i.e.,* the outcome must occur after the exposure, was not feasible. Instead, the cross-sectional design lend itself

to study exposure and outcomes recorded at the same point in time (or within a short window of time) as a snapshot of the population status of exposure and outcome at this time.[96] For the purpose of describing temporal changes, we extended the study design by conducting a serial cross-sectional study, including the entire study period from 2000-2018 and reporting prevalences in three consecutive calendar periods. Some consideration must, however, be kept in mind when interpreting these results due to the lack of temporality. Moreover, if clinical practice regarding acquisition of urine culture changes over time, *e.g.*, if urine culture becomes more easily available or recommended, it could potentially affect coding practice and the likelihood of being diagnosed with APN; thus, complicating comparison of the estimated prevalences over a longer period of time. These changes and the direction of the associations must, therefore, be interpreted with some caution.

For estimating the clinical outcomes by type of uropathogen, Study II relied on a population-based cohort study design in line with that used in Studies I, III, and IV to estimate the risks of APN and CKD. Cohort studies facilitate observation of a, potentially large, group of individuals, which serves to increase the precision of the estimates. Furthermore, it is possible directly estimate risks and to study outcomes that are rare or separated in time from the exposure.[5] A cohort is defined as any designated group of individuals who are followed over a period of time.[157] In the present studies, the overarching cohort was designated by the common trait of having been diagnosed with APN at a hospital in Denmark during 2000-2018 (Figure 9), and this cohort fostered all other cohorts under study. The study cohort of Study I was defined by calendar time and heterogeneous with respect to potential risk factors for the APN outcome, thereby enabling us to make comparisons of incidence rates between subgroups within the cohort.[5] In Study II-IV, the study cohorts differed by exposure status (*i.e.*, *E. coli* versus. non-*E. coli* spp., APN versus no APN, and AKI versus no AKI), which allowed for comparison of risks of the outcome.

In Study III, we aimed to compare the risk of CKD between patients with APN and individuals from the general population without APN. For this purpose, we used balanced cohort matching (by sex, age, calendar period, and municipality in a 1:10 ratio) as a design option to control confounding (confounding is described in more detail in the following). We, thus, ensured an equal distribution of possible confounding variables in the patient and comparison cohorts.[158,159] Thereby, the cohorts differed with respect to exposure (APN), but were comparable on the matching variables at the start of follow-up. Matching prevents confounding by the matching variables only in the study participants at the start of follow-up, *i.e.,* prior to the occurrence of outcome and competing events and censoring. Thus, matching can by design to some extent control confounding when comparing the crude risks at the start of follow-up.[5]

6.2.2. Sources of error

Despite the many strengths associated with the setting, data sources, and study designs applied in the present studies, observational research inherently has some common limitations, which will be described in the following. Two main types of error, random error and systematic error, must be considered and reduced to obtain results that are as accurate as possible.[96]

Random error

Random error is variability in the data that is not completely determined by measurable factors, but resulting from chance.[96,157] The presence of random error decreases precision of the estimates, which can be quantified by the width of confidence intervals around the point estimates. Thus, a narrow interval indicates high precision, while a wide interval indicates low precision.[96] Precision can be improved by increasing the size of the study population.

Systematic error

In contrast, errors that persist regardless of study size are systematic errors, termed bias. Thus, in large-size studies, bias is the major concern relative to random error. [96] Bias can arise at any step in the process of conducting a study by error in the conception and design of the study or in collecting, analyzing, interpreting, and reporting data.[157] Bias affects the validity of a study. While the internal validity refers to how accurately the study estimates the actual associations within a given study population, external validity refers to the ability to generalize the results from a given study to other populations.[5,160] Bias can conceptually be divided into three categories, namely selection bias, information bias, and confounding. Whereas selection bias and information bias must be forestalled in the design and data collection phase and cannot be corrected in the analytical phase, confounding can be controlled in all of these phases.[5]

Selection bias

Selection bias arise when the association between the exposure and the outcome differs for participants and non-participants who are eligible for inclusion. This bias can occur if the outcome is associated with the selection of participants or if the outcome affects study participation. The population-based medical registries, free access to health care, and virtually complete follow-up minimize the risk of selection bias.[117]

Yet, the patient cohort in Study III was restricted to include patients with a recorded pCr measurement within three years before hospitalization or on the admission date. This restriction was applied to ensure that included patients were covered by the laboratory databases and could therefore have eGFR measurements recorded during follow-up, which was a prerequisite for the laboratory-based outcome assessment. In doing this, we risked to select a subset of patients for inclusion with a higher *a priori* risk of CKD. However, we assessed the characteristics of patient who were not included based on this

restriction and found that they were largely comparable to included patients by all characteristics but calendar period. Since comparison individuals would be substantially less likely to have a recorded pCr in in relation to the index date than APN patients, we expected that imposing the same restriction in construction of this cohort would lead to overrepresentation of diseased individuals in the comparison cohort. We therefore opted for matching by municipality as a proxy for laboratory register coverage. Yet, when we required only APN patients to have a prior pCr measurement, we designed the study so that APN patients (if they had a baseline eGFR measurement below CKD cut-off) were at higher risk of being diagnosed with CKD within the first 90 days after hospitalization. Therefore, we conducted a sensitivity analysis in which we excluded all individuals with a single outpatient eGFR <60 ml/min/1.73 m^2 prior to index when we assessed the risk of CKD, and we found the association to be robust (Figure S5, Appendix III). Another possible way of handling this in the study design could be by restriction; restriction either of the study period to years with complete laboratory database coverage or restriction of the study population to include only patients and comparisons residing in municipalities that have gained coverage. However, for the current analyses, we opted to include as much information available as possible. The selection of patients and comparison individuals with index in the most recent calendar period would result in a shorter follow-up time, however, we did not expect this shorter follow-up to affect the comparison of CKD risk between the cohorts due to the matched design.

Similar selection of patients with index in the most recent calendar period with laboratory database coverage was present in Study IV, as we included only patients with at least one pCr measurement within 30 after admission (for assessment of CKD status and as a qualifier of laboratory database coverage). We therefore expected these patients to have been more ill following hospital admission, *i.e.*, to have presented with an indication for kidney function assessment, than patient who were not included in our study. Yet, we found observed association was robust as the risk of CKD remained elevated across calendar periods and AKI stages.

Selection bias can also result from loss to follow-up if continued study participation is related to the association between the exposure and the outcome. In Study III-IV considering the risk of a non-fatal outcome, we handled the risk of such a bias from the competing risk of death by using appropriate statistical methods (the Aalen-Johansen estimator).

Information bias

Information bias refers to the systematic error of misclassifying information regarding exposure or outcome. Misclassification can be described as either non-differential, when not related to other variables, or differential, when misclassification of the outcome depends on the exposure (or vice versa). Whereas non-differential misclassification dichotomous outcomes typically dissolve the association between the exposure and the outcome (bias towards the null), differential

misclassification is more unpredictable and can both reduce and increase the association.[5] Many factors related to the patient and the health care available and provided are of importance for correct classification of information. Thus, the classification is liable to influence from, *e.g.*, the health-seeking behavior of the patient, surveillance bias, and the quality of diagnostic coding. Many of the diagnoses in the DNPR have been found to have high validity, *e.g.,* the diagnoses of the 19 conditions included in the CCI score have been shown to have a positive predictive value of 82% or higher.[122,138] Still, some further considerations are in place.

Misclassification of exposure might exert influence in several of the present studies. In Study II-IV, discharge diagnoses of APN might be misclassified, *e.g.*, by inclusion of non-infectious reasons for nephritis or coding errors. However, prior studies validating diagnoses of APN in the DNPR in children and adults demonstrated acceptable positive predictive values of 80% (95%CI: 70-88) and 76%, respectively.[34,161] Moreover, the results from Study II (*e.g.*, the proportion of episodes diagnosed in 2013-2018 with a positive urine culture was 56.7%) indicated that the diagnoses used for inclusion in this study were indeed representative of patients with severe UTI. In Study IV, the AKI exposure could also be subject to misclassification if AKI was not captured (by either of the three AKI criteria) due to missing eGFR measurements. Thus, it is possible that patients with AKI could be misclassified as not having an AKI in which case, the association between AKI and CKD in patients with APN would expectedly be attenuated. However, when stratifying the risk of CKD in the first year of follow-up by calendar period, we found, that the association persisted across calendar period with differing degree of completeness of the laboratory databases (*e.g.,* in 2015-2018, the first-year CKD risk difference was 16 [95% CI: 15-17]). Lastly, also the classification of recovery might be misclassified due to lack of recorded eGFR measurements. Therefore, we only assessed CKD risk in patient with laboratory data documenting recovery.

Misclassification of outcome could also be present in the studies. Similar to misclassification of APN exposure, APN as outcome (Study I) could be misclassified and the degree of misclassification might vary over time due to, *e.g.*, changing diagnostic practices. Thus, it is conceivable that discharge diagnoses became more specific over time with regards to anatomical location of infection, due to, *e.g.*, more accessible and improved diagnostics modalities; as indicated by the concomitant decreasing hospitalization rates for unspecified UTI and unspecified sepsis in the most recent years (Figure S2, Appendix I) and by the results of Study II showing increasing utilization of microbial diagnostics over time (Table S2, Appendix II). Moreover, the CKD outcome (Study III-IV) could be misclassified in a similar way as described regarding the AKI exposure; *i.e.*, missing records of laboratory data would decrease the likelihood of being diagnosed with CKD based on the laboratory-based definition. This misclassification would likely be differential as patients with APN more often had a baseline eGFR measurement; likely due to both laboratory testing in relation to the APN episode and due to other conditions (possible shared risk factors with CKD) requiring monitoring of kidney function. Such a

non-differential misclassification, *i.e.*, surveillance bias, would be expected to augment the association between APN and CKD in comparison with general population individuals without APN. We sought to evaluate the degree of this bias in the sensitivity analysis defining the laboratory-based CKD outcome by severely decreased eGFR <30 ml/min/1.73 m^2 as we expected that surveillance bias would to lesser degree affect this outcome. In this analysis, we found that the association was reduced in size but persisted throughout follow-up and across all strata (Figure S6, Appendix III).

Confounding

Confounding is inherent in observational studies, and if confounding is present, a simple comparison of exposed and unexposed groups will not validly reflect the isolated effect of an exposure.[5] A confounder is a variable that is associated with both the exposure and the outcome without being an intermediate step on the exposure-outcome pathway.[162] Confounders can be identified based on existing knowledge and construction of DAGs.[159] In this way, in Study III, we constructed a DAG for identification of potential confounders in the association between APN and risk of CKD. When identified, and if data allows, known, presumed confounders can be controlled by design, *e.g.*, by matching, or in the analysis of data by, *e.g.*, standardization (Study I), stratification (Study I-IV), or adjustment for confounding variables in regression analyses (Study III). Thus, we applied several of these strategies for controlling known confounders. Still, residual confounding due to misclassified information on the confounding variables could still remain. As an example, diabetes mellitus might not be fully adjusted for if the information we used for assessing the presence of this confounder (diagnosis codes and redeemed prescriptions) did not capture all patients that truly have diabetes mellitus. To minimize the risk of residual confounding as much as possible in Study III, we adjusted for confounding variables in the Cox model and stratified the cumulative incidences and the Cox model by potential important confounders.

In contrast to known or measured confounding, unmeasured confounding refers to confounding by variables that are not observed. Unmeasured confounding is an important limitation of observational studies relying on data from medical databases as information not only on clinical signs and symptoms might not be available but also much information on lifestyle factors such as obesity, smoking, and alcohol consumption is not routinely recorded.

6.2.3. Statistical considerations

Although all statistical methods applied in the present studies are well-described in the literature, a few statistical considerations require further discussion.

First, matching does not necessarily eliminate the need to control the matching variables in the statistical analysis. If the exposure and the matching variables affect the risk of disease or censoring (competing risks, *i.e.*, death, and loss to follow-up), the original balance produced by matching will

not be retained for the risk set available for the analysis over time.[5] Thus, an association of exposure and matching variables among persons under observation and the observed person-time might arise over the course of follow-up, *e.g.*, by depletion of persons susceptible to the outcome under study.[159,163] Therefore, risk factors used for matching must also be controlled in the analysis in order to valid estimates for comparison of risks, and we did so by including the matching variables sex, age, and calendar time as confounding variables for adjustment in Study III.

Second, as previously described, we applied a landmark analysis approach for the analyses in Study IV (Figure 8).[143] This was done to clearly mark when follow-up time started (*i.e.*, after 30 days and 90 days, respectively) to ensure that no follow-up time was included before the time for exposure assessment (*i.e.*, AKI and recovery from AKI, respectively) had passed as this would result in immortal person-time and the associated bias.[5]

7. Main conclusions

7

Based on the four studies comprising this PhD thesis, we conclude that:

I. The hospitalization rate for acute pyelonephritis nearly doubled in Denmark from 2000 to 2018. While the increase was evident in both sexes and age groups, it was largely driven by a prominent increase among young children which was not explained by enlarging comorbidity burden.

II. Microbiological testing was increasingly integrated in the diagnostic workup in patients with hospital-diagnosed APN. The bacterial spectrum was largely stable over time with *E. coli* as the main causative pathogen, and non-*E. coli* spp. were mainly causing APN in patients of male sex, older age, with diabetes mellitus or CAKUT.

III. A first-time hospitalization with APN was associated with an increased risk of CKD across patient characteristics. The absolute CKD risk was, however, very low (≤1%) in all study participants younger than 30 years. Patients with non-*E. coli* spp. APN had slightly longer hospital stay and higher 30-day mortality than patients with *E. coli* APN.

IV. Occurrence of AKI concomitant with APN was associated with increased short- and long-term risk of *de novo* CKD. Nearly 5% of patients with APN developed AKI within 30 days following hospital admission and in almost one third of these patients, kidney function impairment advanced to CKD within 10 years. The risk of CKD remained high in patients with occurrence of AKI despite documented recovery from AKI.

8. Clinical implications and future perspectives

8

Our findings of increasing incidence of hospitalization for APN together with the observed increased risk of *de novo* CKD in these patients warrant continued efforts targeted towards preventing this serious infection and its potential short- and long-term consequences. Such preventive measures may include identification of patients with conditions conveying increased risk of APN, including diabetes and other pre-existing urinary tract diseases, and optimal management of these conditions. Also, it is imperative to thoroughly consider the indications for and duration of urinary catheterization, which is known to be a major risk factor for APN, yet still widely used.[21,164,165] it The present studies point to a need for paying special attention to UTIs in young children in order to improve our understanding of the underlying reasons for the prominent increase observed in hospitalization rate for APN.

Early initiation of efficient treatment, including antibiotic treatment, is crucial for mitigating adverse outcomes of APN, and should be initiated without delay in patients with suspected APN. Condition-specific microbiological data can advance empirical antibiotic selection towards a personalized approach.[112] To guide empirical antibiotic treatment, up-to-date local microbiological data are needed. According to our findings, empirical treatment should be aimed at covering the most common causative organism, *E. coli*, yet considering possible non-*E. coli* APN in patient with characteristics such as male sex, older age, and diabetes. However, including the antimicrobial susceptibility patterns in uropathogens causing APN are needed in future studies to guide the most optimal strategies for empirical antibiotic treatment.

The augmented risk of CKD following APN, in particular in patients developing concomitant AKI, furthermore brings attention to a potential under-recognized need for follow-up reaching beyond the acute hospitalization, *e.g.*, by repeated creatinine measurements, to detect early signs of deteriorating kidney function in groups of patients previously considered at low risk of developing long-term sequelae from APN.

Finally, future extensions of the present studies could benefit from assessing the usefulness in different populations of the newly-developed full age spectrum equations for assessing eGFR in both children, adolescents, and adults. We found the absolute risk of CKD to be very low in children and young adults with APN, however, a large potential may lie in evaluating the effects of amending the CKD definition to include age-specific thresholds for CKD. This, proposedly, might facilitate earlier identification of CKD onset in young individuals, at a point when progressive kidney damage may still be preventable.[166] Thus, studies in this field may prove essential for the optimization of current methods of evaluating kidney function in all ages with a potential for notable clinical impact.

9. Summary

9

Acute pyelonephritis (APN), a severe manifestation of urinary tract infection, has an annual incidence around one per 1000 persons. Following APN, a patient may develop kidney scarring and impairment of kidney function, and some studies suggest that this can lead to chronic kidney disease (CKD). The incidence of CKD related to APN is, however, scarcely described in the existing literature. With this thesis, containing four registry-based Danish studies, we describe the temporal changes in hospitalization rate for APN, the use of microbiological diagnostics and temporal changes in microbiological findings, and examine the risk of *de novo* CKD following APN and the extent with which acute kidney injury (AKI) marks an increased risk of CKD in patients with APN.

Study I included 61,439 APN hospitalizations in 52,479 patients. From 2000 to 2018, the hospitalization rate of APN per 10,000 person years increased from 6.4 (95% CI: 6.1-6.7) to 12.9 (95% CI: 12.5-13.3) in the female population and from 2.6 (95% CI: 2.4-2.8) to 4.0 (95% CI: 3.8-4.3) in the male population. The increase occurred in both sexes and all age groups, yet largely driven by a prominently increasing rate among young children, which was not explained by increasing comorbidity burden.

Study II described the microbiological findings in 5,002 patients with hospital-diagnosed APN in the North Denmark Region. The proportion of microbiologically confirmed APN increased over time from 2000-2018. Overall, 85% of all APN episode was accompanied by urine culture, while 52% had a positive urine culture. *Escherichia coli* accounted for 80% of all APN episodes, and non-*E. coli* spp. were mainly causing APN in patients of male sex, older age, with diabetes or congenital anomalies of the kidney and urinary tract. Patients with non-*E. coli* spp. APN had slightly longer hospital stay and higher 30-day mortality than patients with *E. coli* APN.

Study III examined the risk of *de novo* CKD following APN, using a matched cohort design from 2000-2018. In 20,797 patients with APN, the absolute risk of CKD was 4% (95% CI: 4-5) in the first year of follow-up and 7% (95% CI: 7-8) in year >1-15, compared with 1% (95% CI: 1-1) and 6% (95% CI: 5-6) in 207,687 general population comparison individuals. The corresponding adjusted hazard ratio was 3.86 (95% CI: 3.58-4.16) in the first year and 1.79 (95% CI: 1.69-1.91) in year >1-15.

Study IV examined if AKI in 41,243 patients with first-time hospitalization for APN was associated with increased risk of *de novo* CKD. Nearly 5% of patients with APN developed AKI following hospital admission and in 30% of these patients, kidney function impairment advanced to CKD. The risk difference for CKD between 1,730 patients with AKI and 17,817 patients without AKI was 15% (95% CI: 14-16) in the first year and 19% (95% CI: 17-22) in year >1-10. The risk of CKD remained high in patients with occurrence of AKI despite documented recovery from AKI.

10. Dansk resumé

10

Akut pyelonefritis (APN), en alvorlig form for urinvejsinfektion, har en årlig forekomst på ca. én pr. 1000 personer. Efter APN kan patienter udvikle ar i nyrevævet og svækkelse af nyrefunktionen, og nogle undersøgelser tyder på, at dette kan føre til kronisk nyresygdom (CKD). Forekomsten af CKD relateret til APN er dog sparsomt beskrevet i den eksisterende litteratur. Med denne afhandling, som indeholder fire danske register-baserede studier, beskriver vi de tidsmæssige ændringer i indlæggelsesraten for APN, brugen af mikrobiologisk diagnostik og tidsmæssige ændringer i mikrobiologiske fund, samt undersøger risikoen for nyopstået CKD efter APN og i hvilket omfang akut nyreskade (AKI) markerer en øget risiko for CKD hos patienter med APN.

Studie I inkluderede 61.439 indlæggelser med APN blandt 52.479 patienter. Fra år 2000 til 2018 steg indlæggelsesraten for APN pr. 10.000 personår fra 6,4 (95% CI: 6,1-6,7) til 12,9 (95% CI: 12,5-13,3) i den kvindelige befolkning og fra 2,6 (95% CI: 24. -2,8) til 4,0 (95% CI: 3,8-4,3) i den mandlige befolkning. Stigningen forekom i begge køn og alle aldersgrupper, men blev i vid udstrækning drevet af en markant stigende rate blandt små børn, hvilket ikke kunne forklares af øget forekomst af følgesygdomme.

Studie II beskrev de mikrobiologiske fund hos 5.002 patienter med hospitalsdiagnosticeret APN i Region Nordjylland. Andelen af mikrobiologisk bekræftet APN steg over tid fra år 2000 til 2018. Samlet set var 85% af alle APN-episoder ledsaget af urindyrkning, mens 52% havde en positiv urindyrkning. *Escherichia coli* blev påvist i 80% af alle APN-episoder, og non-*E. coli* spp. forårsagede hovedsageligt APN hos patienter af mandligt køn, ældre alder, med diabetes eller medfødte urinvejsmisdannelser. Patienter med non-*E. coli* spp. APN havde hospitalsophold af lidt længere varighed og en højere 30-dages dødelighed end patienter med *E. coli* APN.

Studie III undersøgte risikoen for nyopstået CKD efter APN ved hjælp af et matchet kohortedesign fra år 2000 til 2018. Hos 20.797 patienter med APN var den absolutte risiko for CKD 4% (95% CI: 4-5) i det første opfølgningsår og 7% (95% CI: 7-8) i år >1-15, sammenlignet med 1% (95% CI: 1-1) og 6% (95% CI: 5-6) i 207.687 sammenligningspersoner fra baggrundsbefolkningen. Den tilsvarende justerede hazard ratio var 3,86 (95% KI: 3,58-4,16) i det første år og 1,79 (95% CI: 1,69-1,91) i år >1-15.

Studie IV undersøgte, om AKI hos 41.243 patienter med førstegangsindlæggelse for APN var forbundet med øget risiko for nyopstået CKD. Næsten 5% af patienterne med APN udviklede efterfølgende AKI, og 30% af disse patienter udviklede CKD. Risikodifferencen for CKD mellem 1.730 patienter med AKI og 17.817 patienter uden AKI var 15% (95% CI: 14-16) i det første år og 19% (95% CI: 17-22) i år >1-10. Risikoen for CKD forblev høj hos patienter som genvandt nyrefunktionen efter AKI.

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12. Appendices

12

Paper I

Appendix I

Paper II

Appendix II

Paper III

Appendix III

Paper IV

Appendix IV

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