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Survival and Renal Function after Acute Kidney Injury,

Epidemiological and Biomarker Studies

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Survival and Renal Function after Acute Kidney Injury, Epidemiological and Biomarker Studies

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By Claire Stigare

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To my family

“You should never, never doubt something that no one is sure of.”
Roald Dahl, Charlie and the Chocolate Factory

ABSTRACT

Acute Kidney Injury (AKI) occurs frequently in the critically ill and has an extremely high short-term mortality. The long-term risks of death, Chronic Kidney Disease (CKD) and End Stage Renal Disease (ESRD) have not been fully established and have never been investigated in the Swedish intensive care (ICU) population.

Nephrological surveillance and intervention could improve outcome for AKI survivors at particular risk of persistent renal dysfunction; these patients need to be identified and their renal function, reflected in the glomerular filtration rate (GFR) ought to be followed. However, the performance of endogenous biomarkers in estimating GFR in the recovery period has not been evaluated. Sarcopenia during ICU stay confounds creatinine´s use during admission and this effect could persist beyond ICU discharge.

All studies were cohort in design. In studies I and II, we used the Swedish intensive care registry (2005-2011) comprising 130,134 adult patients and applied epidemiological techniques. Studies III and IV were clinical studies with AKI patients recruited from the Karolinska University Hospital´s adult ICU. Study IV was a cohort nested within this local database.

In study I, we found that AKI patients had significantly higher crude mortality at one (48.4% vs 24.6%) and five years (61.8% vs 39.1%) compared to non-AKI patients. CKD and ESRD were significantly more common in AKI survivors at one year (adjusted incidence rate ratio (IRR) 7.6 and 22.5 respectively) than in the non-AKI group.

Study II examined the impact of pre-existing renal dysfunction on survival and development of ESRD. Five-year mortality was highest in patients with Acute on Chronic disease (AoC) being 71.3%, for the CKD group it was 68.2%. AoC and CKD were associated with an increased risk of ESRD, adjusted IRR were 259, and 96.4 respectively compared to the no renal disease group. Risk factors independently associated with ESRD in one-year survivors were: AoC, CKD, AKI, congestive cardiac failure and elevated admission serum potassium.

Study III investigated the proportion of AKI survivors developing Chronic Kidney Disease according to creatinine, and cystatin C estimated GFR (eGFR) three months after ICU discharge. We found that a quarter of patients fulfilled CKD criteria using creatinine and two-thirds using cystatin C based GFR estimation methods. Age, discharge cystatin C, discharge creatinine and female gender were predictive of creatinine defined CKD at follow-up.

Study IV compared the performance of creatinine and cystatin-C eGFR to estimate Iohexol clearance (mGFR) 9 months post ICU discharge. We found that creatinine equations overestimated mGFR, and demonstrated greater accuracy (82.6% versus 60.8% of estimates within 30% of mGFR (p30)) compared to cystatin C; which underestimated mGFR. Combined CKD-EPI-creatinine-cystatin-C formula showed minimal bias, and the greatest precision and accuracy (P30 =87%). Concordance between creatinine and cystatin-C eGFR increased from discharge to nine-month follow-up.

We conclude that patients with AKI are at increased long-term risk of death and renal dysfunction. Patients with pre-existing renal dysfunction have the highest risk of developing ESRD. We recommend implementing systematic follow-up of renal function at three months using combined creatinine and cystatin C eGFR estimation. At risk patients may be identified using our predictive models.

LIST OF SCIENTIFIC PAPERS

I. **Evolution of chronic renal impairment and long-term mortality after de novo acute kidney injury in the critically ill; a Swedish multi-centre cohort study.**
C Rimes-Stigare, P Frumento, M Bottai, J Mårtensson, C-R Martling, S Walther, G Karlström, M Bell.
Published: Critical Care 2015 May 6;19:221

II. **Long-term mortality and risk factors for development of end-stage renal disease in critically ill patients with and without chronic kidney disease.**
C Rimes-Stigare, P Frumento, M Bottai, J Mårtensson, C-R Martling, M Bell.
Published: Critical Care 2015 19:383

III. **Using Creatinine and cystatin-C to describe Acute Kidney Disease and Chronic Kidney Disease in AKI survivors.**
C Rimes-Stigare, B Ravn, A Awad, K Torlén, C-R Martling, M Bottai, J Mårtensson, M Bell.
Submitted manuscript

IV. **Evaluating performance of Creatinine and Cystatin C for estimating Iohexol measured GFR in AKI survivors.**
C Rimes-Stigare, B Ravn, A Awad, K Torlén, C-R Martling, E Löfberg, M Bottai, J Mårtensson, M Bell.
Submitted manuscript

CONTENTS

LIST OF ABBREVIATIONS

ADQI	Acute Dialysis Quality Initiative
AIC	Akaike information criteria
AKD	Acute Kidney Disease
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
AoC	Acute on Chronic
APACHE II	Acute Physiology and Chronic Health Evaluation II
AUC	Area under (receiver operated) curve
BIC	Bayesian information criteria
CI	Confidence interval
CKD	Chronic Kidney Disease
CKD-EPI-cr	CKD-EPI creatinine-based GFR estimation formula
CKD-EPI-cy	CKD-EPI cystatin C-based GFR estimation formula
CKD-EPI-cr-cy	CKD-EPI combined creatinine & cystatin C GFR estimation formula
COPD	Chronic obstructive pulmonary disease
CRRT	Continuous Renal replacement therapy
DAMPS	Damage associated molecular patterns
DR	Swedish cause of death register
ECC	Extra corporeal circulation
eGFR	Estimated Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
ICD 10	international classification of diseases version-10 codes
ICU	Intensive Care Unit
IGFBP7	Insulin growth factor binding protein 7
IHD	Intermittent Haemodialysis
IQR	Interquartile range
IR	Incident rate
IRR	Incident rate ratio
KDIGO	Kidney Disease Improving Global Outcomes group
L-M	Lund-Malmö creatinine-based GFR estimation formula
MDRD	Modified Diet in Renal Disease GFR estimation formula
mGFR	Measured Glomerular Filtration Rate
MRR	Mortality rate ratio
NPR	National patient register

PAMPS	Pathogen-associated molecular patterns
P30	Accuracy to within 30% of measured value
RIFLE	Risk, Injury, Failure, Loss, End stage, AKI classification
ROC	Receiver operated curve
ROS	Reactive oxygen species
RRR	Relative risk ratio
RRT	Renal replacement therapy
SAPS	Simplified Acute Physiology Score II or III
SD	Standard deviation
SIR	Swedish Intensive Care Register
SNR	Swedish Renal Register
TGFß	transforming growth factor beta
TIMP-2	Tissue Inhibitor Metalloproteinase 2
uHGF	urinary hepatocyte growth factor
WHO	World Health Organisation

CHAPTER 1. INTRODUCTION

BACKGROUND

The establishment of the speciality of intensive care (ICU) and the advances achieved have enabled critically ill patients to survive disease which would previously have proved fatal. Initially, we were content to measure success by immediate survival, but as ever greater numbers of patients are discharged alive from intensive care, our focus should now be directed to the long-term sequelae of critical illness. In the case of acute kidney injury (AKI), survival may be accompanied by formidable complications including remote organ disease, the development of chronic renal dysfunction, impaired quality of life and elevated long-term mortality. For many AKI survivors, ICU discharge heralds only the nascence of their problems, and we should strive to improve the long-term outcome for these patients.

AKI research has primarily focused on aetiology, injury biomarkers, and short-term mortality studies. The long-term consequences of AKI have not been fully established. National outcomes studies have never previously been conducted in the Swedish ICU population, and a causative relationship has not been firmly established between AKI and the subsequent development of chronic renal impairment. The pathophysiological processes involved in renal recovery or its failure have not been entirely elucidated. To improve long-term outcome, patients at greatest risk of persistent renal dysfunction need be identified, and we should determine when and how to follow them up and how to intervene.

This thesis aimed to determine the long-term mortality from AKI in adults and the risk of developing chronic kidney disease (CKD) and end stage renal disease (ESRD) in the Swedish ICU population. We identified predictors of death, CKD and ESRD after AKI. The second part of the thesis sought to establish the extent of renal dysfunction in AKI survivors three months post discharge. We aimed to determine the best method of estimating glomerular filtration rate (GFR) at follow-up by evaluating the performance of the endogenous markers creatinine and cystatin C against Iohexol measured GFR in AKI survivors.

AKI DEFINITION

Acute kidney injury is a syndrome characterised by a sudden decline in renal function, manifesting as a reduction in GFR and diagnosed by a rise in serum creatinine or a decrease in urine output. AKI results in accumulation of products of nitrogen metabolism and impaired volume and electrolyte homeostasis. Symptoms depend on the severity. AKI may be asymptomatic (detected by laboratory data only) in mild cases, but the spectrum may extend to nausea, neurological impairment, respiratory distress and even cardiovascular collapse. AKI is common in intensive care populations, affecting around half of the patients admitted to critical care and incidence is increasing.

HISTORICAL PERSPECTIVE

The kidneys situated in the retro-peritoneum are neither as glamorous as the heart or lungs nor as immediately dramatic in their dysfunction. Perhaps this is why knowledge of the kidneys and their acute failure have been acquired slowly. Renal dysfunction though can be equally as life-threatening as cardiopulmonary failure. Chiang Kai-Shek and Wolfgang Amadeus Mozart may be among those who succumbed to acute kidney injury.

Humans have known of the existence of the kidney and its association with disease since at least the 13th century B.C, as revealed by excavations in Kition, Cyprus where a clay votive representing the kidney was uncovered[1]. Renal function took many centuries to elucidate; indeed, Aristotle believed it was the bladder that produced urine. Galen (119-200) correctly identified the kidney as the site of urine production and suggested that blood was cleaned by the kidneys [2]. He proposed a classification of anuria, based on whether or not the bladder was empty. Precise understanding of renal function remained elusive for over a millennium until the period of the renaissance and the age of enlightenment when anatomical studies by Leonardo da Vinci among others and physiological work by Morgagni began to demystify the organ. Morgagni (1682-1771) proposed a system to classify causes of anuria or ischuria. Richard Bright's ground-breaking 1827 report in which he characterised the presentation and natural history of renal diseases paved the way for modern nephrology (the eponymously named disease is recognised now to be a group of chronic and acute conditions)[3]. The chemist, Thomas Graham's experiments, culminated in him describing and demonstrating the process of dialysis using an ox bladder in 1854; together Bright and Graham predicted that this discovery could be used to treat renal patients.

In the latter part of the 19th century William Dickinson, a physician at Great Ormond Street Hospital (1832-1913) worked extensively with nephrological conditions and particular acute nephritis. He provides us with one of the first and most lurid descriptions of septic AKI, in this case, caused by post-streptococcal glomerulonephritis. "*A man approaching middle age... becomes ill all over, with shivering and headache, oedema spreads quickly over the whole body, and the urine has nearly stopped. Urine becomes black with blood, and loaded with a dark sediment. ... The pulse is hard and full, the skin hot and dry, ... If the renal mischief be very intense, the urine may be reduced to 2 oz. or 3 oz. a day ... This condition cannot last long. If the secretions do not speedily increase, the patient will be poisoned by the elements of the urine which are kept in the blood. He will become comatose and die ... The kidneys (at post-mortem) will be gorged with blood, greatly increased in bulk*"[4].

In the twentieth-century case reports of the life-threatening complications of crush injuries emerged first after the Messina earthquake of 1908 and later from those treating casualties from both the first and second world wars. A truly ground-breaking paper by Bywaters and Beall described the syndromes now known as rhabdomyolysis and reperfusion injury which

they observed in patients trapped under rubble in the London Blitz of 1941[5]. This exquisite paper not only described the symptoms, sign and post-mortem findings but also presented the results of treatment with fluid resuscitation. The publication was a catalyst for research into acute renal failure and dialysis. A physician and Dutch resistance worker, Willem Kolff is credited with using the first artificial kidney in a human. He produced a home-made device consisting of washing machine parts, cans and sausage castings in 1945. In 1951 the term "Acute Renal Failure" was proposed by Homer Smith and rapid advances both in dialysis technology and in the understanding of acute nephrology followed; eventually led to Kramer introducing continuous renal replacement therapy (CRRT) into a German ICU in 1977[6].

Acute kidney injury was previously known as acute renal failure, and for many years a unified definition and categorisation system was lacking. At the onset of the millennium, 35 different definitions were simultaneously circulating. Consequently, this lack of consensus hindered research and slowed progress towards understanding and treating the condition.

AKI CLASSIFICATION

2004 marked the introduction of the RIFLE classification system proposed by Acute Dialysis Quality Initiative Group (ADQI) which led to a consensus regarding definition and grading. RIFLE is an acronym for the severity and duration of renal failure and represents Risk, Injury Failure, Loss and End Stage disease[7]. The system uses the degree of creatinine rise above baseline occurring within the timescale of one week and an individual's volume of urine production to grade severity. RIFLE has subsequently been refined, first by the Acute Kidney Injury Network (AKIN) group in 2007, who altered the time spectrum for AKI to 48 hours and added a minimum absolute serum creatinine value[8]. The system was further modified by KDIGO working group in 2012 as summarised in Figure 1.

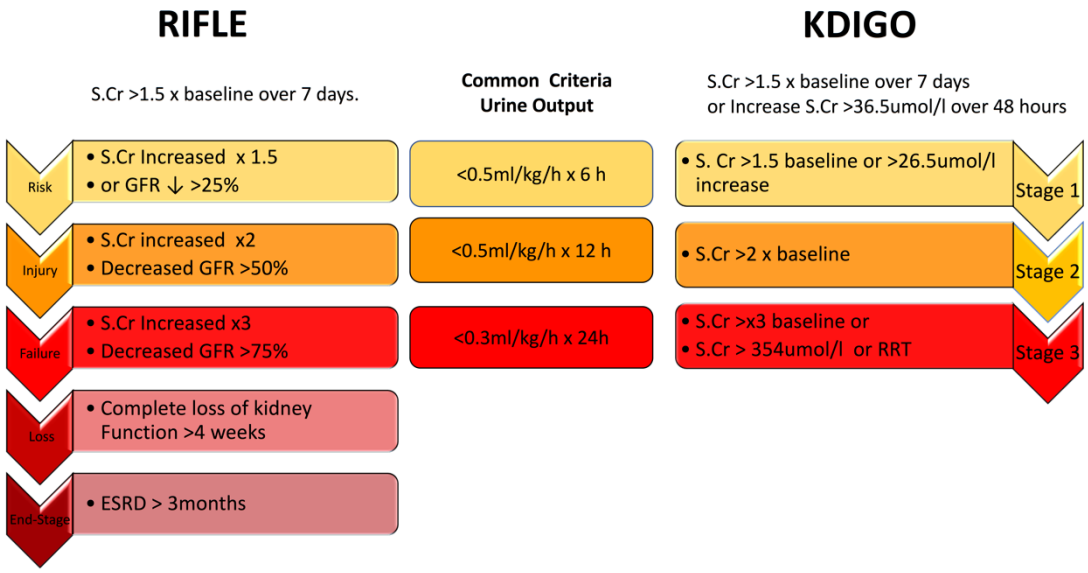


Figure 1 AKI staging according to RIFLE and KDIGO criteria[9]..

RIFLE and its modified successors have significantly improved understanding, awareness of and research possibilities for AKI. RIFLE had been validated in over half a million study patients, by 2010[10-13]. Validation studies include a multi-centre cohort study of over 9000 patients in which each RIFLE grade was independently associated with hospital mortality[14]. Analysis of a large cohort by Hoste et al. demonstrated an association between ascending

RIFLE grade and increasing mortality[15]. Uchino, in a study of over 20,000 found a near linear relationship between RIFLE grade and elevated hospital mortality, which was three and tenfold higher in RIFLE R and F patients respectively than in their non-AKI group. The three classification systems were evaluated as predictors of hospital mortality in a cohort of nearly 50,000 people, KDIGO and RIFLE were found to have areas under the receiver operated curves of 0.77 and 0.78 respectively, while the discriminative ability of AKIN was inferior (0.69) [16].

INCIDENCE

The incidence of ICU AKI varies in the literature depending on the population studied, criteria used and accuracy of reporting[17]. Thakur's observational study of 325,000 patients found a total incidence of AKI of 22% of which 2% experienced severe AKI[11]. In contrast, a 2015 cross-sectional study involving 97 centres worldwide with 1800 patients used KDIGO criteria and described an incidence of 57% [18]. AKI incidence reported by FINNAKI was 39.3%, 14.1% had stage 3 AKI, with 10.2% requiring RRT[19].

The incidence of AKI in the ICU setting is increasing; Wald et al. described a 4-fold increase in dialysis-requiring AKI between 1996 and 2010 and studies from Long and Sakhuja also report rising AKI incidence over time[20-22]. This may be explained by, increased awareness and a standardised definition of AKI which as has led to improved diagnosis and reporting. Concomitantly the demographics of those admitted to ICU are changing, patients are older and tend to have higher burdens of comorbid disease; due partly to greater prevalence of diseases such as diabetes and CKD in the general population[23,24]. Additionally, more liberal admittance criteria to ICU are being applied as ever sicker patients are treated successfully.

A national US study examined over 18 million hospitalisations with AKI and found that incidence increased fivefold between 2001 to 2011. Contemporaneously prevalence of comorbidities in this population also rose including hypertension (22.9% to 61.2%), obesity (2.9% to 14.1%), and anaemias (20.2% to 35.9%)[25].

AETIOLOGY

AKI used to be defined anatomically depending on the site of pathology as 1) prerenal usually resulting from reduced renal perfusion due to hypotension or reno-vascular disease, 2) post renal, mainly caused by mechanical obstruction downstream of the kidneys or 3) intrinsic with the latter being the most common cause in critically ill populations. Modern classification is by aetiology. Sepsis, ischaemia, and nephrotoxin induced AKI account for the greatest proportion of cases on ICU in developed countries. Sepsis predominates and is a precipitating factor in around 50% of patients[12,26-28]. In many cases though AKI is multifactorial.

Septic AKI

Septic AKI results from exposure of the kidney to pathogen-associated molecular patterns (PAMPS) and damage associated molecular patterns (DAMPS), reactive oxygen species and reactive nitrogen species. Insult exposure leads to a pro-inflammatory state and endothelial damage which results in increased microvascular permeability and interstitial oedema. Tubular injury predominates. Histologically, AKI is characterised by loss of the brush border of the apical surface of the tubule, loss of cell polarisation, disruption of the cell's cytoskeleton and sloughing of cells into the tubular lumen causing dilatation and obstruction. Micro-vascular ischemia and activation of the clotting cascade may be responsible for micro thrombi seen in the glomeruli.

Historically septic AKI has been assumed to be characterised by acute tubular necrosis occurring secondary to disturbance of renal perfusion. However, histological examinations such as those conducted by Brun and Munck in 1957 have failed to confirm the large-scale presence of tubular necrosis[29]. Indeed, Lerolle's cadaveric studies demonstrated the presence of apoptosis and glomerular leucocyte infiltration in the renal biopsies of patients who died of sepsis with AKI [30]. Hotchkiss et al. found neither necrosis nor apoptosis at autopsy in the kidneys of 20 patients with sepsis and multi-organ failure. However, lymphocyte infiltration was marked, and apoptosis of other organs including the spleen and colon was common[31]. It is now believed that adaptive mechanisms occur within the kidney in response to sepsis which while temporarily reducing renal function, work to avoid cell death and allow for potential later renal recovery. These mechanisms may include energy conservation, GFR reduction and the occurrence of vascular shunting and resemble the adaptions observed in the kidneys of mammals which hibernate[32]. Tubular cells may conserve energy by entering a state of cell cycle arrest in which mitosis is ceased[33,34]. Where cell death does occur, it appears to occur in a regulated fashion and be dominated by apoptosis [31,35].

Ischaemic AKI is characterised by hypo-perfusion, in contrast, renal blood flow may fluctuate during the course of sepsis. Limited research is available in humans but results from studies which do exist and animal models suggest that blood flow to the kidneys is often maintained and may even paradoxically increase in sepsis[36-39]. However, a number of intra-renal mechanisms contribute to GFR reduction and in some cases, total cessation of glomerular filtration occurs. This may be beneficial because as GFR decreases, energy-dependent processes such as sodium chloride absorption will be reduced and energy consumption in the stressed nephron may diminish[40]. Additionally, the kidneys (and particularly the tubuli) will be protected from further exposure to toxins. Haemodynamic changes are facilitated by alterations in the relationship between afferent and efferent arteriolar vascular tone, triggered by activation of the renal sympathetic system and by detection of increased sodium chloride load at the Macula Densa. Vascular shunting from afferent to efferent arterioles may contribute to reduced GFR because blood may bypass the glomerulus altogether further reducing renal energy consumption and toxin exposure [41,42]. Venous congestion is likely to be deleterious to renal recovery and outcome because it contributes to increased intra-capsular pressure and reduces trans-renal perfusion. Potential treatments for septic AKI may then be focused on influencing the immune response to sepsis, reducing inflammation and perhaps apoptosis as well as maintaining renal perfusion and avoiding fluid overload.

Nephrotoxin exposure: A multitude of drugs and toxins can cause AKI, among the most common causes in ICU patients, are exposure to radiocontrast medium, commonly administered during radiological examinations including angioplasty. Glycopeptides and aminoglycoside antibiotics may precipitate interstitial nephritis, and drugs acting on the renin-angiotensin system can cause impaired renal autoregulation.

Rhabdomyolysis results from muscle breakdown after crush injury and exposes the kidney to high concentrations of myoglobin which exceed the kidney clearance capacity. Oxidation of myoglobin's haem molecule to its ferric state causes free radical formation and lipid peroxidation. Additionally, in acidic conditions myoglobin precipitates and cause tubular obstruction. AKI is exacerbated in crush injury syndrome by the extravascular sequestration of fluids resulting in hypovolemia and by renal vasoconstriction in response to myoglobinuria[43].

AKI following cardiac surgery

The aetiology of AKI occurring after **cardiothoracic surgery** is multifactorial, some causes are common to other ICU populations while others are unique to the cardiothoracic cohort. Lau et al. described how perioperative inflammation, ischemia-reperfusion, haemodilution, and embolism might all result from exposure to the extracorporeal circuit (ECC) during cardiac bypass and contribute to AKI[44]. Contact with the ECC promotes leucocyte as well as endothelial cell activation and triggers an immune cascade, resulting in a systemic inflammatory response and the release of cytokines and reactive oxygen species (ROS) many of which can cause interstitial inflammation and tubular injury[45,46]. Ischaemic injury tends to occur in those areas of the kidney where oxygen delivery and tension are lowest, the outer medulla and the proximal tubule are particularly vulnerable. ECC exposure may also result in erythrocyte lysis which can lead to the release of redox active iron[45]. When the iron binding capacity is exceeded free iron is oxidised, and nephrotoxic oxygen free radical species can be released, a state further exacerbated by the production of ROS during reperfusion injury[47,48]. The mechanism of **ischaemic AKI** is more purely exemplified by the model of injury resulting from aortic aneurysm rupture and repair, and we choose this model when investigating how outcome varied according to aetiology.

MORTALITY

Short-term outcomes, such as ICU, hospital and 90 days mortality are well studied, but few publications have addressed longer-term outcomes. Comparison of studies is difficult because populations are heterogeneous. Study settings and AKI aetiologies in the published literature are diverse. Variations in RRT modality, dose and time to initiation have further impact on results[49]. Additionally, the presence in many cohorts of patients with pre-ICU renal disease is likely to affect outcome. A summary of AKI outcome studies is given in Table 1.

Study	Year	No of patients	Setting for AKI	In-hospital / 30-day mortality%	Follow-up (Years)	Total Mortality %
Korkelia[50]	2000	62	ICU	45	5	64.5
Morgera (93-98) [51]	2002	979	ICU	69	5	84.5
Luckraz[52]	2005	98	Cardiac + CRRT	42	5	48
Ahlström[53]	2005	703	ICU	41	5	69.5
Landoni[54]	2006	126	Cardiac Surgery	67	3.5	76.2
Triverio[55]	2009	206	ICU	53	3	67
LaFrance[56]	2010	82,711	Hospital non-RRT requiring		2	29.8
Carl[57]	2010	130	ICU	58	1	76.4
Long[58]	2013	3686	Hospital		1	48
Lopez-delgado[59]	2013	2940	Post cardiac surgery		5	Risk 25% Failure 77%
Rydén[60]	2014	27,929	AKI post CABG		5	17%
RENAL(Gallagher)[61]	2014	1464	ICU		4	63
Poukkanen[62]	2015	774	ICU	26.5	1	39.8
Eriksson[63]	2015	103	Trauma	17.5	1	26.2
Long[20]	2016	10,419	Hospital		1 year	49%

Table 1. Summary of long-term survival studies after AKI

Three-month mortality
In patients with severe AKI, mortality at 90 days ranges between 39% in FINNAKI cohort of 2900 patients in 2013 up to 73.5% in Metcalfe's study of Scottish patients from 2002[19,64]. Three-month mortality in the randomised control "RENAL" trial was 44.7%, the Swedish "SWING" study (1995-2004) reported 50.6%, and in Gammelager´s Danish ICU-cohort it was 54.7%. [65-67]. The discrepancy in results may be due both to temporal changes and to Metcalfe's inclusion of patients with chronic renal failure and to dialysis modality. Metcalfe used both IHD and CRRT whereas, in other studies (RENAL and SWING), CRRT was used exclusively or almost exclusively.

One-year Mortality
At one year, total mortality ranges from 48% in Longs Icelandic study, 63.8% in Bagshaw's Canadian population-based report up to 72% amongst Wong's liver transplant cohort[20,68,69].

Longer-term follow-up
Five-year mortality was reported as 48% by Luckraz and as 63% in four year extended follow-up of the RENAL trial[52,61]. The highest death rate recorded was 84.5% in Morgera's study[51]. This variation in mortality may be as a result of time bias and differing patient populations. Morgera's was in a mixed ICU population, treated between 1993 and 1998, while Luckraz investigated patients undergoing cardiopulmonary bypass 1997-2005.

Temporal mortality trends.
Evidence suggests that mortality from AKI has decreased over the last decade. Using a national cohort of 18 million AKI admissions in the United States, Brown et al documented a decline in in-hospital mortality from 21.9% in 2001 to 9.1% in 2011[25]. Likewise in Longs Icelandic study, one-year survival improved from 47% in the years 1993-97 to 57% in the later quinquennial 2008-2013[20]. It should be noted that ICU mortality as a whole has also decline over the previous decade[70].

RENAL OUTCOMES

Definitions
Acute Kidney Disease (AKD) was proposed by ADQI in 2017 to describe prolonged renal dysfunction after AKI and prior to CKD. AKD is considered present if KDIGO AKI criteria stage 1 or greater continues to be fulfilled 7 to 90 days after AKI debut[71].

Chronic kidney disease is defined in KDIGO 2012 guidelines as an "abnormality of kidney structure or function, present for more than three months, with implications for health". CKD may be diagnosed by the presence of markers of kidney damage or by GFR less than 60ml/min/1.73m^2[10]. Figure 2 details this classification. For this thesis, CKD is defined solely by its GFR definition.

End Stage Renal Disease refers to severe chronic renal disease, grade 5 with a GFR under 15ml/min/1.73m^2 or with dialysis dependence.

Obtaining data on the incidence of ESRD in AKI survivors is relatively simple because this is a well-documented hard endpoint. The extent of kidney dysfunction as a whole though is far harder to ascertain because renal function is rarely routinely followed and thus dysfunction is underdiagnosed[72]. Definitions of renal recovery also vary, ranging from the absence of dialysis requirement to return to baseline GFR or creatinine. Furthermore, baseline values and pre-ICU CKD status are often lacking in study data. Moreover,

determining renal function in critically ill and post ICU patients may be difficult as will discussed later.

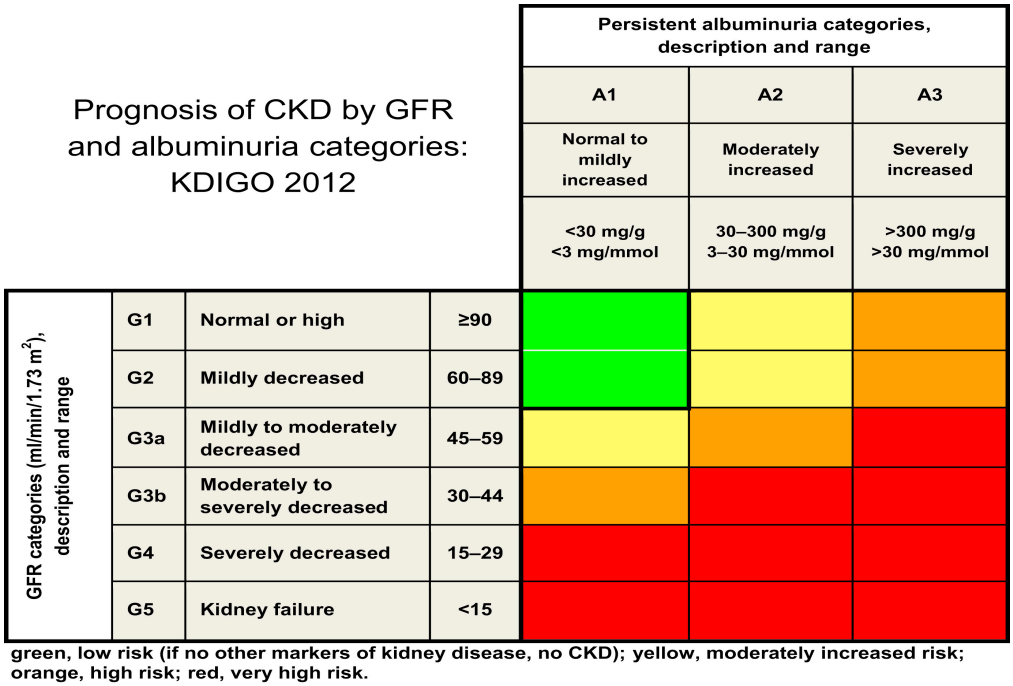


Figure 2. KDIGO CKD categorisation
Reproduced with permission from Elsevier. KDIGO. Kidney International Supplements, Jan 2013-3:1-150[10].

Incidence of ESRD

Early follow-up

The majority of studies show that AKI survivors are dialysis-free at 90 days post admission, but dialysis dependence rates vary widely [73]. ESRD was reported in the Swedish SWING study (which excluded pre-existing CKD patients) to be 8.3% in those who had received CRRT [65]. Gammelager´s Danish cohort found ESRD incidence to be 8.5% at six months. this was lower than the 11.5% of AKI survivors reported to be dialysis dependent by the FINNAKI group. Perhaps this reflects competing risks of death and ESRD: because FINNAKI had the lowest mortality of all studies, a greater number of patients with severe AKI may have survived to be at risk of ESRD [74,75].

Longer-term ESRD risk.

Triverio and Landoni revealed similar rates of ESRD amongst survivors at 3 and 3.5 years post-AKI (10.1 and 9.1% respectively)[54,55]. Concordance exists between Schiffl, Luckraz (both five years) and Pannu´s (34-month) follow up, where dialysis dependence rates were all between 2% and 2.2%[52,76,77]. The post RENAL study with a 42 month mean follow-up, observed incidence of ESRD to be between 5.1 and 5.8% (depending on CRRT intensity)[61]. The highest five year ESRD rates(22.7%) were recorded by Korkeila who conversely described relatively low mortality (64.5%) in a mixed ICU population who had received both IHD and CRRT, again competing risks could be at play[50].

Risk of developing Chronic Kidney Disease

Previously AKI and CKD were seen as separate entities and exposure to AKI was not thought to be associated with subsequent CKD development. It is only recently that a causal link has been proposed. In the few clinical studies of post ICU renal function which have

been conducted, CKD has been shown to be significantly more common amongst AKI-survivors than in non-AKI patients, occurring in between 20-40%[78-80]. Schiffl reported that of AKI survivors with renal impairment at discharge, GFR improved in 26%, returned to baseline in 10.7% and deteriorated in 8.6% [76]. All renal function improvement occurred during the first year, and no-one with full renal recovery at discharge subsequently deteriorated[76]. Long-term renal function was reported by Bell; permanent renal failure increased from 8% at 90 days to 14% at seven-year follow-up in patients surviving AKI after CRRT[81].

Macedo, followed AKI survivors over four years, stabilisation was seen in all patients by 18 months and in 83% by 12 months. At 18 months, 54% had recovered renal function to a GFR greater than 60, while 4.8% developed ESRD[82]. Heung et al. studied AKI in hospitalised patients, 91% had AKI grade 1[83]. At one year 31.8% developed CKD (GFR under 60). Risk of developing CKD stage 3 was associated with the speed of creatinine decline. Adjusted relative risks for fast, intermediate or slow/unknown recovery were 1.43, 2.00, and 2.65 respectively[83]. A British study, evaluated the long-term risk of CKD development among 396 dialysis requiring AKI patients, who at one year had recovered GFR to > 60ml/min/1.73^2; the investigators found a very low risk of CKD progression at five years. This might suggest that surveillance of renal function beyond one year in recovers is unnecessary[84]. In the longest follow-up (8 years) of hospitalised AKI patients, Ponte found renal function improvement in 31%, stabilisation in 50% and deterioration in 19%[85]. Duran recorded CKD grade 3 or greater in 80% of dialysis-requiring AKI survivors. Chawla found AKI grade was a "robust" predictor of progression to CKD[86].

Predictors of non-recovery and ESRD

Factors associated with non-renal recovery in the literature include advancing age[82,83,86], hospital discharge serum creatinine or eGFR [82,83] comorbidities [85,87] including congestive heart failure[88], hypertension [87]and diabetes [86]. Long-term dialysis requirement was also predicted by the presence of pre-existing renal dysfunction in Harel's cohort [87]. Low serum albumin levels, AKI severity (RIFLE) and mean hospital creatinine levels were predictors in Chawla's study[86]. Interestingly Macedo found that grade of AKI, maximum creatinine, and renal replacement therapy (RRT) requirement was not associated with the risk of non-recovery[82].

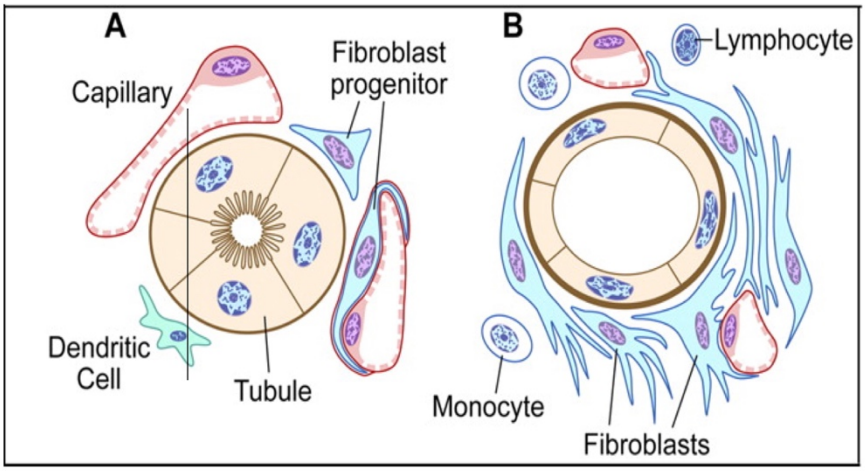
In an investigation of 390 hospitalised AKI patients who were dialysis-dependent at discharge, Brahmbhatt el al. reported that 56% developed ESRD, univariate analysis found predictors included: male gender, CKD , hypertension, diabetes and heart failure[89]. Hickson in an American study of patients with persisting haemodialysis requirement at hospital discharge found that recovery "was not rare" and was most likely in those with higher baseline eGFR, however patients with heart failure were the least likely to recover[90].

RECOVERY

A number of physiological processes may be involved in renal repair after AKI, all of which promote the return of normal renal architecture. Cells in the proximal tubule undergo differentiation and dedifferentiation to replace cells which have undergone apoptosis or necrosis. Recovery is promoted by up-regulation of inflammatory inhibitors including haemoxygenase in tubular epithelial cells and concomitantly a down-regulation of toll-like receptors[91]. Kidney injury molecule is secreted in the proximal tubule and acts with Neutrophil Gelatinase Associated Lipocalin (NGAL) to inhibit apoptosis and increase cell proliferation[91,92]. Clearance of necrotic and apoptotic cells is carried out by macrophages and dendritic cells in the proximal tubule[93,94].

MALADAPTIVE REPAIR

In some patients, maladaptive repair occurs, this newly described process may be characterised by lack of cell differentiation, capillary rarefication, cellular atrophy and fibrosis as illustrated in Figure 3. Inflammatory cell infiltration, including myofibroblast migration, culminates in tissue fibrosis. Macrophages may contribute especially in the presence of reperfusion injury[91,94-96]. Connective tissue effectively pushes capillaries away from the tubuli causing further hypoxia and promotes a self-perpetuating cycle of hypoxia and fibrosis, augmented by persistent paracrine and pro-fibrotic signalling. The result of this process is tubular atrophy, and ultimately atresia. The likelihood of maladaptive repair occurring post-AKI may be increased in a number of conditions including, diabetes, hypertension and CKD. The risk is also elevated in older patients and those exposed to ongoing hypoxia.



Manjeri A. Venkatachalam et al. Am J Physiol Renal Physiol 2010;298:F1078-F1094

Figure 3. Representation of renal recovery (a) normal process (b) maladaptive repair. Reproduced from Ventkatachalam et al. Am J Physiol Renal Physiol 2010;298: F1078-F1094 .

Persistent hypoxia occurring as a result of prolonged or repeated renal insult particularly affects the deep cortex and medulla where even under physiological conditions oxygen tension is low. Hypoxia may cause oedema, endothelial swelling and vasoconstriction resulting in oxidative stress. Microvascular rarefaction can result from prolonged hypoxia.

In CKD, a chronic pro-inflammatory and hypoxic state with architectural derangement may exist, further insult with AKI may accelerate and accentuate this situation. Reduced renal mass means patients have decreased renal reserve. In both CKD and the ageing kidney, epigenetic changes alter the balance of expression of pro and anti-inflammatory genes[97].

Diabetes. Renal microvascular damage resulting from diabetes has been demonstrated in animal models to exacerbate hypoxia in AKI and to increase inflammation. Diabetic rats have been observed to have an increased incidence of interstitial fibrosis post AKI compared to controls[98,99]. This model offers a possible explanation of why people with diabetes are at increased risk of developing CKD after AKI[99].

In **the ageing kidney,** growth factor production declines, synchronously low-grade activation of the immune system occurs. This coupled with increased production of pro-inflammatory factors creates a milieu which renders the senescent kidney vulnerable to

hypoxia and prone to fibrosis. This picture is similar if less dramatic than that seen in patients with CKD[100].

Hypertension post-AKI may injure the kidney and accelerate progression towards CKD, because barotrauma resulting from arterial hypertension in the microvasculature may lead to arteriolosclerosis and auto-regulatory impairment. The consequence maybe hypoxia and fibrosis[97,101].

PRE-EXISTING RENAL DISEASE AND LONG-TERM OUTCOME.

Impact of prior CKD on recovery

Intriguingly in some ICU cohorts, patients with pre-existing renal disease have been observed to have lower mortality although greater post-AKI dialysis dependence than de novo AKI subjects[102-104]. One study noted that patients with pre-existing CKD, received earlier nephrological referral; one might speculate that patients under surveillance benefit from prompt detection of critical illness and earlier intervention[103]. However, other studies have shown that subjects with CKD and particularly Acute on Chronic disease (AoC) have higher mortality rates compared to those with AKI. Analysis of 9450 surgical patients found long-term survival to be significantly worse for those with AoC than for patients with de novo AKI (hazard ratio 3.3)[105].

Mortality in patients with pre-existing ESRD

Analysis of results for ICU patients with ESRD is interesting because 90-day mortality is reported as being relatively low compared to that of AKI patients. Two cohorts report 42% and 44.6% of patients dying respectively[81,106]. Ostermann and co-workers found that in their cohort, patients with ESRD were less likely to be mechanically ventilated and they suffered by fewer organ failures[107]. Those ESRD patients admitted to critical care may be representative of a healthier sub-selection of the entire ESRD population. An investigation of 41,972 German and UK critical care patients found lower mortality in ESRD than AKI-patients (hospital mortality was 34.5% versus 61.6%, $P< 0.0001$)[107]. Two reviews and two subsequently published original studies found survival to be better for ESRD-patients than for AKI groups[108-112].

Risk of ESRD in patients with prior CKD

A large cohort found AoC-patients (without renal recovery at discharge) to have a hazard ratio of 213 for developing ESRD, compared to patients with preserved kidney function[105]. Ishani et al. report a HR. of 41.2 for AoC (79.5 cases per 1000 patients) compared to controls in a cohort of over 233,000 elderly hospitalised patients[113]. Another community-based study of more than 39,000 individuals discovered dialysis requiring AoC increased the risk of developing ESRD by 30% compared to CKD without AKI[114].

Clearly, irrespective of ICU admission, patients with CKD have a far higher underlying risk of progression to ESRD than the general population. This risk is proportional to GFR reduction and has been quantified in CKD-patients in the community in two studies as being between 4.14 and 6.37 events per 1000 person-years[115,116]. This is a significantly lower risk than that experienced by CKD patients after treatment on ICU.

Ali et al. described renal recovery in hospitalised patients with AoC and noted significantly lower recovery (return to baseline creatinine) than in the de novo AKI group. Full recovery was seen in 35% and partial in 16%. Interestingly in the AoC group, the proportion with full recovery did not differ according to RIFLE category[117].

The consequences of AKI on remote organ function.

AKI in the acute phases has been demonstrated to be associated with remote organ dysfunction most particularly with acute respiratory distress syndrome and circulatory failure. Cardio-renal and pulmonary-renal syndromes may be the result of fluid overload, endothelial damage, and immunological effects. Recent research has now demonstrated that AKI also increases the long-term risk of remote organ dysfunction and is associated with cardiovascular disease and an increased risk of stroke[118-121]. Further, AKI survivors have been observed to have a higher incidence of bone fractures and elevated risk of neoplasm particularly in the gastrointestinal, respiratory and genito-urinary tracts, than non-AKI ICU survivors. Gastrointestinal bleeds are more common after AKI, and the risk of severe infection, including tuberculosis and sepsis is increased[122,123].

AETIOLOGY AND OUTCOME

Limited literature concerning long-term outcome and aetiology exists. Short-term studies generally report higher hospital mortality for patients with sepsis compared to other AKI causes, with mortality from septic AKI ranging from 53.3% to 64.8% [27,124,125]. This may partly be due to greater disease severity and multi-organ involvement in sepsis, reflected in higher disease severity scores [27,126]. Bagshaw's review of a joint Australian and New Zealand ICU register including over 117,000 found ICU mortality, hospital mortality and length of ICU and hospital stay to be higher in patients with septic versus non-septic AKI (29.7% versus 21.6%; odds ratio 1.53, $P < 0.001$)[127]. The prognosis for renal recovery after septic AKI is unclear; few studies address outcome beyond discharge and concordance is lacking between published studies. Hamzic-Mehmedbasi's investigation of 100 patients, identified non–septic aetiology as an independent predictor of renal recovery measured using creatinine clearance at discharge[128]. However, the study reports that time to initiation of RRT was significantly longer in septic AKI compared to other aetiologies. Zang also found that patients with septic AKI had lower renal recovery rates than those with other AKI aetiologies (74.4% vs 82.8%, P=0.05)[124]. Conflictingly, a number of studies have found that septic AKI survivors may experience better renal recovery than patients with other causes of AKI. Cruz found renal recovery (return to within 20% of baseline creatinine) to occur more often after septic AKI than non-septic AKI, in a study of 117 patients. [125]. In a Canadian cohort of 1753 patients, a trend towards lower creatinine and RRT-dependence at hospital discharge was seen in the septic AKI group. Cho in a Korean study also reported higher in-hospital mortality and requirement for RRT on ICU in septic versus non–septic AKI patients but found that septic AKI survivors were more likely to recover renal function to within 20% of baseline creatinine (81.0% versus 59.3%, p=0.012) than non-septic AKI[129]. Long-term follow-up of AKI according to aetiology has not previous been investigated, hence we wished to address this question in our research.

FOLLOW-UP AFTER AKI

Automatic nephrological follow-up of AKI survivors is neither praxis nationally in Sweden nor many other countries. The reported incidence of nephrological follow-up is surprisingly low 8.5-17% in observational studies[72,130]. Thus, we know relatively little about the true renal function of survivors and the potential consequences of intervention. In a randomised study Harel reported a lower incidence of all-cause mortality in dialysis-requiring AKI patients with early nephrological referral (less than 90 days) compared to those with no follow-up (8.4 versus 10.6%, hazard ratio 0.76). Silver observed that referral led to intervention in 70% of patients[130].

Which interventions might improve long-term outcome?
Many factors in our management of patients with AKI on ICU could influence outcome, including prevention, early detection, avoidance of nephrotoxins, optimisation of cardiac output and renal perfusion, optimisation of fluid balance and intervention with RRT. RRT treatment choices such as modality; continuous versus intermittent, dose, choice of anticoagulant and timing of initiation may also influence outcome. In the recovery period optimisation of blood pressure, blood glucose and electrolyte balance may be important. Medication review, treatment of anaemia lifestyle and nutritional advice may all be influential in reducing or slowing CKD progression. Establishing an effective and economically viable follow-up programme requires careful patient selection and determination of when and how to best assess renal function.

BIOMARKERS OF RENAL FUNCTION

Renal function tests.
Renal function is quantified by GFR which measures the volume of plasma that is cleared of a substance per unit time (ml/min) and is usually corrected to a body surface area of $1.73m^2$. Its measurement requires intravenous injection or infusion of a tracer which is freely filtered by the glomerulus and is neither secreted nor reabsorbed by the tubule. Inulin fulfils these criteria but is expensive and very time-consuming to measure. Its use has been superseded by somewhat simpler methods involving the single intravenous injection of radiocontrast agents such as Iohexol and Iothalamate; they have been shown to be comparable in accuracy to Inulin[131]. These methods require repeated measurement of plasma concentration to determine the speed of renal elimination, and although less cumbersome than Inulin, they are expensive and inconvenient to perform. In everyday clinical practice, endogenous surrogate markers of GFR which are simpler and cheaper to measure are required to screen large numbers of patients.

Creatinine
Plasma creatinine levels are most often used to approximate renal function and formulas are available which commonly use age, sex, and race to improve estimation of GFR. Creatinine is produced from the breakdown of creatine phosphate mainly in skeletal muscles. It is close to being an ideal GFR marker because it is freely filtered. However, it is also secreted in the tubuli. Several equations for estimating GFR from creatinine are in use. These include the Modified Diet in Renal Disease (MDRD) formula which was developed from a cohort of over 1600 CKD patients. The 4-variable equation is well validated and known to underestimate GFR at higher levels. The CKD-EPI equation was proposed in 2009, the development dataset of over 8200 subjects included CKD and non-CKD patients. In validation assessments, it has been shown to have greater accuracy than MDRD[132-134]. An equation developed in Sweden by Grubb, Björk and colleagues has refined the estimation further. The Lund-Malmö equation has been demonstrated to outperform both MDRD and CKD-EPI at low GFR in Swedish populations[135]. Creatinine-based GFR estimates are seen as satisfactory markers of GFR in steady state populations[136,137].

Cystatin C
Cystatin C, a cysteine protease inhibitor is a low molecular weight protein which is produced in all nucleated cells and is filtered freely by the glomerulus, reabsorbed in the proximal tubule and almost entirely catabolised. Levels are affected by thyroid function, corticosteroid use, smoking and the presence of cancer.

GFR estimation in ICU populations.
The use of creatinine to assess renal function in ICU populations is problematic because

catabolism and sarcopenia are commonly observed in critically ill patients, particularly those with prolonged admission[138,139]. Consequently, creatinine production and serum concentration may decline during ICU admission[138,140,141]. Further, secretion in the proximal tubuli increases at GFR under 30, thus giving rise to falsely elevated estimates of GFR. Therefore, creatinine's use as a proxy of GFR is confounded in the critically ill. Cystatin C may be a superior renal function marker in this population because its measurement is not affected by muscle mass. Ravn et al. compared daily cystatin C and creatinine measurements in over 3000 critically ill patients[141]. They found a divergent pattern of mean concentration of the biomarkers during ICU admission with creatinine falling and cystatin C rising with increasing duration of stay. Cystatin C had a strong linear association with one-year mortality and identified nearly twice as many patients as having eGFR under 60 as creatinine did. Creatinine was a poor prognostic marker of death. Cystatin C may be more useful for identifying renal dysfunction in ICU[141]. How long the catabolic state continues after ICU discharge and how well creatinine and cystatin C perform in estimating GFR in the recovery period after ICU has never been assessed.

CHAPTER 2. SUBJECTS AND METHODS

ETHICAL CONSIDERATIONS

The Stockholm regional ethics committee, approved studies I-IV, which were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

REGISTERS

The Swedish state has a long tradition of meticulous record keeping which began in the 16th century with parish registers and Karl IX degree of 1686 saw the establishment of national records[142]. The population register has existed in its current form since 1946 and identification of individuals was facilitated by the creation of the personal number system. This is a unique 12-digit code which is assigned at birth or upon immigration by the Swedish tax authority. It is required for all interactions with administrative agencies and healthcare providers and allows linkage of national registers. Sweden has been a pioneer in healthcare and social science research owing in part to access to these high-quality registers which have almost complete coverage of the population and unprecedented accuracy.

The cause of Death Register (DR) established in 1952, includes details of time and reason for death of all Swedish citizens and residents who have been issued with a national identification number. It is administrated by the Swedish tax authority and considered to be very reliable. All deaths reported to the tax authority are registered, and cause is recorded in 97% of cases[143]. Exclusions include deaths of temporary visitors, asylum seekers, and stillbirths. Dates of death obtained from DR were used in studies I-IV.

The National Patient Register (NPR) comprises in- and outpatient databases. The in-patient register includes information on all hospital discharges in Sweden, and complete coverage (100%) has been achieved since 1987[144]. The out-patient register records clinic visit-diagnoses and interventions. Validation in 2011 indicated that 77% of all outpatient episodes were recorded, and private health care contacts accounted for the majority of omissions[144]. Diagnoses have been coded according to the WHO International Classification of Disease System (ICD 10) since 1996. NPR was used in studies I and II to ascertain the cohort's premorbid status, classified according to the Charlson comorbidity index[145]. Patients with pre-existing renal disease (Charlson definitions) were identified here, as were subjects who developed CKD (ICD-10 codes N18 and N19) after ICU admission.

The Swedish renal register (SNR) was established in 2007 and contains the start and finish dates of all individuals receiving treatment for ESRD. The database records if patients received treatments with haemo- or peritoneal dialysis and whether they were recipients of renal transplants. There are around 3700 patients receiving dialysis in Sweden at any point in time. SNR provided data on patients with ESRD prior to and post ICU admission for studies I-IV.

The **Swedish Intensive Care Register (SIR)** which was established in 2001and receives data from ICU´s in district, county and tertiary referral centres including general, cardiothoracic, neurosurgical and burns injuries units. Since its establishment, the number of units submitting data has increased, and by 2011, 91% of all patients admitted to Swedish, ICUs were included in SIR[146].

The submission of data to SIR relating to patient characteristics and administrative details of ICU admission is mandatory therefore this data is complete. Registration of other variables, however, has been optional and data is sometimes absent. Examples of non-mandatory data include interventions, surgical codes, complications and disease severity scoring systems from which admission laboratory data was obtained. Pre-morbid biochemistry data was not available.

SIR uses personal identification numbers to identify patients in the register and admissions to ICU. A temporary number is assigned for patients without a valid national identity number at admission. Data is prospectively collected and transferred electronically to SIR. Logical defects are identified by a validation script and returned to the admitting unit for re-evaluation. Data is then entered on to the master database.

From 2008 to 2011 SIR used three disease severity scoring systems, including APACHE II (Acute Physiology and Chronic health evaluation) 2008-2010, SAPS (Simplified Acute Physiology Score) version II (2005 -2009) and SAPS III (2008-2011). In some patients, APACHE admission reason and ICD-10 discharge diagnoses were also available.

Local databases

Centricity Clinisoft is an electronic patient data system and was introduced to the ICU at Karolinska in 2005. The database acquires data from ICU apparatus including, ventilators, infusion pumps and CRRT machines as well as from monitors. Laboratory results are also collected, together with manually entered data such as diagnosis codes, interventions and medications.

Excrete is a database in excel format established to contain the records of patients recruited and followed in the studies comprising part III and IV.

Table 2. Summary of studies I-IV

	Study			
	I	**II**	**III**	**IV**
Data Sources	SIR, SNR, NPR, DR	SIR, SNR, NPR, DR	Excrete database Clinisoft, DR, SNR	Excrete database Clinisoft
Design	Cohort	Cohort	Prospective cohort	Nested cohort Prospective
Study populations	Swedish ICU patients	Swedish ICU patients +/- CKD	ICU patients with AKI Karolinska hospital	AKI survivors renal dysfunction three-months
Study Period	2005-2011	2005-2011	2008- 2011	2008- 2011
Exposure	AKI versus non-AKI	CKD +/-AKI	AKI	AKI + non-recovery three-month.
Outcome	Death CKD & ESRD	Death CKD & ESRD	CKD at three months Death	Performance Cystatin C & Creatinine eGFR versus mGFR
Statistical analysis	Kaplan Meier, Poisson Laplace regression	Polynomial logistic regression	Cox regression Kaplan Meier Logistic regression	ROC Inter-rater analysis concordance analysis Bland Altman
Maximum Follow-up	Mortality 7 yrs. CKD & ESRD 6 yrs.	Mortality 7 yrs. CKD & ESRD 6 yrs.	2 years	2 years

STUDIES I AND II

Definitions

AKI criteria for Study I and II.

Data available in SIR precluded classification of AKI according to RIFLE. Severe de novo AKI was considered present if at least one of the following criteria was fulfilled:

1. IHD or CRRT received during ICU admission according to SIR.
2. The diagnosis "acute renal failure" was recorded within the APACHE II score.
3. ICD 10 code "acute kidney failure" N17 was designated at discharge.
4. Serum creatinine of over 354 μmol/L (KDIGO grade 3) recorded on admission in APACHE II, SAPS-II or SAPS-III scoring systems.

Classification of patients according to renal status

Premorbid GFR estimates and creatinine were not available. Pre-ICU renal status was classified as:

- **No renal disease**
- **CKD**, if ICD-10 codes for CKD (using Charlson criteria) were present in NPR[147]
- **ESRD** if registered in the SNR prior to ICU admission.
- **Acute on Chronic** (**AoC**) if patients were registered as having CKD before admission and met one of the AKI criteria 1-3 above.

Secondary outcome: Presence in registers three months or more after ICU discharge led to classification as:

- **Post-ICU-CKD** if ICD-10 codes N18 or N19 were recorded in NPR,
- **Post-ICU-ESRD** if patients were recorded in the SNR.

Aetiology: AKI aetiology was categorised into septic AKI, ischaemic AKI and non-specified cause using ICD-10 codes. AKI after ruptured abdominal aortic aneurysm (AAA) was chosen to represent ischaemic-reperfusion-injury aetiology. AKI and any of ICD-10 codes: I712, I714 I716 or I719 resulted in classification to this group. Similarly, subjects were considered to have had septic AKI if ICD-10 codes on discharge included at least one of the following: A401-A418 or A41. All other AKI-patients were classified as unspecified AKI.

Study design. This was a cohort study using prospectively collected data from SIR which was cross-matched with national patient registers (SNR, NPR, and DR).
Subjects: The population base consisted of all patients admitted to Swedish ICU´s, (submitting cases to SIR), data was extracted between January 2005 and January 2011.
Inclusion: First admissions of all adult subjects.
Exclusion:

- Patients aged under 18 years
- Incomplete records, i.e. cases where ICD-10, intervention codes, and scoring systems were all absent since AKI-classification was not possible.
- Study I: Patients with CKD and ESRD prior to admission were excluded.

Outcome measures

- **Primary**: one-year mortality.
- **Secondary**: incidence of ESRD (and CKD in Study I).

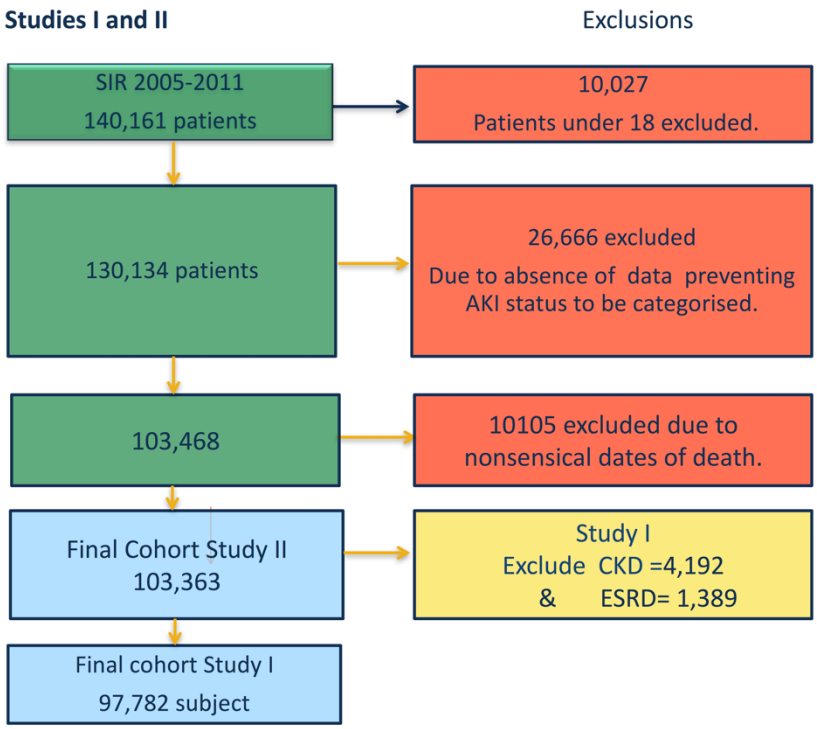


Figure 4. Flowchart of patient selection, studies I & II

STUDY III

Definitions

AKI defined according to RIFLE (this classification system was current at study inception) [148]. Baseline creatinine was obtained from laboratory review up to 3 months prior to ICU admission; the lowest value was used as the baseline. Where baseline was absent, an estimated creatinine was obtained using the Modified Diet in Renal Disease (MDRD) formula with expected GFR of 75 mL/min/1.73m^2 (eGFR 75 method)[7].

Study design. Prospective cohort study
Study Population: Patients admitted to the central ICU at Karolinska University Hospital Solna 2008-2011 with Acute Kidney Injury.
Inclusion: Adult patients with AKI during admission and alive at discharge.
Exclusion: Patients aged under 18 and over 100 and those who died before three-month follow-up. Only first admissions were analysed. Patients with CKD or ESRD prior to ICU were excluded. Patients admitted February to April 2010, and those discharged at weekends or public holidays were not recruited due to lack of research staff availability.
Measurement: Recruited patients were referred either to nephrology or ICU clinics at three months when serum creatinine and cystatin-C were sampled. A turbidimetric method was used to determine Cystatin-C (Gen- tian Cystatin C UDR-Kit for Beckman-Coulter Synchron and UniCel Systems, Ref A52761). Creatinine was measured with a modified Jaffe method (CREm, Creatinine, Ref 472525).

Notes of recruited patients not attending follow-up were reviewed. Creatinine and cystatin C obtained during contact with primary care and outpatient services were collated. Values from acute admissions were not used due to the risk of later episodes of AKI being misclassified as AKD or CKD. The *complete Excrete database* was then cross-matched

with the DR and SNR to obtain dates of death and ESRD diagnoses; thereafter data was anonymised.

Values of creatinine were transformed to eGFR using the (MDRD), Lund Malmö (L-M) and CKD-EPI creatinine formulas while for cystatin C, CKD-EPI cystatin-C (CKD-EPI-cy) was used. The CKD-EPI combined equation (CKD-EPI-cr-cy) was employed to derive joint estimates and all equations are presented in Figure 5. Patients were subsequently classified as having CKD at three months if GFR was <60ml/min/1.73m^2 according to KDIGO stages of CKD [149]. Urinalysis was not available therefore we describe GFR >90 and between 60 and 90ml/min/1.73m^2 instead of Grades I and II. We classified patients as having acute kidney disease (AKD) if their follow-up creatinine was >1.5 times their baseline creatinine and follow-up occurred between two and seven months[71].

Outcome measures:
Primary CKD at three months according to Creatinine and Cystatin C eGFR.
Secondary analysis: Incidence of AKD at three months. Identifying predictors of CKD at three months. Two-year mortality.

GFR estimating formulae

Key: PCr = plasma creatinine value, Pcy= plasma cystatin

Revised Lund-Malmö equation[151]

$eX - 0.0158 \times Age + 0.438 \times \ln(Age)$

Female pCr < 150 μmol/L: $X = 2.50 + 0.0121 \times (150 - pCr)$

Female pCr≥150 μmol/L: $X = 2.50 - 0.926 \times \ln(pCr/150)$

Male pCr < 180 μmol/L: $X = 2.56 + 0.00968 \times (180 - pCr)$

Male pCr≥180 μmol/L: $X = 2.56 - 0.926 \times \ln(pCr/180)$

CKD-EPI Study equation for caucasians [151]

Female pCr ≤ 62 μmol/L: $44 \times (pCr/62) - 0.329 \times 0.993^{Age}$

Female pCr > 62 μmol/L: $144 \times (pCr/62) - 1.209 \times 0.993^{Age}$

Male pCr ≤ 80 μmol/L: $141 \times (pCr/80) - 0.411 \times 0.993^{Age}$

Male pCr > 80 μmol/L: $141 \times (pCr/80) - 1.209 \times 0.993^{Age}$

MDRD Study equation for caucasians[151]

$175 \times (pCr/88.4) - 1.154 \times Age - 0.20\,3 \times 0.742$ (if female)

CKD_EPI Cystatin C equation 2012

$eGFR = 133 \times \min(P_{cys}/0.8, 1)^{-0.499} \times \max(P_{cys}/0.8, 1)^{-1.328} \times 0.996^{Age} \times 0.932$ [if female]

CKD-EPI Cystatin C and Creatinine equation 2012 [152,153]

$eGFR = 135 \times \min(P_{Cr}/\kappa, 1)^{\alpha} \times \max(P_{Cr}/\kappa, 1)^{-0.601} \times \min(P_{cys}/0.8, 1)^{-0.375} \times \max(P_{cys}/0.8, 1)^{-0.711} \times 0.995^{Age} \times 0.969$ [if female] $\times 1.08$ [if black]

Figure 5. GFR estimating formulae

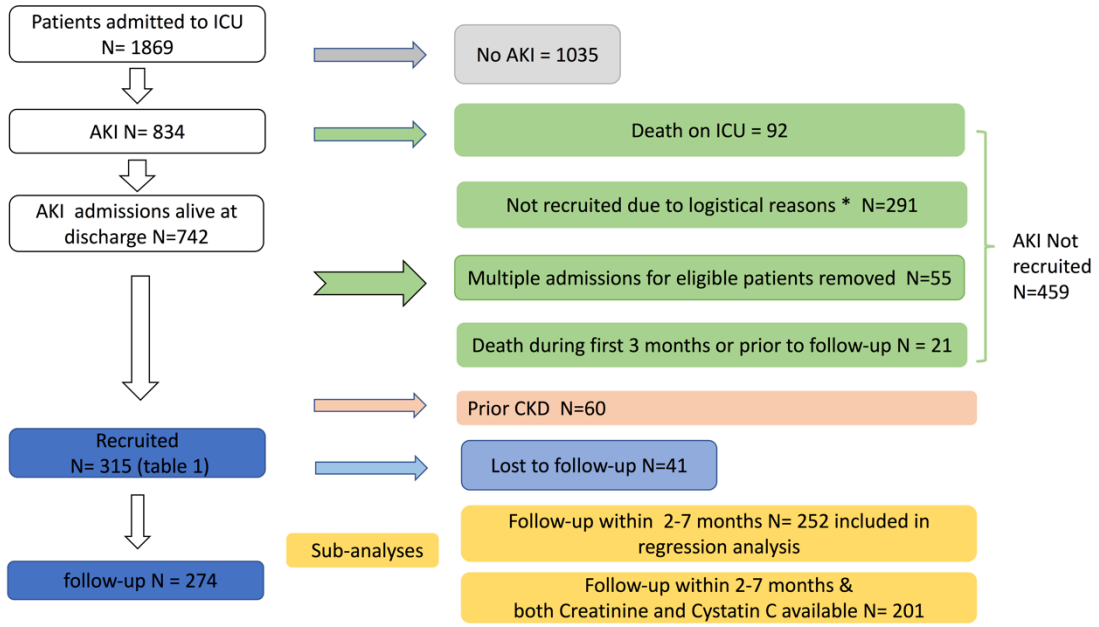


Figure 6. Flowchart showing patient selection for study III.

STUDY IV

Definitions

Renal dysfunction at three months post ICU: defined as creatinine elevation greater than 1.5 times baseline or absolute cystatin C values greater than 1.25mg/l.

Study design: A nested cohort study.
Study population AKI survivors with persistent renal dysfunction three-months post ICU discharge. The cohort was selected from the source population detailed in paper III, patients with prior CKD were however included in this study.
Inclusion: Patients attending three months follow-up (range 2-7 months) with persisting renal dysfunction.
Exclusion: Second follow-up beyond <150 and >500 days, patients excluded if only one biomarker was measured at nine months.

Measurement: Serum creatinine and cystatin C were taken and 4-point Iohexol clearance was performed; by first obtaining baseline blood samples and then administering a 5ml intravenous bolus of Iohexol (Omnipaque). Three subsequent blood samples were taken in the contralateral arm. Iohexol concentration was measured using Ultra High-Performance Liquid Chromatography, and a clearance curve was constructed for each patient and the elimination phase used to determine GFR.
The notes of AKI patients without renal dysfunction at three months were reviewed and subsequent values of creatinine, cystatin C and Iohexol obtained within 150-500 days of ICU discharge were collated and used for comparison concordance analysis only.

Outcome measure:
Primary: Performance of creatinine and cystatin C eGFR formulas in estimating mGFR.
Secondary: Temporal trend in concordance between creatinine and cystatin C eGFR from discharge to follow-up.

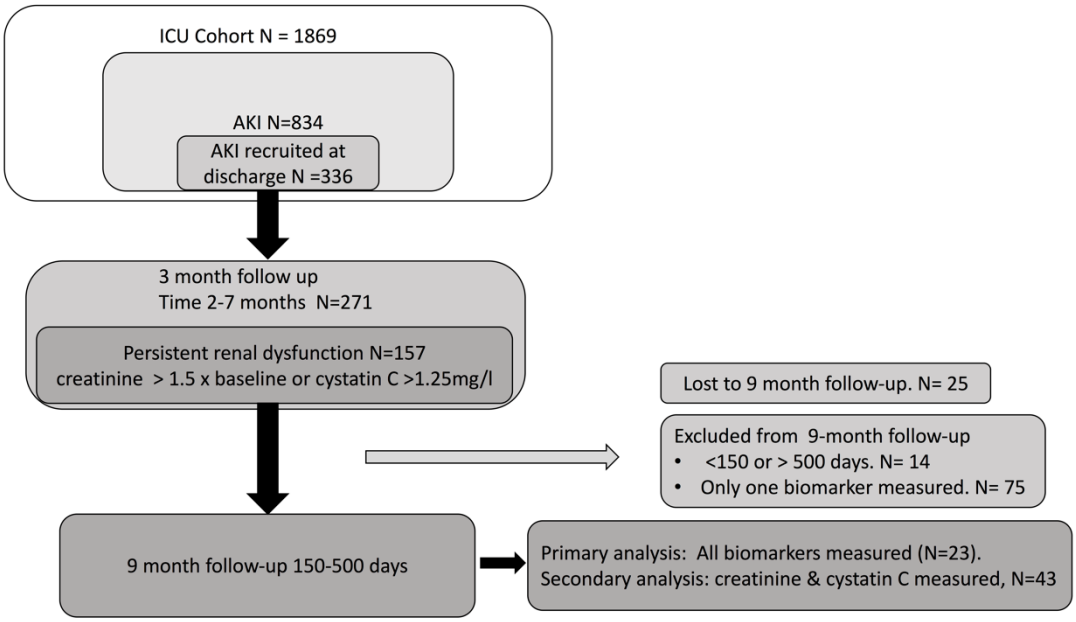


Figure 7. Flowchart showing patient selection for study IV

STATISTICAL ANALYSIS

All studies

Continuous data are reported as medians with interquartile range (IQR). Categorical data are expressed as counts and percentages. The Mann-Whitney test compared distributions of continuous variables at baseline. Sign test, assessed equality of matched pairs, and Fisher´s test and Student T-test compared means of binary variables. A two-sided P-value <0·05 was considered to be significant. Stata version 12 was used to perform all analysis (StataCorp LP, College Station, Tx, USA).

In all multivariate analyses, potential confounders were considered on the basis of prior knowledge of AKI and CKD and on whether the addition of covariates to the models changed estimates of relative risk by >10%. Multivariate analyses were performed using stepwise backward elimination technique, and we tested for collinearity.

Studies I and II

Survival analysis was performed using Kaplan Meier method; log-rank test verified equality of survivor functions between subgroups. Mortality data was extracted up to 31 December 2011 while data from all other registers was available until December 2010 (maximum follow-up seven and six years respectively). We considered time from ICU-admission to death or end of follow-up whichever occurred first. Using Schoenfeld's residuals evidence of non-proportionality of survival curves was found. Therefore, Poisson regression was used to obtain mortality and incidence rates and ratios (MRR and IRR) estimates. We used Laplace regression to estimates survival percentiles.

Study I. We considered the different endpoints of CKD, ESRD and death as competing events and constructed a univariate model for the cumulative risk of developing either CKD, ESRD or of dying at any time point.

Study II. Polynomial logistic regression was used to identify predictors of ESRD at one year in one-year survivors. A competing risk model was used because there was a high early mortality and death may have precluded progression to ESRD, i.e. some patients may

have developed ESRD had they survived. The model included patients with at least one-year of follow-up and no censored data. This was a two-step process. First, a polytomous model was constructed with four-levels of outcome, death, ESRD, ESRD and death or no adverse outcome and relative risks are reported. The polytomous (competing risk) model then enabled prediction of the probability of the binary outcome ESRD. This dichotomous variable was created by combining the risk ratios of outcomes ESRD/no-ESRD thus we report a relative risk ratio. Sensitivity and specificity were investigated using the receiver operating characteristics curve (ROC). The area under the curve (AUC) was used to assess discrimination.

Study III Modelling.
Death was considered a censoring event, without which we could have observed patient's three-month values. We created a model using Cox regression weighted for the inverse probability of dying after discharge and before three months; adjusted for covariates found to be independently associated with death before follow-up. This model was used in all regression analysis.
Logistic regression was used to identify covariates which affected the risk of the binary outcomes CKD (GFR <60 mL/min/1.73m^2) according to each of creatinine and cystatin-C and for AKD. Odds ratios are presented. The models were assessed using Somers´ d as well as *Bayesian*- and *Akaike information criterion* (*BIC*, *AIC*). Survival probabilities were calculated using the Kaplan Meier method and differences between groups were assessed using log-rank test.

Study IV
Agreement between nine months eGFR according to each biomarker and Iohexol mGFR was graphed using Bland Altman plots. We assessed each biomarker´s performance characteristics and we used the following definitions for these metrics:

- **Accuracy (P30)** = proportion of Creatinine or Cystatin C eGFR estimates lying within 30% of Iohexol measured values[150].
- **Bias Absolute** = median difference between estimated and measured GFR.
  Relative = percentage difference between estimated and measured GFR.
- **Precision** IQR of median difference (bias). Sign test compared precision and bias.

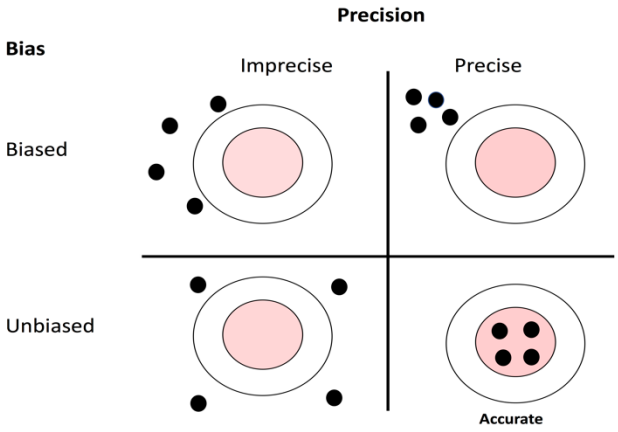


Figure 8. Depiction of performance characteristics

The predictive performance of creatinine and cystatin C based GFR for estimating an Iohexol mGFR under 60 mL/min/1.73m^2 at nine-months was evaluated. We calculated AUC of the ROC curve. **Concordance coefficient**s were calculated according to Lin´s method and used to compare Inter-rater reliability between creatinine and cystatin C GFR estimates observed at: discharge, three and nine-month follow-up[151]. **Inter-rater agreement** between eGFR methods was evaluated using Cohen's Kappa coefficients, classification statistics are reported using a 0.2 cut off level.

Modelling and diagnostic test studies II and IV

The performance of a screening test or statistical model for a dichotomous outcome may be evaluated by using sensitivity, specificity analysis and ROC curve. Table 3 illustrates how sensitivity, specificity and predictive values are derived from a contingency table.

Table 3. Contingency table evaluating diagnostic test performance.

Test	Disease	
	Present	Absent
Positive	True positive (a)	False Positive (c)
Negative	False negative (b)	True negative (d)

- Sensitivity is the probability of the disease being present if the test is positive. $\frac{a}{a+b}$
- Specificity is the probability of the disease being absent if the test is negative. $\frac{d}{c+d}$
- Positive predictive value is the probability of having a positive test if the disease is present. $\frac{a}{a+c}$
- Negative predictive value is the probability of the test being negative if disease is absent $\frac{d}{b+d}$

The disadvantages of these tests are that they require a single threshold (cut off) for defining disease. Additionally, predictive values are affected by disease prevalence. The ROC curve may be used to assess a range of cut off points and display these graphically. Sensitivity (true positive rate) is plotted against 1-specificity for different threshold levels of diagnostic tests, and the user may then decide which cut off represents the most optimal trade-off between sensitivity and specificity, since an increase in sensitivity results in a decrease in specificity. Test accuracy is reflected in how close the curve is to the left-hand border, and away from the 45-degree diagonal line, the area under the curve gives a numerical value of accuracy. ROC curves may be used to: 1. Assess the diagnostic performance of a test to discriminate present or absence of disease as used in study II 2. compare the performance of 2 or more tests and in study IV we compared the ability of creatinine and cystatin c based mGFR to predict Iohexol mGFR under 60ml/min/1.73m^2.

CHAPTER 3. RESULTS

STUDY I

Baseline Characteristics

Overall 5.4% of patients were identified as having de novo AKI (Table 4). AKI patients were older, had longer length of ICU stay and were more often male than the no-AKI group. Prevalence of comorbidities was higher among AKI patients; a greater proportion had cardiovascular disease, diabetes, liver disease and cancer compared to non-AKI cohort.

Mortality

During follow-up of 97,782 patients without prior renal disease, 35.3% of patients died. One year survival was 51.3% in AKI patients and 75.4% in the non-AKI group, detailed in Table 5. Crude survival centiles are presented in Table 6. Short-term mortality was particularly high for AKI patients with 20% dying by ICU day 4, whereas in the non-AKI cohort the first 20% died by 118 days. Mortality rate was 2.9 times higher for the AKI group compared to those without AKI (Table 7). This ratio remained elevated but diminished after adjustment.

Incidence of CKD and ESRD

CKD incidence post ICU (Table 7) was significantly higher in AKI patients, being 6% at one year and 10.5% at five years compared to the non-AKI population (0.44 and 1.8% respectively for one and five years) yielding an adjusted IRR of 7.6.

The incidence of ESRD (Table 7) after ICU was low but significantly higher in the AKI population compared to the non-AKI cohort with an adjusted IRR of 22.5. At five years, 3.9% of AKI patients had developed ESRD in contrast to 0.3% in non-AKI patients (Table 8). The Kaplan Meier estimates of the cumulative incidence of death, CKD and ESRD is presented in Figure 9d.

Table 4. Baseline characteristics of patients in SIR cohort. Studies I and II.

Baseline Characteristics	Study I / Study II								
	No renal disease N = 92509	**AKI N =5273**	**P**	**ESRD (N=138 N=1389**	**P**	**Chronic only N=3194**	**P**	**Acute on chronic N=998**	**P**
Median age (years) IQR	63 (45-75)	70 (61-78)		63 (52-72)		74 (64-81)		72 (64-81)	
Length of ICU stay (hours) IQR	23 (12-53)	68 (26-189)	<0.001	26 (15-58)	<0.001	27 (15-63)	<0.001	64 (25-156)	<0.001
Women (N) (%)	40411 (43·6)	2086 (39.6)	<0.001	500 (36.0)	<0.001	1140 (35.7)	<0.001	343 (34.4)	<0.001
Highest Potassium (mmol/l) IQR	4·1 (3·9-4·5)	4.7 (4.1-5.5)	<0.001	4.8 (4.2-5.5)	<0.001	4.4 (4.0-5.0)	<0.001	4.9 (4.3-5.7)	<0.001
Highest Sodium (mmol/L) IQR	139 (136-142)	137 (134-141)	<0.001	138 (135-140)	<0.001	139 (136-142)	0.65	138 (135-141)	<0.001
Lowest arterial pH IQR	7·36 (7·30-7·41)	7.29 (7.19-7.38)	<0.001	7.35 (7.2-7.4)	<0.001	7.34 (7.25-7.4)	<0.001	7.28 (7.17-7.36)	<0.001
Maximum Creatinine (µmol/L), IQR	80 (63-105)	254 (164-422)	<0.001	460 (275-673) 660	<0.001	185 (129-277) 1738	<0.001	363 (238-553)	<0.001
Maximum urea (mmol/L) IQR	6·7 (4·3-10·1) 2717	17.5 (11-26) 532	<0.001	19.4 (15-25) 62	<0.001	16.6 (11.9-24.5) 104	<0.001	27 (19.3-36.6)	<0.001
Apache II score IQR	14 (8-20)	25 (20-32)	<0.001	22 (17-28)	<0.001	19 (14-26)	<0.001	27 (21-33)	<0.001
SAPS II score IQR	25 (0-41)	55 (42-70)	<0.001	35 (16-52) 269	<0.001	40 (23-53)	<0.001	56 (43-68)	<0.001
SAPS III score IQR	52 (43-63)	68 (59-77)	<0.001	60 (51-71)	<0.001	64 (55-72)	<0.001	69 (60-78)	<0.001
Charlson comorbidity score mean * (SD)	1·8 (2·2)	2.6 (2.5)	<0.001	5.2 (2.5)	<0.001	5.8 (2.6)	<0.001	5.6 (2.6)	<0.001
Charlson score renal removed mean (SD)	1.78 (2.2)	2.6 (2.5)	<0.001	3.2 (2.4)	<0.001	3.8 (2.6)	<0.001	3.6 (2.6)	<0.001
Comorbidity ((%)									
Myocardial infarction	(12·9)	(18)	<0.001	(28.0)	<0.001	(32.8)	<0.001	(29.0)	<0.001
Congestive cardiac failure	(13·5)	(25.1)	<0.001	(31.5)	<0.001	(48.9)	<0.001	(43.6)	<0.001
Peripheral vascular disease	(9·6)	(13.4)	<0.001	(25.0)	<0.001	(23.8)	<0.001	(21.4)	<0.001
Cerebro-vascular disease	(16·9)	(15.3)	<0.001	(22.3)	<0.001	(24.7)	<0.001	(17.7)	0.497
COPD	(14·1)	(15.3)	0.010	(10.8)	<0.001	(21.8)	<0.001	(18.8)	<0.001
Cancer	(17·0)	(24.0)	<0.001	(15.7)	0.183	(23.1)	<0.001	(23.3)	<0.001
Metastatic disease	(3·6)	(4.7)	<0.001	(1.7)	<0.001	(4.0)	0.246	(2.8)	0.199
Uncomplicated Diabetes	(14·2)	(26.2)	<0.001	(38.7)	<0.001	(38.0)	<0.001	(39.3)	<0.001
Diabetes + complications	(5·0)	(9.9)	<0.001	(35.9)	<0.001	(25.2)	<0.001	(26.9)	<0.001

Values are median (IQR), if not otherwise stated.
P values compared to no renal disease group *Charlson score is not age adjusted

Table 5. Time specific survival probability according to AKI status.

Group	Survival probability (%)					
	30 day (CI)	90 day (CI)	180 day (CI)	1 year (CI)	3 year(CI)	5 year(CI)
No AKI	84·0 (83·8-84·3)	80·6 (80·4-80·9)	78·5 (78·4-78·7)	75·4 (75·1-75·6)	67·2 (66·9-67·5)	60·8 (60·5-61·3)
AKI	61·9 (60·6-63·2)	56·5 (55·1-57·8)	54·0 (52·6-55·3)	51·3 (50·0-52·6)	43·2 (41·8-44·7)	38·2 (36·4-40·0)

· CI= 95% Confidence interval.

Table 6. Crude survival centiles according to AKI status.

Group	Crude Survival (days) for each given centile (95% CI)			
	5th	10th	20th	30th
No AKI	1·0 (0·42-2·1)	5·9 (4·7-7·3)	118·5 (110-129)	748 (727-767)
AKI	0·63 (-1-2·4)	1 (-0·8-3·8)	4 (-0·3-8·3)	11 (3·7-18·4)

CI= 95% Confidence interval.

Table 7. Multivariate Poisson regression showing the association between: AKI & I) mortality II) CKD III) ESRD.

Outcome	Group	N	Deaths (N)	Person-years	Mortality rate deaths/ person-year 95% CI)	Crude MRR	Adjusted MRR*	Adjusted MRR#
Mortality	No AKI	92509	31530	$2{\cdot}3 \times 10^5$	0·135 (0·134-0·137)	1	1	1
	AKI	5273	2943	$7{\cdot}6 \times 10^3$	0·387 (0·374-0·402)	2·87 (2·76-	2·14 (2·06-	1·15 (1·09-
			Events (N)		**IR event/ person-year (95% CI)**	**Crude IRR (95% CI)**	**Adjusted IRR † (95% CI)**	
CKD	No AKI	92509	649	1.7×10^5	0·0038 0·0035-	1	1	
	AKI	5273	193	4.9×10^3	0·0340 0·0341-0·0452	10.3 (8·8-12.1)	7.6 (5·5-10·4)	
ESRD	No AKI	92509	116	1.7×10^5	0·0007 0·0006-	1	1	
	AKI	5273	65	5.2×10^3	0·0125 0·0098-	18·6 (13·7-	22·5 (12·9-39·1)	

MRR =mortality rate ratio. IR= Incident rate, IRR = Incident rate ratio

*Model 1: Adjusted for age, gender, myocardial infarction and diabetes mellitus with complications.

#Model 2: Adjusted for age, gender, SAPS3 score, myocardial infarction, cerebrovascular disease, diabetes mellitus with complications, moderate-to-severe liver disease, cancer and dementia.

† Model 3 (secondary outcome) *Adjusted for SAPS3 score, age, gender and diabetes.

Table 8. The time-specific probability of developing ESRD and CKD by AKI status.

Group	Outcome	Estimate of percentage of patients who develop outcome at specific time points·					
		1 year	CI	3 years	CI	5 years	CI
No AKI	ESRD	0·08	0·06-0·10	0·20	0·16-0·23	0·3	0·25-0·38
AKI		2.0	1.6-2.7	3.0	2.2-4.0	3.9	2·7-5.5
No AKI	CKD	0·44	0·39-0·49	1.1	1.0-1.2	1·8	1·6-1·9
AKI		6.0	5.1-7.0	8.7	7.5-10.2	10.5	13.0

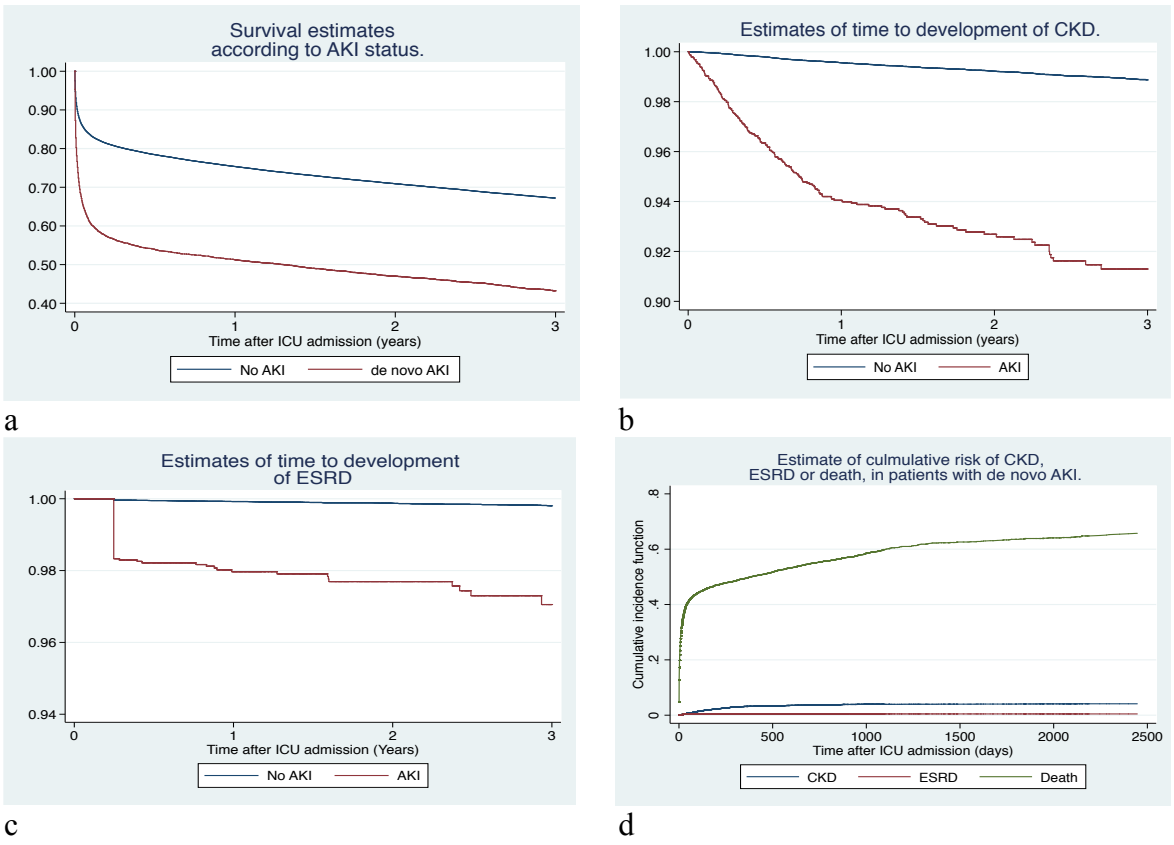


Figure 9. Kaplan Meier Curves showing estimates of a) survival b) CKD, c) ESRD, d) Cumulative incidence of Death, CKD and ESRD.

STUDY II

Baseline Characteristics

Overall 4.1% of patients had pre-existing CKD of which 23.8% developed acute on chronic disease during ICU admission as shown in Table 4. The proportion with pre-existing ESRD was 1.34%. Patients with ESRD and CKD had a significantly shorter length of stay than AKI and AoC groups. Illness severity scores were highest in the AoC group, and ESRD had the lowest severity scores of all the renal disease groups. Interventions were generally under-reported, AoC and AKI had the highest incidence of invasive ventilation and emergency surgery. CKD patients had a significantly greater number of comorbidities compared to all other groups, this included cardiovascular disease and diabetes. The ESRD group had less congestive cardiac failure, COPD and malignancy than all other renal dysfunction groups.

Mortality

In this cohort of all eligible ICU patients (103,363) one-year mortality was highest for patients with AoC disease, being 54.3% compared to 48.7% for AKI, 47.7% for CKD and 40.3% for the ESRD group (Table 9). Five-year mortality was greatest in CKD and AoC patients (71.3% and 68.2% respectively). Mortality rate ratios compared to the no-renal disease cohort were 3.53 for AoC, 2.87 for AKI and 2.99 for CKD (Table 10). The risk of death for the group with ESRD was elevated compared with those without renal disease, yielding a MRR of 2.08, but lower than for other renal disease groups. MRR estimates were reduced in both multivariate models but remained elevated compared with controls in all renal dysfunction groups. Full adjustment revealed MRR for ESRD to be higher than for all other groups. Laplace survival centiles (Table 11) showed that 30% of patients in CKD and AoC groups had died by 31.0 and 31.4 days respectively. It took 109 days for the first 30% of ESRD patients to die.

Incidence of ESRD

The risk of developing ESRD after ICU admission was highest among patients with AoC and prior CKD being 19.7% and 9.1% respectively at one year (Table 12) and rising to 21.1 and 25.5% at five years. Adjusted IRR for AoC was 259 and for CKD, 96.4 compared to the referent no renal disease group (Table 13).

Competing risk analysis showed that significant predictors of ESRD one-year post ICU discharge were: the presence of AoC, CKD and AKI, congestive heart failure, elevated admission potassium (Table 14). Patients with AoC were 356 times more likely to develop ESRD than the no renal disease cohort, and the CKD RRR was 266. The AUC for this predictive model was 0.937. Age was not associated with the likelihood of ESRD and was modelled using 20-year interval cubic splines. Patients aged over 80 are less likely to be accepted to receive chronic dialysis treatment than younger patients.

Table 9. Times specific mortality estimates by group

Group	Mortality probability % (CI)			
	90 days	**1 year**	**3 years**	**5 years**
No renal disease	19.3 (19.1-19.6)	24.6 (24.4-24.9)	29.1 (28.8-29.4)	39.1 (38.7-39.5)
AKI	43.5 (42.2-44.9)	48.7 (47.4-50.1)	53.0 (51.6-54.4)	61.8 (60.0-64.6)
Chronic only	36.8 (35.1-38.5)	47.7 (45.9-49.4)	55.7 (53.9-57.4)	71.3 (69.1-73.4)
Acute on Chronic	46.2 (43.2-49.3)	54.3 (51.3-57.4)	58.6 (55.5-61.8)	68.2 (64.2-72.2)
ESRD	29.0 (26.7-31.4)	40.3 (37.8-42.9)	47.0 (44.4-49.7)	62.9 (59.8-66.1)

Table 10. Multivariable Poisson regression analysis, the association between renal group and death.

Group	N	Deaths	Person-years	Mortality rate deaths/person-year (95% CI)	Crude MRR (95% CI)	Adjusted MRR* (95% CI)	Adjusted MRR# (95% CI)
All	103363	37836	2.5×10^5	0.151 (0.150-0.153)			
No renal disease	92509	31530	$2{\cdot}3 \times 10^5$	0·135 (0·134-0·137)	1	1	1
AKI	5273	2943	$7{\cdot}6 \times 10^3$	0·387 (0·374-0·402)	2·87 (2·76-2·97)	2·14 (2·06-2·22)	1·15 (1·09-1·21)
Chronic only	3194	2002	4.9×10^3	0.405 (0.387-0.423)	2.99 (2.86-3.13)	1.75 (1.71-1.86)	1.26 (1.17-1.36)
Acute on chronic	998	619	1.3×10^3	0.478 (0.442-0.518)	3.53 (3.26-3.33)	2.36 (2.18-2.56)	1.38 (1.24-1.54)
ESRD	1389	782	2.8×10^3	0.281 (0.26-0.30)	2.08 (1.94-2.23)	2.13 (1.98-2.30)	1.46 (1.29-1.67)

*Model 1: Partly adjusted for age, gender, myocardial infarction and Diabetes Mellitus with complications.
#Model 2: Fully adjusted for age, gender, SAPS3 score, myocardial infarction, cerebrovascular disease, diabetes mellitus with complications, moderate-to-severe liver disease, cancer and dementia.
MRR are relative to patients in the no renal disease group.

Table 11. Crude survival centiles derived from Laplace regression.

Group	Crude Survival (days) for each given centile (95% CI)			
	5th	**10th**	**20th**	**30th**
No renal disease	1.0 (0-2.0)	5.9 (4.6-7.3)	117 (108-125)	745 (725-765)
AKI	0.76 (0-2.4)	1 (0-3.6)	4 (0.8-7.0)	11 (7.5-14.5)
Chronic only	1.0 (0-4.4)	2.0 (0-7.4)	9.0 (2.5-15.5)	31.0 (23.7-38.3)
Acute on chronic	1 (0-4.9)	1.2 (0-7.8)	4.6 (0-12.8)	13 (3.6-22.4)
ESRD	1.0 (0-7.8)	2.8 (0-10.8)	20.3 (10.0-30.7)	109.0 (72.2-145-7)

Table 12. Times specific estimates of risk of developing ESRD

Group	Estimates of likelihood of developing ESRD at specific time points %, ci			
	90 days	1 year	3 years	5 years
No renal disease	0.04 0.03-0.06	0.08 0.06-0.10	0.20 0.16- 0.25	0.30 0.24-0.38
AKI	1.67 1.25-2.22	2.03 1.56-2.65	2.95 2.18-3.98	3.88 2.72-5.51
Chronic only	5.95 4.98-7.10	9.13 7.88-10.57	16.56 14.38-19.03	21.09 17.92-24.73
Acute on chronic	15.82 12.93-19.28	19.71 16.45-23.52	25.45 20.92-30.76	25.45 20.92-30.76

Table 13. Multivariable Poisson regression for risk of developing ESRD.

Group	Patients (N)	Events (N)	Person-years	IR event/ person- year (CI)	Crude IRR (95% CI)	Adjusted IRR * (CI)
No renal disease	92509	116	1.7×10^{5}	0.0007 (0.0006-0.0008)	1	1
AKI	5273	65	5.2×10^{3}	0.0125 (0.0098-0.0160)	18.6 (13.7-25.2)	24.1 (13.9-42.0)
Chronic Only	3194	237	3.4×10^{3}	0.069 (0.0611-0.0788)	103 (82.5-128.6)	96.4 (59.7-155.6)
Acute on chronic	998	111	803.1	0.1382 (0.1147-0.1665)	205.1 (158.1-266.1)	259 (156.9-429.1)

IR= Incidence rate, IRR= incidence rate ratio

*Adjusted for (Simplified Applied Physiology Score version 3 (SAPS3) score, age, gender and diabetes and dementia.

Table 14. Competing risks model, predicting risk of ESRD in one-year ICU survivors. Polynomial multivariable logistic regression analysis.

Covariate	Relative risk ratio * (95% ci)	P value
Female gender	1.12(0.48-2.63)	0.787
Congestive heart failure	0.091 (0.011-0.690)	0.020
Admission serum potassium high (>4.59)	4.6 (1.30-16.40)	0.018
AKI	30.4 (5.98-154)	<0.001
CKD	265.7 (55.1-1280)	<0.001
AoC	356.6 (69.9-1811)	<0.001

Reference category = Male, no comorbidity (according to Charlson index), admission potassium (3.9-4.59), no renal disease.

*Risk relative to the reference category.

SUBGROUP ANALYSIS. AKI AETIOLOGY- UNPUBLISHED DATA

Baseline characteristics

Patients with ischaemic AKI were older compared to other aetiological groups and this group, had the highest proportion of male patients, 80.5% (Table 15). The septic AKI group had longer lengths of stay, higher admission creatinine and urea and the highest illness severity scores; compared to ischaemic AKI they had greater comorbid burden according to Charlson score. Although the highest mean Charlson score was seen recorded in the unspecified AKI group.

Mortality

Mortality rate was highest for patients with AKI of septic aetiology (Table 16). In crude analysis septic AKI had a MRR of death of 1.23 compared to unspecified AKI aetiology. In the adjusted analysis in model I this rose to 1.3 but declined to 0.88 in model II which adjusted for SAPS score. Ischaemic AKI had crude and adjusted MRR of 1.09 and 2.03.

Incidence of CKD and ESRD

CKD Incidence did not differ significantly between aetiology in adjusted or crude analysis (Table 16). Only three patients developed ESRD of those with ischemic AKI. Patients with septic AKI had lower crude and adjusted IRR for ESRD compared to patients with an unspecified cause.

Table 15. Baseline characteristics of de novo AKI patients according to aetiology.

Baseline Characteristics	**AKI Aetiology**				
	Unspecified N= 3599	**Septic N= 1597**	**P**	**Ischaemic N=77**	**P**
Age (years)	70 (61-78)	70 (60-78)	0·129	74 (67-78)	0·024
ICU stay (hours)	54 (24-141)	116 (39-283)	<0·001	104 (48-257)	<0·001
Female N (%)	1374 (38·2)	697 (43·6)	<0·001	15 (19·5)	<0·001
Highest Creatinine (µmol/l)	275 (165-457)	239 (164-371)	<0·001	179 (132-246)	<0·001
Highest Urea (mmol/L)	18 (10-29)	17 (12-24)	0·530	12 (10-18)	0·360
Apache II score	24 (19-31)	27 (22-33)	<0·001	20 (18-29)	0·211
SAPS II score	52 (40-67)	60 (47-75)	<0·001	55 (39-65)	0·789
SAPS III score	66 (57-75)	71 (63-81)	<0·001	58·5 (52-70)	0·047
Charlson score * (SD)	2·7 (2·49)	2·55 (2·50)	0·008	1·83 (1·64)	0·005
Comorbidity N (%)					
Myocardial infarction	719(20·0)	242(15·2)	<0·001	25(32·5)	0·007
Congestive heart failure	953(26·5)	353(22·1)	<0·001	18(23·4)	0·603
Peripheral vascular disease	499(13·9)	140(8·8)	<0·001	65(84·4)	<0·001
Cerebral vascular disease	580(16·1)	212(13·3)	0·005	16(20·8)	0·274
COPD	547(15·2)	249(15·6)	0·738	12(15·6)	0·873
Liver disease	231(6·4)	73 (4·6)	0·009	0 (0)	0·014
Diabetes (uncomplicated)	988 (27·5)	372 (23·3)	0·002	12 (15·6)	0·020
Diabetes complicated	373 (10·3)	144 (9·2)	0·145	6 (7·8)	0·572
Cancer	830 (23·1)	423 (26·5)	0·008	9 (11·7)	0·019

Values are median (IQR), if not otherwise stated, or n (%).
*Charlson score is not age adjusted

Table 16. Multivariate Poisson regression primary and secondary outcomes according to AKI aetiology

Group	**Patients (N)**	**Events (N)**	**Person-years**	**Event rate/ person-year (95% CI)**	**Crude Relative risk (95% CI)**	**Adjusted Relative risk (95% CI)**	
A. Primary outcome death.					MRR	MRR Model 1*	MRR Model 2#
Unspecified Aetiology	3599	1966	$2{\cdot}4 \times 10^5$	0·364 (0·348-0·381)	1	1	1
Septic AKI	1597	932	$2{\cdot}1 \times 10^3$	0·449 (0·421-0·479)	1·23 (1·14-1·33)	1·3 (1·20-1·41)	0·88 (0·79-0·99)
Ischaemic AKI	77	45	113	0·396 (0·29-0·53)	1·09 (0·81-1·46)	0·811 (0·59-1·10)	2·03 (1·39-2·97)
B. Secondary outcome CKD					IRR	IRR Model 3†	
Unspecified aetiology	3599	143	$5{\cdot}0 \times 10^3$	0·028 (0·024-0·033)	1	1	
Septic AKI	1597	47	$2{\cdot}0 \times 10^3$	0·024 (0·018-0031)	0·84 (0·61-1·17)	0·90 (0·65-1·25)	
Ischaemic AKI	77	3	99·6	0·030 (0·01-0·093)	1·06 (0·34-3·33)	0·6 (0.19- 2·03)	
C. Secondary outcome ESRD					IRR	IRR Model 3†	
Unspecified aetiology	3599	51	$5{\cdot}3 \times 10^3$	0·0096 (0·007-0·012)	1	1	
Septic AKI	1597	11	$2{\cdot}1 \times 10^3$	0·0053 (0·003-0·009)	0·55 (0·29-1·06)	0·57 (0·29-1·1)	
Ischaemic AKI	77	3	110	0·027 (0·009-0·085)	2·84 (0·89-9·1)	2·1 (0·6-7·8)	

*Model 1 Adjusted for diabetes, peripheral vascular disease, gender, age, myocardial infarction, and emergency surgery·
#Model 2 Adjusted for diabetes, peripheral vascular disease, gender, myocardial infarction, emergency surgery and SAPS3
†Model 3 adjusted for diabetes, peripheral vascular disease, gender, age, myocardial infarction and emergency surgery·

STUDY III

The incidence of AKI was 41.4% in the ICU cohort of 1869. Among AKI patients, ICU mortality was 11.1%; 315 patients with exposure to AKI on ICU were recruited, 41 were lost to follow-up. Follow-up occurred at a median time of 101.5 days (IQR 89.5-126).

Baseline characteristics
Baseline characteristics of recruited AKI patients are shown in Table 17.In this AKI cohort, 36.9% fulfilled RIFLE F criteria. Baseline creatinine was measured in 53.3%. The recruited AKI group differed from the non-recruited AKI group (which included AKI patients who died on ICU) in having shorter median lengths of stay, lower median daily diuresis and a smaller proportion received invasive ventilation.

Incidence of CKD
Median follow-up creatinine was 76umol/l (IQR 59-96) cystatin C was available in 211 patients with a median of 1.33mg/l (IQR 1.09-1.73). Lund-Malmö formula gave the lowest median GFR estimate of the creatinine-based equations and is nearer to the cystatin C mean GFR (74.6 and 51.4mL/min/1.73m^2) (Table 18). Estimates obtained using the composite cystatin C and creatinine formula (64.5mL/min/1.73m^2) lie in between those obtained from individual markers.

Criteria for *KDIGO* CKD stage 3 or greater, were fulfilled by 63.7% of patients according to cystatin C-based estimates, 30.8% using L-M and 25.8% according to both MDRD and CKD-EPI creatinine-based formulae (Table 19). The combined equation identified 42.2% of patients as having CKD.

Incidence and predictors of AKD
In this study, 18.9% fulfilled AKD criteria at three months. The probability of AKD increased with increasing cystatin C value, and female gender (Table 20).

Predictors of CKD:
CKD diagnosis based on cystatin C eGFR was associated with elevated discharge cystatin C level, older age and pre-morbid diabetes (Table 21). The risk of developing CKD according to creatinine eGFR in both logistic regression models was associated with increasing age and female gender (Table 22). Model I, included discharge cystatin C and performed slightly better when assessed using Somers´s d, AIC and BIC than model II, which instead included discharge creatinine. Univariate sensitivity analysis showed that the likelihood of diagnosis with CKD according to cystatin C and the likelihood of AKD increased significantly if known baseline creatinine was used rather than estimated baseline, this effect disappeared in multivariate analysis.

Mortality
Overall one-year mortality for all AKI patients was 28.2%, and in recruited AKI patients alive at three months, it was 9.8% (Table 23). Mortality increased linearly with RIFLE grade (Table 24). The two-year mortality for patients classified as AKD was 16.7% versus 11.2% without AKD, P=0.277.

Mortality according to CKD classification.
No statistically significant differences were seen between patients classified as having CKD according to each biomarker and those without CKD. Neither did mortality differ between those identified as CKD by creatinine or by cystatin C; nor did mortality vary according to AKD status.

Table 17. Base characteristics of recruited patients

Baseline Characteristics of All recruited AKI-patients (315) Median values		**Maximum RIFLE level attained N (%)**		
		Risk 118 (37.5)	**Injury 80 (25.4)**	**Failure 117 (37.1)**
Median age (years) IQR	64 (52-72)	65 (50-72)	63 (52-73)	63 (53-72)
Sex, female N (%)	128 (40.6)	47 (39.8)	34 (42.5)	47 (40.2)
Median length of stay days, (IQR)	6 (3-12)	5 (3-10)	4.5 (3-9)	8 (4-16)
SAPS 2 score, (IQR)	48 (37-64)	45 (36-57)	44 (32-60)	56 (41-72)
Invasive ventilation, N (%)	126 (40)	56 (47.5)	23 (28.8)	47 (40.2)
Dialysis on ICU, N (%)	71 (22.5)	0 (0)	0 (0)	71 (60.7)
Minimum daily diuresis (ml) (IQR)	770 (365-1418)	988 (502-1676)	785 (465-1580)	770 (365-1417)
Maximum Urea (mmol/l) (IQR)	15.3 (8.7-24.8)	9.1 (6.4-15.6)	14.0 (8.5-20.2)	24.4 (17.4-32.4)
Baseline Creatinine				
N measured (%)	168 (53.3)	71 (60.1)	46 (57.5)	51 (44)
Measured (umol/l) IQR.)	64 (49-75)	63 (43-72)	65.5 (50-76)	66 (55-81)
Estimated (MDRD[a]) (umol/) (IQR) N= 146	88 (71-97)	88 (71-94)	88 (71-97)	88 (71-97)
Admission Creatinine (umol/l) (IQR)	135 (106-205)	115.5 (77-130)	135.5 (95-177)	241 (149-320)
Maximum Creatinine (umol/l) (IQR)	163 (123-260)	121 (94-139)	178 (144-206)	291 (231-378)
Last ICU Creatinine (umol/l) (IQR)	104 (71-149)	77.5 (59-116)	102 (74-157)	136 (96-211)
Admission cystatin C (mg/l) (IQR)	1.58 (1.1-2.28)	1.19 (0.87-1.58)	1.37 (1.02-1.80)	2.36 (1.73-2.91)
Maximum cystatin C (mg/l) (IQR)	2.12 (1.42-2.98)	1.50 (1.16-2.02)	2.12 (1.58-2.51)	3.18 (2.44-3.73)
Last ICU cystatin C (mg/l) (IQR)	1.63 (1.2-2.2)	1.31 (1.04-1.70)	1.62 (1.25-2.12)	2.16 (1.59-2.73)
Discharge Creatinine/ cystatin ratio (IQR)	7.1 (5.3-9.2)	6.8 (2.9-14.8)	7.0 (3.1-14.5)	7.3 (5.4-10.0)
Comorbidity N (%)				
COPD	48 (15.3)	22(18.0)	12 (14.6)	19 (15.3)
Diabetes Mellitus I & II	59 (18.8)	16 (13.1)	17 (20.7)	30 (24.2)
Cardiovascular disease	105 (33.4)	46 (39.0)	24 (30.0)	35 (30.2)
Hypertension	138 (44.0)	53 (44.9)	34 (42.5)	51 (44.0)
Liver failure	10 (3.2)	1 (0.9)	3 (3.8)	6 (5.2)
Haematological malignancy	20 (6.4)	5 (4.3)	7 (8.8)	8 (6.9)
Other malignancies	94(30)	44 (37.3)	25 (31.3)	25 (21.5)
Heart failure	40 (12.8)	14 (11.9)	14(17.7)	12 (10.3)

[a]=MDRD based estimate

Table 18. GFR estimates according to Creatinine and Cystatin-C equations.

N= 201 (mL/min/1.73m^2)	**Median**	**IQR.**	**Minimum**	**Maximum**	**P Compared to L-M[a] estimate**
Lund-Malmö	74.6	55.9-94.3	18.5	132.2	Reference
MDRD [a]	81.6	58.6-106.8	7.0	225.2	<0.001
CKD-EPI-Cr	86.0	59.6-101.4	6.6	139.6	<0.001
CKD-EPI-Cy	51.4	35.8-69.9	9.1	138.3	<0.001
CKD-EPI-Cr- Cy	64.5	46.7-83.5	7.28	137.5	<0.001

Table 19. KDOQI CKD stage according to creatinine and cystatin-C based equations.

N= 201 ***KDOQI*** **CKD stage**	**GFR Estimating formula**				
	Creatinine Based formulae				
	L-M[a]	MDRD[a]	CKD-EPI	CKD-EPI Cystatin C	Combined CKD-EPI Creatinine & Cystatin C
	N (%)				
GFR >90 *	61 (30.4)	80 (39.8)	86 (42.8)	28 (13.9)	42 (20.9)
GFR 60-90*	78 (38.8)	69 (34.3)	63 (31.3)	45 (22.4)	74 (36.8)
3	55 (27.4)	45 (22.4)	44 (21.9)	97 (48.3)	71 (35.3)
4	7 (3.5)	6 (3.0)	7 (3.48)	24 (11.9)	12 (6.0)
5	0 (0)	1 (0.5)	1 (0.5)	7 (3.5)	2 (1.0)
CKD Stage 3,4 5	62 (30.8)	52 (25.8)	52 (25.8)	128 (63.7)	85 (42.2)

a Lund-Malmö formula

b MDRD = Modified Diet in Renal Disease formula.

*no urinalysis available

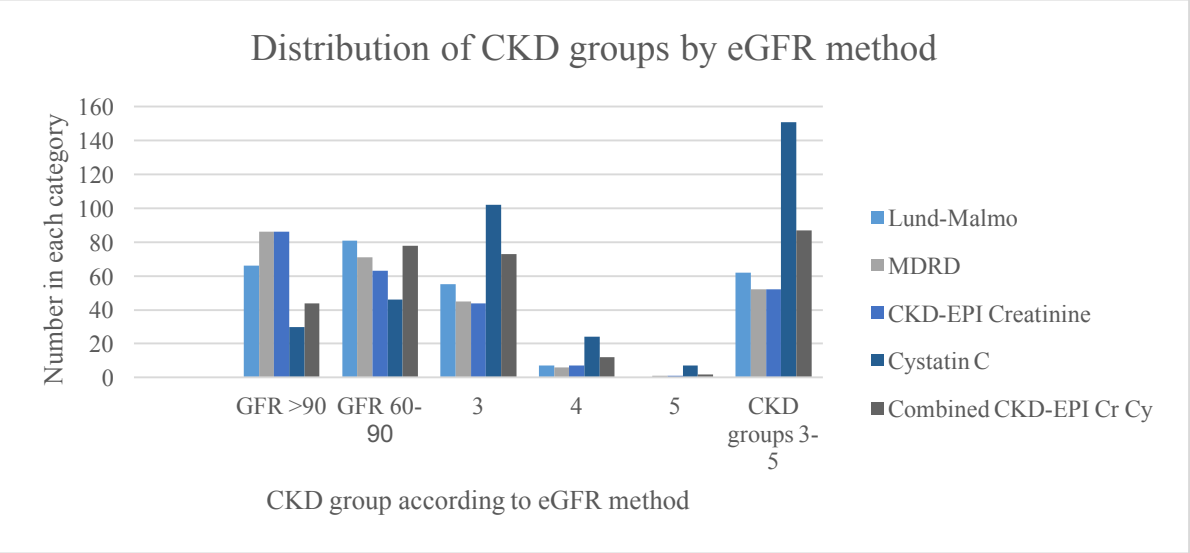


Figure 10. Categorisation of patients by CKD group at follow-up according to the method of GFR estimation. N= 201 (Follow-up 2-7 months).

Table 20. Logistic regression model; the risk of AKD at three-month follow-up.

Probability of AKD at follow up.		
Covariate	**Odds ratio (95% CI)**	**P**
Discharge Cystatin C (mg/l)		
0-2	1.0 (ref)	
2-3	1.5 (0.74-3.15)	0.248
>3	3.1 (1.01-9.48)	0.047*
Gender		
Male	1.0 ref	
Female	2.1 (1.05-4.07)	0.034*

Table 21. Logistic regression model, the probability of CKD according to Cystatin-C based GFR estimate.

Probability of CKD according to CKD-EPI cystatin C-based eGFR < 60ml/min/1.73m^2 at follow up.		
Covariate	**Odds ratio (95% CI)**	**P**
Discharge Cystatin C (mg/l)		
0-2	1.0 (ref)	
2-3	3.2 (1.4-7.1)	0.005*
>3	7.8 (2.4-25.3)	0.011*
Age (years) (25 centile distribution)		
<52	1.0 (ref)	
52-64	5.8 (1.9-17.9)	0.002*
64 -72	12.3 (4.1-37.4)	<0.001 *
>72	54.7 (14.2-211)	<0.001 *
Comorbidity		
No diabetes	1.0 ref	
Diabetes I & II	2.8 (1.1-8.2)	0.042

Table 22. Logistic regression models. Risk of CKD based on Creatinine eGFR.

Probability of CKD according to MDRD creatinine-based eGFR < 60ml/min/1.73m^2 at follow-up.				
Covariate	**Model 1**		**Model 2**	
	Odds ratio (95% CI)	**P**	**Odds ratio (95% CI)**	**P**
Discharge Cystatin C(mg/l)				
0-2	1.0 (ref)			
2-3	2.3 (1.1-4.8)	<0.031*		
>3	4.6 (1.4-15.2)	<0.013 *		
Discharge Creatinine (umol/l)				
<100			1.0 (ref)	
100-200			2.3 (1.1-4.7)	0.025*
200-300			2.9 (1.0-8.2)	0.050
>300			4.7 (0.5-44.4)	0.179
Age (years) (25 centile distribution)				
<52	1.0 (ref)		1.0 (ref)	
52-64	1.9 (0.5-8.6)	0.363	2.2 (0.5–9.9)	0.293
64 -72	8.0 (2.1-30.9)	0.003*	8.8 (2.1-34.8)	0.002*
>72	11.8 (2.9-30.9)	<0.001 *	14.1 (3.6-55.1)	<0.001*
Gender				
Male	1.0 (ref)		1.0 (ref)	
Female	3.0(1.5-6.1)	0.002*	3.4 (1.7-6.9)	0.001*

Table 23. Kaplan Meier mortality estimates for ICU cohort

Time after admission (days)	**All N=1869**	**No AKI N=1035**	**AKI not recruited N=459**	**AKI recruited N=315**	**CKD N= 60**	**All AKI N=774**
30	14.2	10.4	31.8	0.0	20.0	18.8
90	17.0	13.0	36.6	0.32	25.0	21.8
360	22.2	16.6	40.7	9.8	40.0	28.2
730	25.4	18.7	42.5	17.0	55.0	32.1
P vs no AKI		<0.001	<0.001	0.282	<0.001	<0.001

NB. AKI not recruited included AKI-patients who died on ICU or before follow-up

Table 24. Mortality estimates according to RIFLE grade for all AKI patients in the ICU cohort.

Time after admission (days)	**Max RIFLE Grade all AKI patients in the ICU cohort**		
	Risk N= 420	Injury N= 143	Failure N= 262
30	12.4	22.4	27.1
90	15.3	25.9	30.5
360	21.4	31.5	38.9
730	25.8	36.5	44.8
P		vs Risk 0.007	vs Risk <0.001

STUDY IV

In the primary analysis, we studied 23 patients in whom both endogenous biomarkers and Iohexol were measured at nine-months. In secondary analysis, we studied 43 patients where both creatinine and cystatin-C were available. Lack of availability of research staff to oversee nine-month follow-up, meant Iohexol, and both biomarkers were not measured in all patients. Median follow-up time was 224 days (IQR 196-301).

Baseline characteristics
The nine-month follow-up cohort had a median age of 66 years, and 34.9% were female (Table 25). They differed significantly from all other AKI survivors recruited at discharge in having a lower median daily diuresis, maximum ICU and discharge creatinine, and cystatin C values were all significantly higher ($p=<0.05$). 20.9% had pre-existing CKD.

Nine-month follow-up
Median values of creatinine and cystatin C taken at each time-point are detailed in Table 26 and Figure 12. Median creatinine did not statistically differ between discharge and three-months post-ICU ($P=0.126$) nor between three and nine-months ($P=0.522$). Median cystatin C decreased significantly from discharge to three-months ($P<0.000$) and diminished again from three and nine-months ($P=0.017$).

EGFR was under 60ml/min/1.73m2 in 52% of patients when estimated using creatinine derived formulae (CKD-EPI and Lund-Malmö equations), in 82% using cystatin C, in 56% with the combined formula and 69.6% when measured using Iohexol (Table 27). Figure 11 displays a boxplot of eGFR and measured Iohexol GFR (mGFR) values obtained at three and nine-months in the 23 patients with all measured biomarkers.

Comparison of creatinine and cystatin C formula in estimating measured GFR.
Compared to Iohexol GFR, the creatinine derived formulae CKD-EPI, and L-M tended to overestimate Iohexol GFR as displayed in Bland-Altman plots Figure 13. Variation between CKD-EPI and Iohexol was most pronounced at higher GFR whereas with L-M the difference was constant over the range of observed values. Cystatin C tended to underestimate Iohexol mGFR, and the difference between the two measures was greatest at high measured filtration rates. The composite formula underestimated Iohexol GFR at the lowest and the highest values.

Performance characteristics of biomarkers in estimating Iohexol mGFR
The composite formula performed better at estimating Iohexol mGFR than other formulae, displaying the lowest level of bias, the highest level of precision and greatest accuracy (87% p30) detailed in Table 28. Lund-Malmö and CKD-EPI creatinine eGFR demonstrated a high degree of accuracy (82.6%). Cystatin C was least accurate (60.8%) and underestimated eGFR particularly at higher GFR values.

ROC analysis showed creatinine and cystatin C to have very high sensitivity and specificity to 4 point Iohexol measurement at GFR threshold 60ml/min/1.73m^2 displayed in Figure 14. Creatinine yielded an (AUC) of 0.938, cystatin C 0.910 and the composite CKD-EPI-cr-cy an area of 0.955.

The greatest concordance with Iohexol measurement at nine months was seen with the composite creatinine and cystatin C formula and the least observed with cystatin C formula (0.81, CI 0.64-0.95).

Secondary analysis: concordance between GFR estimates

In 43 patients, concordance increased between creatinine (L-M) and cystatin C based eGFR from 0.28 (CI 0.05-0.50) to 0.48 (CI 0.31-0.65) at three months and to 0.63 (CI 0.48.-0.76) at nine months (Table 29). The analysis was repeated using data from the source AKI population of patients recruited at discharge (Table 30). Patients contributed data if both biomarkers were analysed at any of the time points between discharge and nine-month (336 at discharge, 51 at 9-months). We included out-patient data taken outside of the study at nine months. A similar pattern of increasing concordance was observed being 0.49 at discharge and 0.65 at nine-month follow-up.

Inter-rater agreement at nine months was greatest between Iohexol and creatinine (MDRD formula) being 68% (kappa 0.54) (Table 30). Cystatin C and combined CKD-EPI-Cr-Cy both had an observed agreement of 60 when compared to Iohexol with kappa values of 0.38 and 0.43 respectively.

Creatinine concentration at nine months.

All nine-month creatinine values available from patients originally recruited to the "Excrete" study were analysed. These included values from 99 patients with (some of whom were excluded from main analysis because cystatin C was not concurrently taken) and 71 without renal dysfunction at three-month follow-up (lab data collated from outpatient visits). Patients with CKD were excluded. In total, nine-month creatinine was available in 67.1% of the three-month follow-up attendees and median creatinine between three and nine months was statistically unchanged P=0.178. In total 28.7% had eGFR under 60ml/min/1.73^2 at 9-months according to CKD-EPI creatinine.

Table 25. Baseline characteristics of patients in study IV.

Baseline characteristics (Median unless stated otherwise) N=336	**Patients followed to nine-months N = 43**		**All other AKI patients recruited at discharge N =293**		**P**
	Median	IQR	Median	IQR	
Age (years)	66	56-73	64	52-72	0.220
Sex, female N (%)	15	34.9	119	40.6	0.509
Median length of stay days,	5	3-11	6	3-12	0.581
SAPS 2 score	51	42-66	47.5	(37-63)	0.074
Invasive ventilation, N (%)	18	41.8	25	39.9	0.868
Dialysis on ICU, N (%)	13	30.2	68	23.2	0.341
Minimum daily diuresis(ml)	500	260-975	791	370-1510	0.026
Maximum Urea (mmol/l)	19.5	10.6-30.8	15.6	8.45-25.2	0.092
Baseline creatinine Measured, N (%) per group Baseline creatinine umol/l	 29 86.0	 67.4 64-97	 156 72	 53.2 64-88	 0.058 0.101
Admission creatinine (umol/l)	185	120-273	135	106-209	0.054
Maximum creatinine (umol/l)	197	156-323	161	123-262	0.023
Maximum RIFLE Grade ICU	3	1-3	2	1-3	0.027
Last ICU creatinine (umol/l)	138	107-188	102	70-152	0.003
Admission cystatin-C (mg/l)	1.95	1.35-2.56	1.57	1.08-2.28	0.010
Maximum cystatin-C (mg/l)	2.57	1.87-3.39	2.12	1.41-3.04	0.018
last ICU cystatin-C (mg/l)	2.14	1.64-2.56	1.61	1.2-2.2	0.001
Comorbidities	**N**	**%**	**N**	**%**	
COPD	8	18.6	45	15.4	0.654
Diabetes Mellitus I & II	9	20.9	57	19.5	0.838
Cardiovascular disease	11	25.6	103	35.3	0.232
Hypertension	25	58.1	127	43.5	0.100
Liver failure	4	9.3	7	2.4	0.040
Haematological malignancy	6	14.0	18	6.2	0.103
Other malignancies	12	27.9	89	30.5	0.859
Heart failure	4	9.3	41	14.1	0.480
Chronic Kidney Disease	9	20.9	12	4.1	<0.001

Table 26. Biomarker values and GFR estimates from baseline to nine-month control.

Measurement time	Variable	Median	IQR	Equation	Median GFR ml/min /1.73m^2	IQR	% GFR <60*
Baseline	Creatinine measured (umol/l)	77	61-110	L-M	69.0	52.6-89.3	31.0
				CKD-EPI	76.9	69.2-94.6	18.6
	Estimated (MDRD)	88	71-97	L-M	67.3	61.6-69.6	21.4
				CKD-EPI	74.2	70.2-77.0	0
Discharge	Creatinine (umol/l)	138	107-188	L-M	40.3	33-61	74.4
				CKD-EPI	41.1	31.2-57.1	37.2
	Cystatin-C (mg/l)	2.14	1.64-2.56		25.7	20.4-40.3	90.7
	Combined formula				46.1	41.6-61.2	74.4
Three-month follow-up	Creatinine (umol/l)	105	83-163	L-M	52.7	35.7-77.8	55.8
				CKD-EPI	55.8	32.7-87.3	53.5
	Cystatin-C (mg/l)	1.81	1.42-2.69		32.8	19.7-48.6	93
	combined formula				42.4	24.6-62.3	72.1
Nine-month follow-up	Creatinine (umol/l)	111	81-148	L-M	52.2	41.7-68.9	62.8
				CKD-EPI	53.1	40.8-76.4	62.8
	Cystatin-C (mg/l)	1.73	1.34-2.45		34.1	20.9-53.6	90.7
	Combined formula				39.8	27.0-62.3	67.4

L-M =Lund-Malmö formula

Table 27. GFR estimates and Iohexol mGFR at 9 months N=23.

GFR method	Median GFR ml/min/1.73m2	IQR	%GFR <60
CKD-EPI Creatinine	57.4	44.9-89.5	52%
Lund-Malmö Creatinine	54.8	42.5-77.5	52%
CKD-EPI cystatin-C	46.2	28.8–54.7	82.6%
CKD-EPI combined formula	53.5	36.2–69.8	56.2%
Iohexol	53	40.0-62.0	69.6%

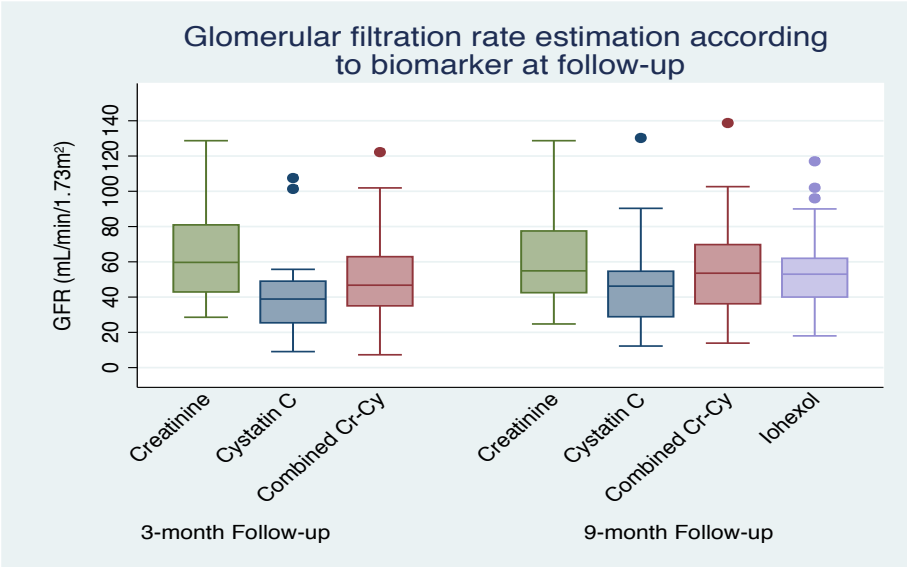


Figure 11. GFR estimates at three and nine months.

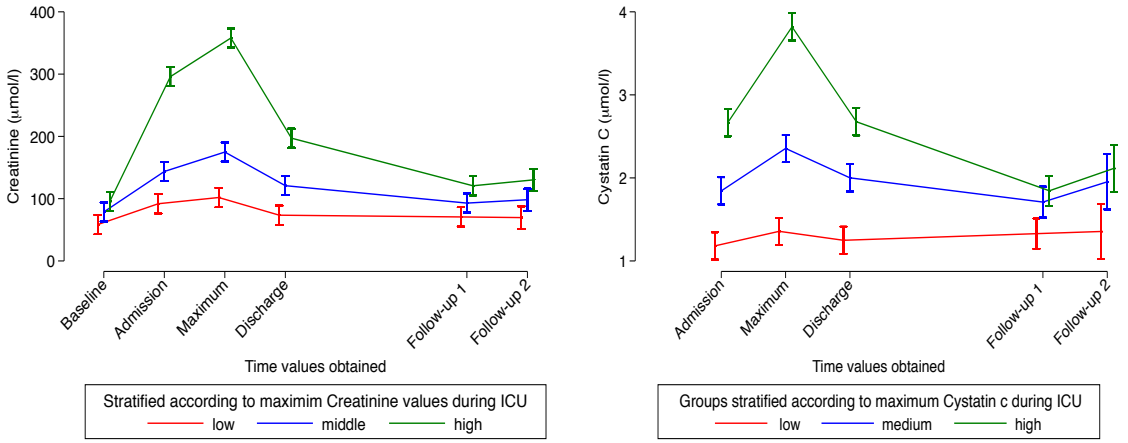


Figure 12. Mean Creatinine and Cystatin C values over study time, stratified by maximum ICU value for each biomarker. Includes all values obtained with-in the study at each time point.

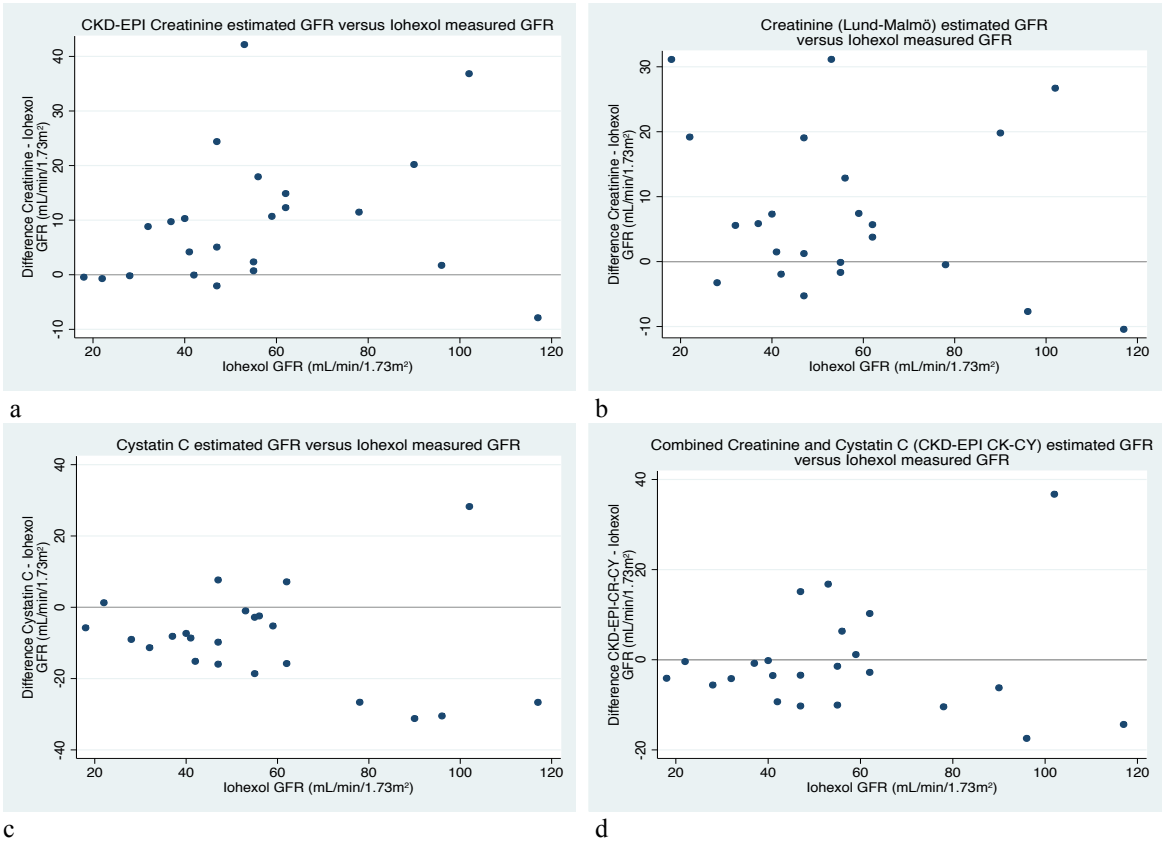


Figure 13. Bland-Altman plots showing difference between estimated GFR according to a) creatinine CKD-EPI, b) creatinine Lund-Malmö formula, c) cystatin-C CKD-EPI formula, d) combined CKD-EPI Cr-Cy formula and Iohexol and measured 4 point Iohexol GFR.

Table 28. Performance of GFR estimators.

Performance measure **GFR =ml/min/1.73m^2**		**GFR estimator vs Iohexol**			
		creatinine		**cystatin-C CKD-EPI**	**Combined CKD-EPI-Cr-Cy**
		CKD-EPI	**Lund-Malmö**		
Bias	Median Absolute difference 95% CI Median Relative difference (%) 95% CI	8.8 0.98-12.1 14.7 4.3-25.3	5.6 -0,39-11.43 11.6 6.5-21.1	-8.6 -15.6—3.4 22.8 16.8-32-7	-3.4 -6.1—2.5 -13.1 7.0-19.6
Precision	IQR range IQR Absolute difference IQR range IQR relative difference (%)	-0.1-14.8 14.9 3.2-26.3 23.1	-1.7-19.1 20.8 4.6-22.9 18.3	-16-0—2.4 18.4 11.5-33.8 22.3	-9.3-1.1 10.4 4.5-21.9 17.4
	Sign test bias=0 Sign test relative diff= 0	0.035 0.000	0.210 0.000	0.0026 <0.001	0.0347 <0.001
Accuracy	P30% CI	82.6 61.3-95.1	82.6 61.2-95.1	60.8 38.5-80.3	87.0 66.4-97.2

Table 29. Concordance between biomarker pairs from discharge to nine-month follow-up.

Time	**EGFR Variable**	**EGFR Variable**	**N**	**Concordance coefficient (ci)**	**Mean difference**	**95% limits of agreement**
Discharge	**CKD-EPI Creatinine**	**L_M Creatinine**	**43**	**0.85 (0.76-0.93)) ***	**-1.6**	**-32.3-28.9**
	CKD-EPI Creatinine	**CKD-EPI Cystatin c**	**43**	**0.37 (0.15-0.59)**	**15.1**	**-39.1-69.4**
	CKD-EPI Cystatin c	**L_M Creatinine**	**43**	**0.28 (0.05-0.50))**	**-16.9**	**-71.2-37.5**
Three-month follow-up	**CKD-EPI Creatinine**	**L_M Creatinine**	**43**	**0.82 (0.73-0.92)**	**1.4**	**-32.1.0-34.8**
	CKD-EPI Creatinine	**CKD-epi Cystatin c**	**43**	**0.55(0.41-0.70)**	**22.9**	**-12.4-58.2**
	CKD-epi Cystatin C	**L_M Creatinine**	**43**	**0.48 (0.31-0.65)**	**-21.7**	**-58.6-15.3**
Nine-month follow-up	**CKD-EPI Creatinine**	**L_M Creatinine**	**43**	**0.94 (0.91-0.97)**	**1.6**	**-16.9-20.1**
	CKD-EPI Creatinine	**CKD-EPI Cystatin c**	**43**	**0.63 (0.50-0.76))**	**20.3**	**-11.3-51.9**
	CKD-EPI Cystatin c	**L_M Creatinine**	**43**	**0.63 (0.48-0.76)**	**-18.7**	**-47.8-10.3**
Versus Iohexol	**CKD-Epi Creatinine**	**Iohexol**	**23**	**0.85 (0.75-0.95)**	**9.7**	**-14.4-33.8**
	L_M Creatinine	**Iohexol**	**23**	**0.86 (0.75-0.96)**	**7.3**	**-16.2-30.7**
	CKD-Epi Cystatin C	**Iohexol**	**23**	**0.81 (0.64-0.95)**	**-9.0**	**-35.5-17.4**
	Joint CKD-Epi Cr-Cy	**Iohexol**	**23**	**0.90 (0.83-0.98)**	**-0.8**	**-23.9-22.6**

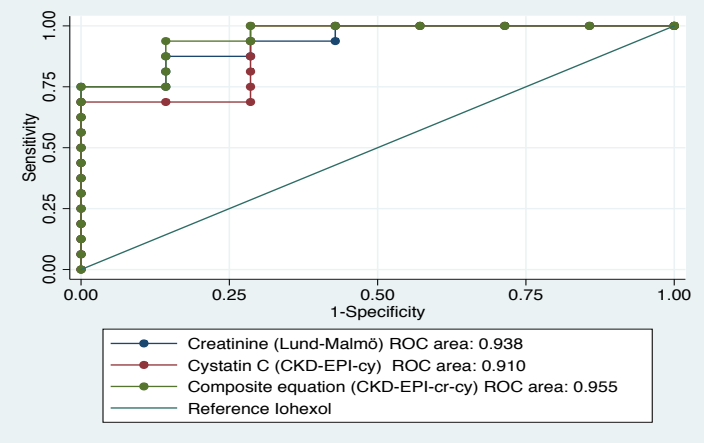


Figure 14.ROC analysis. GFR estimators' ability to predict Iohexol GFR under 60.

Table 30. Inter-rater agreement in classifying patients according to CKD group in AKI patients followed at nine months using Kappa.

GFR Method	**MDRD**			**cystatin-C**			**Iohexol**		
	Agreement %								
	Observed	Expected	Kappa	Observed	Expected	Kappa	Observed	Expected	Kappa
Lund Malmo	84.0	31.5	0.77	48.0	29.6	0.26	64.0	32.3	0.46
CKD-EPI cystatin-C	52.0	27.0	0.34				60.0	35.0	0.38
Combined CKD-EPI creatinine & cystatin-C							60	27.8	0.43
Iohexol	68.0	29.4	0.54						

CHAPTER 4. DISCUSSION

SUMMARY OF FINDINGS

- One year mortality from severe AKI was 49% in the Swedish ICU population.
- Compared to patients without AKI, AKI was independently associated with:
  - A two fold increased risk of death.
  - A 7 and 22 times elevated risk of developing CKD and ESRD respectively.
- Patients with pre-existing CKD and AoC had respectively a 96 and 259 times higher adjusted risk of progression to ESRD after ICU, compared to the no renal disease group.
- Risk factors associated with developing ESRD after ICU were: prexisting CKD, AKI, admission potassium above 4.6umol/l and congestive cardiac failure.
- Renal dysfunction among AKI survivors was common three months after ICU discharge. Its incidence varied depending on measurement method from 26% using creatinine (CKD-EPI) to 64% if cystatin C based eGFR was used.
- Discharge cystatin C was as good as discharge creatinine at predicting three-month creatinine values.
- At nine months the combined creatinine and cystatin C formula best predicted Iohexol mGFR. Creatinine derived formulae overestimated but were more accurate than cystatin C derived equations at estimating GFR.
- Concordance between creatinine and cystatin C eGFR increased between discharge and nine-month follow-up.

EVALUATION OF VALIDITY

Prior to interpretation of this thesis´ findings, an evaluation of the validity of the methods and results should be conducted. **Optimal study design** is fundamental to attaining valid results and we shall consider whether the best possible designs were chosen to answer the research questions? All studies were cohort in design, i.e. these studies were observational, exposure to AKI was naturally assigned and outcomes were followed prospectively by investigators. In Studies I and II, the cohort method allowed us to examine an extremely large population over an extended time and to study relatively common outcomes (death, CKD and ESRD). Studies III and IV were prospective cohort studies and study IV was a nested cohort, where patients were nestled within the group initially recruited to the "Excrete" study. The outcomes, renal function and death were observed after ICU discharge. The two latter studies could have been improved by the presence of a control group consisting of non-AKI ICU survivors. The cohort designs were otherwise, appropriate to address the research questions.

Internal validity may be assessed by considering whether these results have correctly measured the associations between exposure to AKI and outcome in the groups studied. Systematic and random errors shall be considered. **Systematic error** is the consistent deviation of measurements away from the true values. For example, the use of an incorrectly calibrated creatinine assay could consistently overestimate presence of AKI in a population. **Bias** is a form of systematic error, that may lead to an inaccurate estimation of the association between an exposure and an outcome. Selection bias may occur if subjects included in a study differ systematically from those excluded; while information bias may be present if the manner in which data on exposure is obtained differs between groups.

Studies I & II. On excitedly receiving the SIR database and beginning to browse the vast content within, it became clear that diagnosis codes (AKI), intervention codes (CRRT and IHD) and laboratory data, including creatinine, were under-reported or missing. We could not distinguish all patients with AKI by diagnosis codes and describe their AKI grade. Instead, we decided to identify as many patients with severe AKI as possible by using a combination of codes and creatinine values detailed in methods. Other patients with AKI, particularly mild cases, who were not coded, undoubtedly exist in the non-AKI group therefore some degree of misclassification cannot be excluded. Recognition that we were not able to identify all cases of AKI in the cohort meant we did not attempt to describe overall AKI incidence. Similarly, in paper II CKD diagnoses prior to and post ICU are likely to be under-reported and those with the most severe disease may be more likely to have the diagnosis assigned. There was in principle no loss to follow-up in these studies because mortality and renal outcomes were traceable in the comprehensive national databases.

Study III. Patient selection was by convenience sampling at discharge. Recruitment did not occur at times when research staff were unavailable such as weekends. There is some evidence to suggest that weekend discharge may be associated with increased mortality; for this reason the existence of selection bias cannot be excluded[152,153]. We suggest that the absence of research staff at other times ought to have occurred on a random basis and should not be associated with outcome. We were indeed able to compare baseline characteristics for selected and non-selected groups by cross matching the study cohort with the entire ICU database. When baseline creatinine was absent it was estimated using the eGFR 75 approach as recommended by the ADQI group, we used the MDRD formula[7]. This method may introduce bias by categorising patients with undiagnosed pre-existing CKD as having AKI, thus incidence of AKI will be overestimated an incorrect estimation of outcome could occur[154]. Our sensitivity analysis, though found that use of known baseline creatinine measurements rather than estimated values was not independently associated with likelihood of subsequent diagnosis of CKD or AKD.

A degree of loss to follow-up occurred in both **study III and IV**. Patients who did not attend are likely to have differed from those who did, therefore selection bias may exist. However, there was no loss to follow-up pertaining to the outcomes, death and ESRD because these were available in national registers. Loss to nine-month follow-up was encountered in **study IV** due partly to patient factors, which included dementia, substance abuse and feeling too unwell to attend and partly to logistical difficulties. Research staff shortages meant that some GFR estimations and measurements were missed by ordinary staff at follow-up clinics. This led to data loss which ought to have occurred at random. These limitations mean than in paper IV, we do not report the incidence of continuing renal dysfunction at 9 months.

Confounding: Could other factors which were not intermediate steps in the causal pathway account for the association seen between AKI and mortality or the development of CKD? Randomisation is intended to remove differences between the groups being investigated. In cohort studies, randomisation is not possible and one must attempt to deal with confounders in patient selection and in analysis. We attempted to minimise the effect of confounding by a priori, choosing covariates known or likely to influence the association between AKI and our outcomes of interest. Such factors included age, disease severity and comorbidities, we controlled for these potential confounders in regression models. The possibility of residual (unknown) confounding may though still remain.

External validity
Can we generalise our results and make inference about other ICU populations? In studies I and II, the sample cohort was obtained from the extremely large SIR database which covered 90% of the Swedish ICU population at the time. Generalisability to the source population should be very good. These results should also be broadly applicable to other countries with similar health care systems and among patients with comparable comorbid burdens and AKI aetiologies. **Studies III and IV** are single centre investigations performed using patients from a general ICU, generalisability is limited by size particularly in study IV.

Random error describes the influence of chance upon obtaining a point estimate. Random error exists in all studies, and reflects sampling error, i.e. chance variations occurring due to the sampling group differing from the source population. Random error diminishes with increasing sample size whereas, systematic error does not. Estimation precision is effected by random error and described by p values and confidence intervals (CI).

P values evaluate the probability of obtaining the sample data given that null hypothesis is true (no difference in effect between groups exists). A low p value suggests that study data are unlikely if the null hypothesis is true. An arbitrary threshold of 5% (alpha) was specified before each study commenced, as the alpha value under which the null hypothesis would be rejected, as is convention. However, one should be mindful that decisions made on this basis alone can lead to error. **Type I error** occurs when the null hypothesis is incorrectly rejected leading to a falsely positive finding and **type II error** is the act of incorrectly retaining the null hypothesis leading to falsely negative findings.

Confidence intervals (CI) reflect uncertainty associated with sampling methods and provide a range of values likely to contain the population measure of interest. Sample size in **studies I and II** mean than the likelihood of random error is low. The generally narrow CI reflects this. In studies **III and IV** smaller sample size means, random error is more likely and CI are wider. Limited sample size precluded some sub analysis. The two cohorts in this thesis, the SIR and excrete databases illustrate the trade-off between the number of study participants and the granularity as well as validity of individual variables.

To summarise validity: Studies I and II may be affected by some misclassification bias but low random error. The size and national nature of the sample mean the study results should be widely generalizable and validity is high. In **study III** some selection bias may be present. Missing data and loss to follow up, in **study IV** introduced some information and selection bias. Random error is likely to be higher in **studies III and IV** than in the first studies due to relatively small cohort size, and this restricts generalisability particularly of study IV. This later study offers a first indication of the relationship between creatinine and cystatin C and their abilities to predict nine month mGFR in AKI survivors and has generated hypotheses that may result in further research.

DISCUSSION OF FINDINGS

Critically ill patients who survive AKI risk long-term complications in terms of increased long-term mortality and renal morbidity. These risks have never before been described or evaluated in the Swedish ICU population.

Association between AKI and mortality

In **study I**, AKI mortality was similar to that reported in other Scandinavian research [19,58,65] and somewhat lower than in a Danish cohort and other international investigations including the RENAL study from Australia and New Zealand[51,52,61,76]. One year mortality in **study III**, ranged from 21.4% to 38.9% for RIFLE grades Risk to Failure. This represents a lower AKI mortality than observed in other research. An explanation as to why mortality may be lower in the Swedish and particularly the Stockholm cohort than in studies conducted abroad cannot be obtained from the study data. If permitted to speculate, we postulate that this may be a consequence of a high quality, universally accessible health care system and more specifically may reflect differences in AKI treatment traditions between Sweden and other developed countries. Praxis may differ in terms of choice of RRT modality or anticoagulation use, for example; continuous RRT (CRRT) has for a long time been the modality most often administered in Sweden. Of 32 ICU´s reporting to the SWING study (1995-2004), 85.7% used CRRT. This contrasts with practice in some other countries; in the Minnesota Mayo clinics, USA, CRRT was used in 52% of ICU patients requiring RRT between 2007 and 2013[155].

Use of quantile regression allowed us to describe when deaths in our cohort occurred, we found that 10% of AKI patients died by ICU day one and 30% were dead by 11 days. This result emphasises the huge burden of early mortality associated with AKI.

Mortality in patients with pre-existing CKD and ESRD.

In **study II** we found patients with CKD prior to ICU admission had the highest mortality of all the groups studied. This underlines the importance of monitoring the progress of CKD patients after ICU discharge. Patients with acute on chronic disease require particular focus in the future. We found mortality for ESRD patients to be lower than for the CKD group. Examination of baseline characteristics of ESRD patients revealed them to be younger and otherwise healthier than other groups. This corroborates evidence from Ostermann and Chans studies discussed in the introduction[106,107]. We conjecture that patients accepted for admission to ICU are a particularly healthy sub-selection of the ESRD group.

Incidence of CKD and ESRD after AKI.

Study I was conceived when a causal link between AKI and subsequent development of CKD had been proposed but the association was unproven. A lack of long term outcome studies and the absence of consensus on AKI definition meant the association had not been previously studied. This study makes an important contribution in providing evidence of an association. We found the adjusted risk of CKD to be sevenfold and ESRD to be 22 times higher in AKI patients than non-AKI. We may appraise the association between AKI and CKD using the modified Bradford Hill criteria for causality[156].

1. A stong and statistically significant association between AKI and CKD is seen.
2. Consistency. Multiple studies (13) summarised in a metanalysis by Coca found an association between AKI to be an independent risk factor in CKD and ESRD.
3. Hill proposed that associations are more likely to be causal if they are specific, this criterion has subsequently been criticised as our understanding of causality has

improved over time. AKI causes damage to other organs in addition to long-term renal dysfunction.

4. Temporality exists, exposure to AKI precedes CKD development; additionally a bilateral association exists because in patients with pre-existing CKD exposure to AKI can accelerate a decline in GFR and progression to ESRD.
5. Biological gradient. A dose response association has been reported both in Chawla's 2011 study and in Cocas metanalysis, where evidence of increasing effect size with rising severity of AKI was seen in 6 studies[163,16].
6. Plausability and coherence. An association between AKI and CKD is biologically plausible as described by the model of maladaptive repair detailed in the thesis´ introduction. Pathophysiologically the association is coherent.
7. Analogy. In the case of AKI, damage to the kidney undoubtedly occurs and the process of repair or lack thereof may be an analogous mechanism to that of renal function decline in the ageing kidney.

Arguments against this causal model include the assertion that residual confounding may exist, one or several causes may be common to AKI and CKD but remain unrecognised. A further argument is that a link may be due to detection bias, as AKI patients are more likely to be followed-up and renal dysfunction is therefore diagnosed more frequently in this group. On the other hand, follow-up of AKI survivors is notoriously poor being between 8.5-17% in reported studies and CKD diagnoses in register studies are most likely under-reported[130,157,158].

In study II patients with acute on chronic disease on ICU were 259 and CKD patients 96 times more likely to develop ESRD than the group without renal disease. This risk of progression of CKD to ESRD for ICU patients is about 10 times higher than that reported for the natural progression of disease in CKD patients in the community in two studies (detailed in the introduction)[115,116]. Acceleration of CKD and ESRD development has previously been reported[87,97]. Follow-up of CKD and AoC patients after ICU may be particularly beneficial in delaying or hindering disease progression. In the Stockholm region, renal surveillance of CKD patients with GFR 30-60ml/min/1.73m^2 is often performed in primary care and specialist nephrological consultation should not be assumed to occur automatically after ICU.

Incidence of CKD three-months post ICU discharge.

Study III established that renal dysfunction was common three months after AKI on ICU. *If we solely address incidence according to creatinine, CKD is far more common than recorded in our own national registers (studies I and II) where it is under-detected*[159]. It may also be under-diagnosed in the non-AKI population and a control group would have helped quantify this risk and address the assertion that detection bias explains the association between AKI and CKD. CKD incidence may be even higher if the composite equation (with the additional contribution of cystatin C) more correctly estimates GFR, as the results of study IV suggest. Creatinine alone may overestimate mGFR in AKI survivors. Thus, routine use of creatinine alone may result in at risk patients being missed. This study did not demonstrate an increase in two-year mortality associated with having a CKD diagnosis (according to either biomarker) and no difference depending on which biomarker detected CKD. This is likely to be due to lack of power for this analysis, as few deaths occurred in the three-month survivor group and study size was small. A larger, prolonged study is necessary to elucidate whether mortality and morbidity risk differences exist between patients diagnosed with CKD according to each of the endogenous biomarkers.

We applied the newly proposed AKD criteria to the cohort at three months. Incidence was 18.9%. When creatinine CKD criteria (Lund-Malmö equation) were used *at the same time point*, 11.9% more patients were identified as having renal dysfunction. The AKD criteria are modified from those used in RIFLE/KDIGO, which were developed for *diagnosing* AKI, and these may not perform as well in detecting survivors at risk of later renal dysfunction. By applying only, the AKD criteria post AKI, we may risks missing more patients vulnerable to long term renal dysfunction than when using the CKD criteria. We would advocate validation and possible modification of the AKD definition.

Aetiology and outcome

We found that septic AKI patients were sicker (demonstrated by higher severity scores and increased rates of mechanical ventilation) than the cohort with other AKI aetiologies. The septic AKI group has a longer length of ICU stay and higher mortality with a relative risk ration of 1.23. This is in agreement with other studies of aetiology[27,129]. Septic AKI survivors were shown to have a lower risk of developing ESRD than the non-specific AKI group. This is the first long term follow-up of this subset, but is consistent with early trends in renal recovery seen in studies by Bagshaw, Cruz and Ng [27,125,129,160]. This apparent superior renal recovery in septic AKI, may be due to a competing risk phenomenon or may be a consequence of pathophysiological differences in renal injury mechanisms between aetiologies.

Predictive modelling

In this thesis, predictive models were constructed to identify ICU patients at risk of later renal dysfunction. Generally, modelling may be practically useful in identifying groups of patients at increased risk of a particular outcome and implementing prophylactic measures to prevent the outcome in question or to detect disease at an early stage and thereby institute ameliorating treatment. Predictive models cannot determine whether an individual patient will develop a particular disease but rather identify a person or group as bearing particular risk levels corresponding to particular covariate patterns. In this thesis, the models proposed in **studies II and III** may guide physicians when screening patients at ICU discharge. Our models identified those at increased risk of subsequent renal dysfunction, in whom renal follow-up might be most beneficial. We chose predictors in our models based on prior knowledge of AKI and CKD and current knowledge of pathophysiology of maladaptive repair. Variables were entered in to the models if they were potentially causative (i.e. it was biological plausible that they could cause CKD), other predictors were non-causative such as age and gender but are known to be both associated with CKD risk or with other causative factors.

In study II, we propose a competing model for predicting the development of ESRD among one year survivors of AKI. Covariates predictive of ESRD progression were in order of strength of association: acute on chronic renal disease, CKD AKI, elevated serum potassium and congestive heart failure This predictive model demonstrated an area under the curve of 94% and has a level of discrimination not seen as yet with any single biomarker or combination of biomarkers. Validation of this model in other datasets and populations is necessary. This particular model is complex because determination of ESRD is not a biological “diagnosis”, rather it reflects severe renal impairment combined with acceptance to a chronic dialysis program. This means that advanced age and presence of some comorbidities render patients ineligible for ESRD treatment.

We identified risk factors associated with the diagnosis of CKD at 3 months, according to both creatinine and cystatin C in **study III**. The finding that discharge cystatin C was able to predict three-month creatinine at least as well as discharge creatinine is notable. It could

be explained by the confounding effect of muscle mass loss on ICU creatinine levels. Three-month creatinine concentrations may be less likely to be influenced by catabolism and sarcopenia and better reflect renal function than discharge serum concentrations. This assertion is supported by the finding in Paper IV that concordance improved between the two biomarker GFR estimates as time after ICU discharge increased. Conversely, creatinine/cystatin C ratio decreased between discharge and follow-up. This ratio has several applications but in particular has been proposed as a proxy for sarcopenia, for example, it has been demonstrated to distinguish muscle mass loss and disease progression from renal dysfunction in patients with Amyotrophic Lateral Sclerosis [161]. We might have expected the creatinine/cystatin C ratio to rise after discharge had muscle mass loss had a profound effect on ICU creatinine levels for this group. Additionally, length of stay, was not independently associated with follow-up creatinine or cystatin C suggesting that if catabolism affected creatinine concentrations during admission, this confounding effect on its performance as a renal function marker had diminished by three months.

Performance of GFR estimators in AKI survivors, study IV

Endogenous GFR estimators may not perform as well in survivors of AKI with persistent renal dysfunction as in patients at steady state. The performance of the combined equation connotes this formula to be the most useful in AKI survivors. The inaccurate performance of cystatin C was surprising, small study size means this finding could be due to random error but further investigation is warranted. What could cystatin C be signalling? Cystatin C was shown in a community based study in the elderly to predict CKD diagnosis four years later, it was also associated with long term mortality and cardiovascular disease[162]. The association between cystatin C and mortality was demonstrated in Bell´s ICU study in patients with and without renal failure[163]. Cystatin C may be conveying a non-renal signal.

It may be meaningful to comment on **nine-month creatinine levels.** Clearly selection (detection) bias exists for values obtained outside the study in patients without three-month renal dysfunction. Tests were likely to have been conducted in response to clinical problems, therefore these patients may be unhealthier than other survivors in this group. Notwithstanding this, only 5.7% of these patients had a creatinine eGFR under 60 and median creatinine for the entire groups was not significantly changed between three and nine months. This data should be treated with caution but may hint that three-month follow-up is a suitable time to assess renal function and that little deterioration on a group level may be expected up to nine-months.

IMPLEMENTATION AND FUTURE PERSPECTIVES

AKI has been demonstrated to be strongly associated with subsequent development of CKD and death and admission to ICU has been shown to increase/accelerate renal function deterioration in CKD patients. Nephrological surveillance as discussed in the introduction may ameliorate renal function decline and may improve outcome.

The global prevalence of CKD is increasing, and at present is around 13.4%. The costs of ESRD are prohibitive both economically and in terms of quality of life and morbidity for the patients[164-166]. The number of patients receiving dialysis has risen steadily since its introduction in the USA from 10,000 in 1972 to over 468,000 by 2013[167]. In Sweden 9693 patients were receiving care for ESRD in 2016 (this included around 5700 who had received renal transplants)[168]. The economic burden of ESRD is staggering, according to the United States Renal Data System, the annual cost of haemodialysis treatment per patient is around $84,550, the costs for peritoneal dialysis and renal transplant are somewhat lower ($69,910 and $29,920)[167]. The total annual expenditure on ESRD treatment in the United States amounts to 30.9 billion dollars[167]. These figures include the cost of an average 40 days in hospital per year, per patient[169].

ESRD incidence in Sweden is at present stable, but improved survival, means prevalence is increasing. Mortality has decreased from 30% in 1991 to 18.2% for patients with intermittent haemodialysis in 2016[168]. The contribution of AKI to this growing health care problem is important and reducing the incidence of CKD and slowing the rate of progression is paramount. Furthermore, a number of CKD treatments are under development. These include anti-fibrotic therapies, cell proliferation promoters and immune mediator modulators[170,171]. These novel interventions may also have benefice in modifying prognosis for AKI survivors. This thesis may thus serve to identify those patients these treatments may most help.

Interestingly, a recent study performed using data from the Swedish renal register, demonstrated that fast initial decline in eGFR in CKD patients was associated with a higher risk of dialysis initiation and with death[172]. Rate of GFR decline may also be useful to address in forthcoming studies assessing renal function post AKI. Future investigations might additionally aim to assess the ability of novel renal injury and recovery biomarkers to identify patients at risk of CKD. Injury indicators of interest include NGAL and cell cycle arrest markers, Tissue Inhibitor Metalloproteinase 2 (TIMP-2) and Insulin growth factor binding protein 7 (IGFBP7). Of the recovery biomarkers Osteopenia, transforming growth factor beta (TGFß1) and urinary hepatocyte growth factor (uHGF) are among the most propitious[34,173-176].

In conclusion, among the increasing number of ICU patients with AKI, renal follow-up ought to be implemented. Surveillance of all AKI patients is not economically or practically viable and therefore our predictive models are useful in identifying those patients most at risk of CKD and ESRD. We would recommend recalling AKI survivors: who have required RRT, had elevated baseline creatinine, discharge creatinine greater than 200umol/l or discharge cystatin C above 2mg/l, particular if patients are aged over 64, or have diabetes or cardiac failure. We advocate monitoring patients at three months using urinalysis and the compound creatinine and cystatin C formula to estimate GFR. Creatinine alone may underestimate and cystatin C overestimate GFR values.

CONCLUSIONS:

- AKI is associated with an increased short and long-term risk of death.
- AKI is independently associated with an increased risk of CKD and ESRD compared to an ICU control population.
- Pre-existing renal disease significantly increases the risk of death in ICU patients compared to those with normal pre-ICU renal function.
- Acute on Chronic Kidney disease on ICU is associated with an extremely elevated risk of progression to ESRD compared to all other groups.
- Renal dysfunction persisted in a significant number of AKI survivors followed at three months.
- CKD criteria are fulfilled in twice as many patients if three month GFR is estimated using cystatin C as compared to creatinine.
- AKD criteria were met in far few patients than CKD criteria at three months. AKD criteria may need amendment and validation.
- The combined creatinine and cystatin C formula demonstrated the best performance in estimating Iohexol measured GFR at nine months.
- Creatinine and cystatin C eGFR estimates have increasing concordance between discharge and nine month follow-up.

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"I am always ready to learn although I do not always like being taught". Winston Churchill