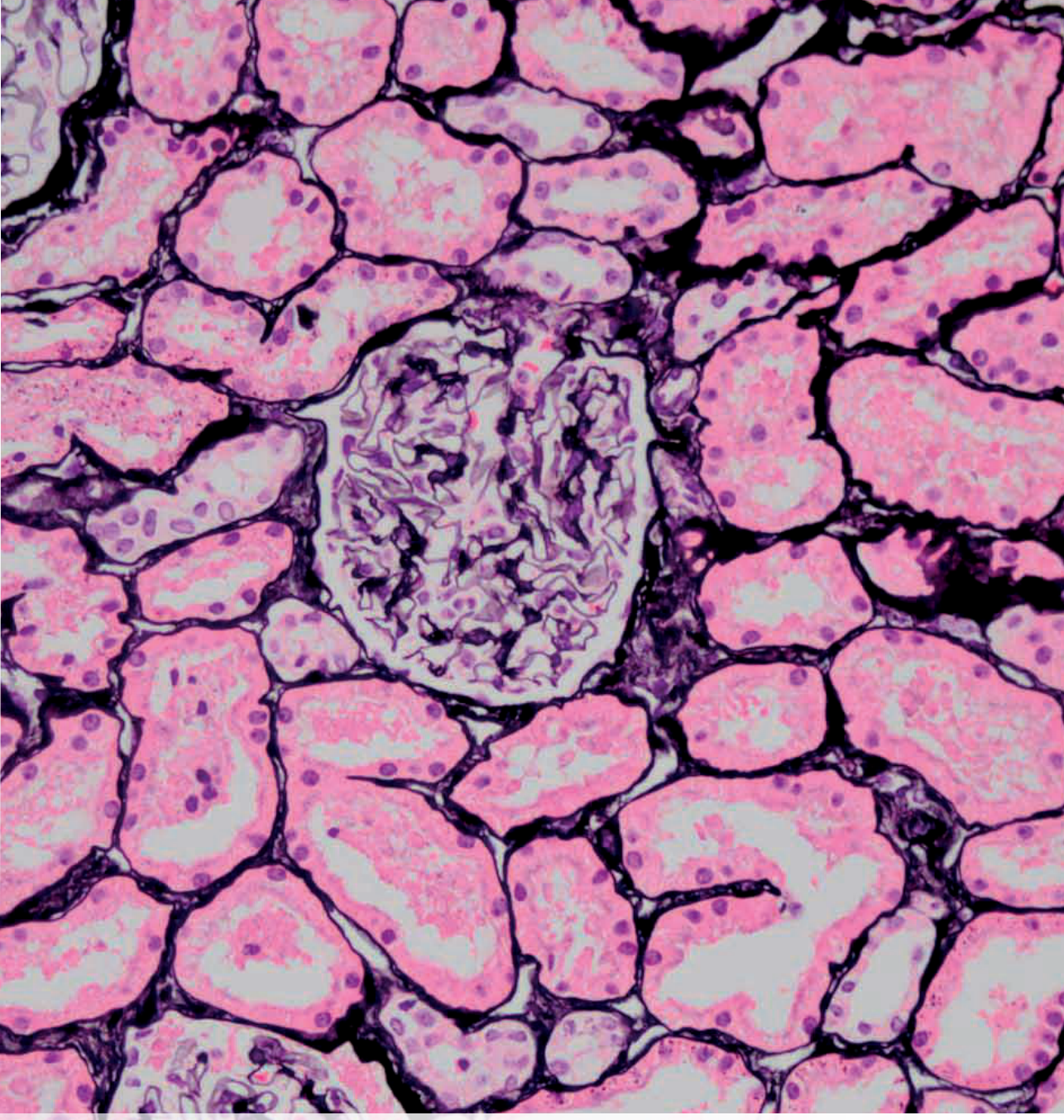

Biomarkers for AKI

Hilde R.H. De Geus

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Hilde RH de Geus

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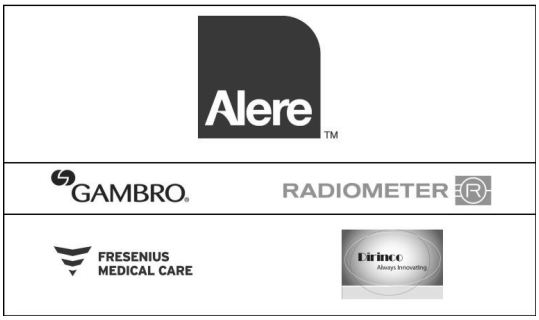

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Biomarkers for AKI

De rol van biomarkers in de predictie van acute nierschade

Proefschrift

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Bismillah, untuk Bapak saya…
(Voor mijn vader…)

Abbreviations

ADQI	- acute dialysis quality initiative
AIC	- akaike information criterion
AKI	- acute kidney injury
AKIN criteria	- modified RIFLE scoring system
ALI	- acute lung injury
AP	- alkaline phosphatase
APACHE	- acute physiology and chronic health evaluation score
AUC	- area under receiver operating characteristics curve
BMI	- body mass index
BUN	- blood urea nitrogen content
CI	- confidence interval
CKD	- chronic kidney disease
CPB	- cardio pulmonary bypass
CPR	- cardio pulmonary resuscitation
CRP	- c-reactive protein
(C) RRT	- (continuous) renal replacement therapy
CVC	- central venous catheter
CVVH	- continuous veno-veneus hemofiltration
CyC	- cystatin-C
EGFR	- estimated glomerular filtration rate
ELISA	- enzyme linked immuno sorbent assay
ESM	- electronic supplementary material
ESRD	- end stage renal disease
FB	- fluid balance
GGT	- gamma glutanyl transpeptidase
α-GST	- alpha glutathione-S-transferase
π-GST	- pi glutathione-S-transferase
HCO_3^-	- bicarbonate
ICU	- intensive care unit
IDI	- integrated reclassification improvement
IL-18	- interleukin 18
IQR	- inter quartile range
KIM-1	- kidney injury molecule 1
kDa	- kilo Dalton
L-FABP	- liver fatty acid binding protein
LTX	- liver transplantation
MDRD	- Modification of Diet in Renal Disease Study Equation

MODS	- multi organ dysfunction syndrome
MOF	- multi organ failure
N	- number
NAG	- n-acetyl-β-D-glucosaminidase
NGAL	- neutrophil gelatinase-associated Lipocalin
NPV	- negative predictive value
NRI	- net reclassification improvement
ODS	- online data supplement
OR	- odds ratio
PPV	- positive predictive value
RIFLE criteria	- risk-injury-failure scoring system
ROC	- receiver operating characteristic curve
SCCM	- society of critical care medicine
SCr	- serum creatinine
SD	- standard deviation
SE	- standard error
SEM	- standard error of the mean
SIRS	- systemic inflammatory response syndrome
SOFA	- sequential organ failure assessment score
SQ	- sieving coefficient
Temp	- temperature
TLR-2	- toll like receptor-2
UP	- urine production
WBC	- white blood cell count

General introduction

General introduction and outline of the thesis

General introduction

Acute Kidxney Injury

Acute kidney injury (AKI) represents an abrupt decrease in renal function that leads to accumulation of nitrogenous waste products such as blood urea nitrogen and creatinine. AKI refers to a complex disorder that comprises multiple causative factors (ischemic, nephrotoxic and septic components with overlapping pathofysiological mechanisms) and occurs in a variety of settings with numerous clinical manifestations that range from minimal elevation in serum creatinine (SCr) to anuric renal failure. In the critical care setting, AKI affects 5-25% of patients and accounts for an overall mortality rate of 50-80% [1]. Once established the treatment of AKI is largely supportive, unsatisfactory and associated with a poor prognosis [2]. Furthermore, AKI is independently associated with an increased risk of death and with a prolonged length of stay [3]. Even small changes in SCr can affect outcome in severely ill patients with multiple-organ dysfunction [4, 5]. Progressive insight in pathofysiological mechanisms of AKI has shown that tubule cell necrosis is rarely encountered in human acute renal failure, indeed the disparity between the severe impairment of renal function and the relatively subtle histological changes in AKI have been bothersome.

Definition of AKI

Over 35 definitions have been used to define AKI in the nephrology literature [6]. This is a result from the well recognized uncertain relationship between "the gold standard biomarkers" (serum creatinine and urine output) and the AKI disease process. Creatinine is the product of the breakdown of creatine to phosphocreatine in skeletal muscle and of the subsequent liver metabolism of creatine to form creatinine. It is released into plasma and filtered by the glomerulus. A small amount is also secreted into the urine through active transtubular transport. Creatinine is not reabsorbed in the tubules or metabolized by the kidney. If filtering of creatinine is deficient, blood levels rise with an inverse hyperbolic relationship with GFR (Figure 1). Thus, SCr is not an injury marker but rather a reflection of functional glomerular filtration. It is an unreliable indicator during acute changes in kidney function for several reasons. First, SCr levels can vary widely with age, gender, lean muscle mass, muscle metabolism, and hydration status. Second, SCr concentrations may not change until about 50% of kidney function has already been lost (Figure 1). Third, tubular excretion, especially at lower rates of glomerular filtration, results in an overestimation of renal function. Finally, during acute changes in glomerular filtration SCr does not accurately depict kidney function until steady state equilibrium has been reached which may require several days. Still, for lack of a better alternative, the Acute Kidney Injury Network (AKIN) introduced the term "AKI" based upon relative changes of SCr during their first consensus meeting in 2005.

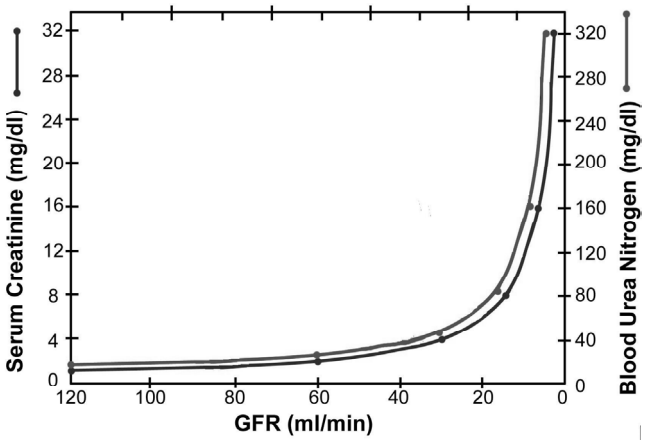

Figure 1: The physiological relationship between serum creatinine concentration and estimated glomerular filtration rate.

RIFLE and AKIN criteria

The acute Dialysis Quality Initiative (ADQI) formulated a consensus definition: the risk, injury and failure, loss, end-stage renal disease (RIFLE) classification for AKI [7]. It is based upon the relative changes in SCr compared to a steady state baseline value and changes in urine output (UP) over time corrected for ideal body weight (Table 1A). Later AKIN, an organisation dedicated to the improvement of outcomes for patients with AKI, proposed small modifications to the RIFLE system [8]. These modifications are based upon the particular inability of SCr to reliably define AKI in the milder forms of the disease. The primary change was to include a 0.3 mg/dl (= 27 µmol/l) rise in SCr even if it was less than 50% increase from baseline. And a 48 hour window was proposed in order for the first criterion to be achieved (staging according would then be based on the worst values during hospital stay) (Table 1B). Several limitations must be recognized using this consensus definition: First, the introduction of urine output criteria may add more confusion since oliguria can be masked by diuretics. Second, the conversion of a continuous variable to a dichotomous outcome is a problem: AKI or no-AKI, a cut-off is arbitrarily applied. Third, an inherent limitation is the need for a steady state baseline SCr value which is often very difficult to establish in emergency medicine.

Biomarkers

The imperfections of SCr urged the need to discover novel injury markers. Therefore, the identification of AKI biomarkers has been designated as a top priority by the American Society of Nephrology. Functional genomics and DNA micro array-based technologies have provided expression profiles of thousands of upregulated genes in case of AKI. These studies have identified novel genes with altered expression and new signal transduction pathways. Their protein products appear in plasma and urine and have been proposed

Table 1: RIFLE (A) and AKIN (B) criteria, definitions for acute kidney injury

A		B		RIFLE and AKIN
RIFLE	Abrupt (1-7 days) and sustained (>24 hrs)	AKIN	Abrupt (48 hrs)	UP (ml/kg/h)
Risk	SCr > 50% rise for 6h	1	SCr > 50% rise or 0.3 mg/dl	< 0.5
Injury	SCr > 100% rise	2	SCr > 100% rise	< 0.5 for 12h
Failure	SCr > 200% rise or SCr > 4 mg/dl with rise of 0.5 mg/dl	3	SCr > 200% rise or SCr > 4 mg/dl with rise of 0.5 mg/dl or anuria for 12h or RRT	< 0.3 for 24 h
Loss	Complete loss of kidney function > 4 weeks with the need of RRT			
ESRD	Persistent need for RRT > 4 months			

UP: urine production; ml: millilitre; kg: kilogram; h: hour; SCr: serum creatinine; mg: milligram; dl: decilitre; RRT: renal replacement therapy and ESRD: end stage renal disease

as promising new tools in the detection of (subtle) cellular injury. In general and ideally, biomarkers should aid in early diagnosis and prediction but may serve several other purposes in AKI. Such as: discerning AKI subtypes (prerenal, intrinsic renal, post renal); identifying AKI etiologies (ischemia, toxins, sepsis or a combination); differentiating AKI from other forms of kidney disease; predicting AKI severity; monitoring the course of AKI; and monitoring the response to AKI interventions. The biomarkers described in this thesis can be divided in three major categories: functional markers, upregulated low molecular weight proteins and constitutive cytoplasmatic enzymes.

Functional biomarkers

Plasma Cystatin-C

Cystatin-C (CyC) is a 13 kDa cysteine protease inhibitor that is synthesized and released into the blood at a relatively constant rate by all nucleated human cells. It is freely filtered by the glomerulus and under normal conditions completely reabsorbed in the proximal tubule. Furthermore there is no evident transtubular secretion. Although it is generally considered less subject to the non-renal variables that impact creatinine, some studies suggest that levels may be affected by various measures, as well as inflammatory processes, use of corticosteroids and changes in thyroid function [9-11]. With a half-life of 2 hrs, plasma CyC reflects glomerular filtration better than SCr in patients with chronic kidney disease [12].

Upregulated low molecular weight proteins

Neutrophil Gelatinase-associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a small (35 kDa) iron trafficking protein that is produced by epithelial cells throughout the human body (kidney, lungs, stomach, and colon) and is furthermore present in specific granulae of human neutrophils [13]. In healthy nephrons it can be detected in the distal tubular epithelial cells and the collecting ducts in small amounts, where it is believed to play a role in bacterial defense mechanisms. In case of AKI, NGAL mitigates iron-mediated toxicity by providing a reservoir for excess iron and may provide a regulated source of intracellular iron to promote regeneration and repair. Besides the transportation of iron, NGAL plays a critical role in kidney development during conversion of kidney progenitors into epithelia and tubules. Administration of NGAL in experimental models before, during or shortly after ischemic injury provides protection at the functional and structural levels with an induction of proliferation and inhibition of apoptosis of tubule epithelial cells [14-17]. In

AKI, plasma NGAL is easily filtered and undergoes a megalin-cubulin mediated re-uptake process in the proximal tubular cells where NGAL is degraded in specific lysosomes [18]. In distal tubular injury NGAL is generated in large amounts which can be detected in the urine [19]. However, urinary NGAL excretion is proportional to albumin excretion in mouse models of diabetic nephropathy and is thus augmented when the proximal transport maximum is exceeded [15, 19, 20]. NGAL measurements may be influenced by a number of coexisting variables such as preexisting renal disease and systemic or urinary tract infections [21].

Kidney injury molecule -1

Kidney injury molecule-1 (KIM-1) is a type-1 transmembrane glycoprotein localized to the apical membrane of exclusively surviving proximal tubular epithelial cells after injury. In normal renal tissue and normal urine it is not detectable (<0.1 ng/ml)[22]. KIM-1 expressing epithelial cells phagocytose intra luminal apoptotic and necrotic cell debris and the KIM-1 ectodomain is shed into the urine [23, 24]. Therefore it has been proposed as a site specific marker in AKI.

Constitutive cytoplasmatic enzymes

Glutathione-S-transferase

Alpha-glutathione-S-transferase (α-GST) and pi-GST (π-GST) are constitutive cytoplasmatic enzymes belonging to a large family of molecules participating in the defense against oxidative stress. The GST enzymes are present in many tissues in the human body and are involved in detoxification of foreign compounds by the addition of glutathione to a wide variety of xenobiotics. In the kidney, α-GST is localized exclusively in the proximal tubular cells, whereas π-GST is detectable in distal tubular cells and glomerular podocytes in Bowman's capsule. Although they are also present in human plasma, glomerular disorders do not result in an increase in urinary concentrations [29-31]. Therefore it is proposed that urinary excretion of GST might be a reflection of the site of tubular injury when the tubular cell wall integrity is damaged.

Renal replacement therapy

Approximately 5% of general ICU patients are treated with renal replacement therapy (RRT) which represents a substantial escalation in the complexity and cost of care for critically ill patients with AKI. Despite the extensive use in critical care practice there is uncertainty about the optimal time and indications for initiation of RRT in the ICU. Studies have shown marked variation of practice between clinicians and across institutions and countries mainly driven by logistic reasons [34, 35]. Currently there exists no broad

consensus to guide clinicians on this issue. With the emerging biomarkers and their predictive properties for AKI and its severity, they might be able to provide some guidance in determining more specific timing.

Aim and outline of this thesis

The aim of this thesis was to asses the predictive value of several biomarkers of different origin for acute kidney injury in adult critically ill patients and to study their biological behaviour. Furthermore, we tried to answer why such experimentally proven highly sensitive markers have less predictive power in general critically ill patients. The basis of this thesis was founded by a prospective cohort study performed at the ICU of the Erasmus University medical center including 700 consecutively admitted adult critically ill patients, recruited from September 2007 till April 2008 for plasma and urine measurements. A sample and databank were created and several research questions were intended to be answered by the information generated form this dataset. In Part A we discern a describing part of the biological and pathofysiological background of biomarkers for AKI and an observational part where we describe the predictive ability of plasma and urine NGAL, plasma and urine Cystatin-C, urine KIM-1, urine Pi- and Alpha GST. Furthermore, we studied several confounding factors that affect the interpretation of biomarker values such as a systemic inflammatory response syndrome in severe sepsis and septic shock, the role of sampling time relative to the injurious renal hit and the site of renal injury. Part B describes a possible role of plasma NGAL in the decision to initiate renal replacement therapy and a clinical nomogram is proposed as a tool for clinicians to aid their decision to start early on (right after ICU admission) with renal replacement therapy.

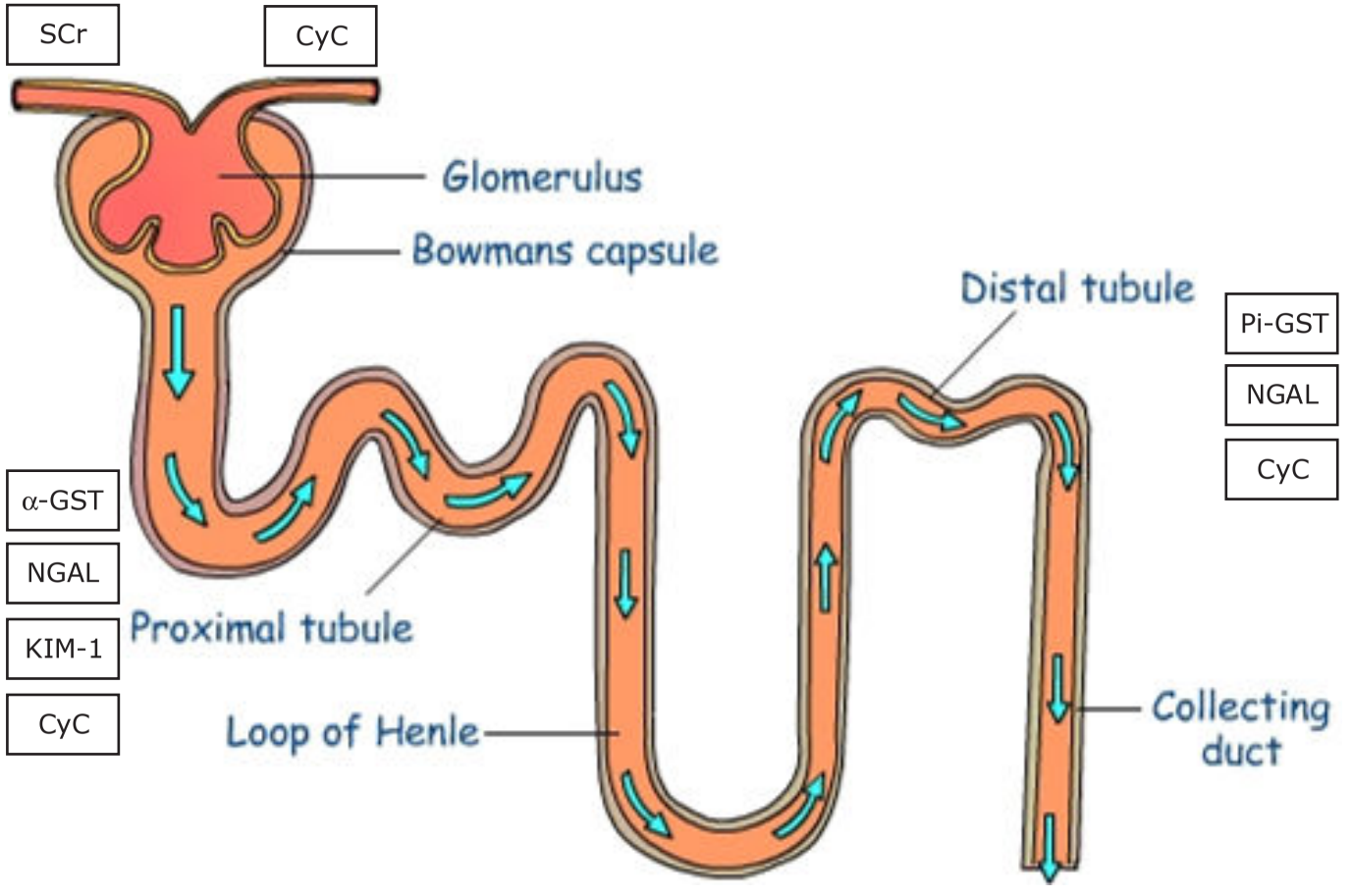

Figure 2: Nephron with expression sites of the biomarkers described in this thesis.

Research questions

1. What is the available evidence on different biomarkers predicting and diagnosing AKI in adult critically ill patients?
2. What is the predictive value of plasma and urine NGAL measured at ICU admission for AKI in general adult critically ill patients? Is there any additional value of both markers above serum creatinine and other clinical parameters? Is there additional value of serial measurements in this prediction?
3. Are plasma NGAL, urine NGAL, plasma CyC and urine CyC capable of differentiating between developing sustained AKI, developing transient AKI or no-AKI in adult patients at ICU entry? Does the prediction for sustained AKI improve when urine NGAL is combined with the other markers?
4. What is the effect of severe sepsis and septic shock on plasma NGAL's diagnostic ability for AKI?
5. How does sampling time relative to the time of the injurious event affect the predictive ability of the urinary biomarkers NGAL, KIM-1, Pi- and Alpha GST?
6. What is the clinical evidence for biomarkers aiding the prediction of need for renal replacement therapy and can we propose an algorithm in which biomarkers may aid in the timing of initiation of RRT?
7. Can we create a clinical scoring system for risk assessment of initiation of RRT that can aid a clinician in his decision to start RRT?
8. What is the effect on plasma NGAL clearance during RRT applying a high cut-off hemofilter during continuous veno-venous hemofiltration?

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Part A

Biomarkers and AKI

Chapter 1

Biomarkers for the prediction of acute kidney injury: A narrative review on current status and future challenges

Hilde RH de Geus, Michiel G Betjes, Jan Bakker

Clin Kidney J. 2012 Apr;5(2):102-108

Abstract

Acute kidney injury (AKI) is strongly associated with increased morbidity and mortality in critically ill patients. Efforts to change its clinical course have failed because clinically available therapeutic measures are currently lacking, and early detection is impossible with serum creatinine (SCr). The demand for earlier markers has prompted the discovery of several candidates to serve this purpose. In this paper we review available biomarker studies on the early predictive performance in developing AKI in adult critically ill patients. We make an effort to present the results from the perspective of possible clinical utility.

Introduction

Acute kidney injury (AKI) represents an acute decline in renal function, which leads to structural changes. AKI is associated with increased mortality, length of hospital stay and costs [1]. This unfavorable outcome might be tied to the late detection of AKI when the elevation of serum creatinine (SCr) is used. Many genes are upregulated in the damaged kidney with the corresponding protein products appearing in plasma and urine. Some of these are candidate markers for more timely diagnosis of AKI. The purpose of this paper is to review the current state of epidemiological data concerning AKI, to evaluate available biomarkers for the prediction of AKI and to describe several potential therapeutic options.

Epidemiology of AKI in critically ill patients

The Beginning and Ending supportive therapy for the Kidney investigators study (BEST Kidney study) has provided recent global insight as to the prevalence of patients with AKI. The reported mortality rate is 60.3%, with sepsis and premorbid renal dysfunction being dominant causes. In this observation, 13.8% of the patients with ARF surviving until hospital discharge required chronic renal replacement therapy (RRT) [2]. AKI and AKI requiring RRT display increasing incidence due to the rising degree of co-morbid conditions, increasing age and severity of illness in critically ill patients [3]. However, there seems to be a steady-state decline in annual in-hospital mortality (from 41.3% in 1988 to 28.1% in 2002). Despite the observed reduction in mortality rates, the rising incidence of AKI comes at a price. Patients tend to survive the ICU but will be discharged with various degrees of chronic kidney disease (CKD), which will increasingly strain the health care system [4]. These data are supported by observations from Australia, where the 10-year trend in the incidence of AKI and the crude hospital mortality rates adjusted for illness severity were likewise investigated. In this study 5.2% of the patients have AKI with an increased incidence over the past decade; however the multivariate adjusted odds of death associated with AKI shows a declining trend. The increased risk of death associated with AKI persisted with the adjustment for several relevant covariates. ARF exerts an independent, profound and specific effect on morbidity and mortality in critically ill patients [5]. Furthermore, outcomes are directly related to the severity of AKI: even small changes in SCr have a detrimental impact on patient long-term survival [1, 6].

Biomarkers for the prediction of AKI

The ability of biomarkers to predict AKI has been studied intensely in several different clinical settings. For a sound interpretation of the reported results, it is important to realize that the studies present a mixture of "AKI diagnosis confirmation" in patients with established AKI and "AKI early prediction" in patients with developing AKI. Obviously, these are two different entities with different clinical impacts. For the clinical application of a new biomarker it should prove to be more accurate with earlier detectability than the current gold standard SCr, which implies "early prediction" only. Therefore, this review focuses on the prediction of developing AKI in adult critically ill patients. There are four major categories of biomarkers (Table 1).

Functional markers

Serum creatinine (SCr)

Serum creatinine (SCr) is a degradation product of muscle cells and represents a surrogate for the efficiency of glomerular filtration. It has poor predictive accuracy for renal injury ,particularly, in the early stages of AKI [7]. In the case of critical illness, SCr concentrations are subject to large fluctuations due to a patient's induced dilutional volume status, the catabolic effects of critical illness, the likelihood of concentration decreases in septic conditions and the increased tubular excretion with diminishing renal function. Furthermore, after an injurious event, the rise in SCr is slow. Therefore, detection of the earliest evidence of AKI necessitates the use of other plasma or urinary biomarkers.

Plasma/serum Cystatin-C (CyC)

Cystatin C (CyC) is a 13-kDa nonglycosylated cysteine protease inhibitor produced by all nucleated cells at a constant rate. In healthy subjects, plasma CyC (pCyC) is excreted through glomerular filtration and metabolized completely by the proximal tubules. Furthermore, there is no evident tubular secretion. Several studies claim the superiority of pCyC against SCr to detect minor reductions in GFR [8]. However, the interpretation of pCyC levels is biased by older age, gender, weight, height, cigarette smoking and high levels of CRP [9, 10]. In addition, CyC levels are supposedly influenced by abnormal thyroid function [11, 12] the use of immunosuppressive therapy [13] and malignancies [14, 15]. In 318 patients included at ICU admission, pCyC predicted developing sustained AKI (n=19) very modestly (AUC= 0.65 [CI 0.58-0.71] in univariate analysis [16]. Herget-Rosenthal described a cohort in whom sCyC was measured at admission in 85 patients with normal GFR. The reported AUC was 0.82 [CI

Table 1: Biomarkers for AKI

BIOMARKER TYPES	BIOMARKERS
Functional markers	SCr, plasma/serum CyC
Upregulated proteins	NGAL, KIM-1, L-FABP, IL-18
Low molecular weight proteins	urine CyC
Enzymes	NAG, α-GST, π-GST, GGT, AP

AKI: acute kidney injury; SCr: serum creatinine; CyC: cystatin C; NGAL: neutrophil gelatinase associated lipocalin; KIM-1: kidney injury molecule 1; L-FABP: liver fatty acid binding protein; IL-18: interleukin 18; NAG: N-acetyl-β-D-glucosaminidase; α-GST: alpha-Glutathione s-transferase; π-GST: pi-Glutathione s-transferase; GGT: Gammaglutanyl transpeptidase and AP: alkaline phosphatase.

0.71-0.92] for acute renal failure two days prior to the event [17]. A recent multicenter study in 151 subjects in a comparative setting found a poorer performance (AUC= 0.72 no CI provided) [18]. Metzger et al. compared the classification performance of a set of urinary proteome analyses with sCyC in 20 general ICU patients, retrospectively, and found low classification accuracy (AUC = 0.67 CI not provided) [19].
In cardio pulmonary bypass (CPB) cohorts, several studies explored the use of CyC for AKI prediction. Haase-Fielitz et al. described 100 cardiac surgical patients among whom 23 subjects were classified as patients without preoperative renal impairment. Their samples were measured at ICU arrival, and the reported AUC= 0.78 [CI 0.58-0.99] did not improve after 24 hours [20]. Koyner et al. reported on 72 patients who were admitted following CPB with 34 subjects developing AKI, which was defined as a 25% increase in pCr or the need for RRT (n=7) within 3 days after surgery. PCyC measured at time of ICU arrival was not a useful early predictor for the composite outcome AUC= 0.62 [0.49-0.75] [21]. A likely explanation is the applied unusual definition of AKI, which indicates less severe grades of AKI among the event group.

Upregulated proteins

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL is a small protein linked to neutrophil gelatinase in specific leukocyte granules [22]. It is also expressed in a variety of epithelial tissues associated with antimicrobial defense [23-26]. In the normal kidney, only the distal tubules and collecting ducts stain for NGAL expression. NGAL's composite molecule binds ferric siderophores, and furthermore, it is a potent epithelial growth inducer, has protective effects in ischemia [27] [28] and is upregulated by systemic bacterial infections [24, 29-32]. In case of AKI, proximal tubule cells also stain for NGAL proteins, which is explained by megalin-cubulin mediated re-uptake of NGAL present in the glomerular filtrate [33] [34]. Urinary NGAL originates from local production in the distal tubules and collecting ducts. However, uNGAL excretion is proportional to albumin excretion in mouse models of diabetic nephropathy and is thus augmented when the proximal transport maximum is exceeded [33, 35, 36]. Siew et al. enrolled their patients within 24 hours after admission and reported a ROC AUC=0.77 [CI 0.64-0.90] for developing AKI in a subgroup of patients with eGFR at admission > 75 ml/min/1.73m^2 for urine NGAL (n=18 vs. 257)[37]. Cruz et al. reported on the development of AKI within 48 hours after first sampling an AUC=0.78 [CI 0.65-0.90]. However, the reported PPV was low (24%), and within 5 days, the AUC was reduced to 0.67 [CI 0.55-0.79] [38]. The first sampling was performed within 24 hours after ICU admission. De Geus et al. came to roughly similar reports with samples at ICU admission in patients with eGFR > 60ml/min/1.73m^2 for both plasma

and uNGAL (AUC=0.75±(SE) 0.103) AUC NGAL=0.79±(SE) 0.085 [39]. It is debatable whether the exclusion of patients with eGFR's below 75 or 60 ml/min/1.73m^2 applied by Siew and de Geus et al. is useful in clinical practice, because a biomarker should also be effective in patients with CKD. In patients with sepsis, the predictive performance for AKI seemed not to be affected, as reported by Martensson for both plasma and urine NGAL (respectively, AUC's= 0.85 [CI 0.67-1.0] and 0.86 [CI 0.68-1.0]) [40]. However, Bagshaw et al report a distinct influence on test characteristics in patients with sepsis [41]. Several studies report results in CPB cohorts: Koyner et al. measured both pNGAL AUC 0.526 [0.388-0.664] and uNGAL AUC= 0.705 [CI 0.581-0.829] [21] at ICU admission. An additional analysis by the same authors stratified their patients according to attained RIFLE stage and reported increased performances when using the harder endpoint of Failure AUC=0.69 [0.57-0.80] and AKIN stage 3 AUC= 0.79 [0.65-0.94] [42]. A large study (n= 426) in CPB patients demonstrated test performance association with the pre-surgery baseline eGFR. Interestingly, only in patients with an eGFR above 60 ml/min was NGAL predictive: AUC=0.68 [CI 0.54-0.81] [43]. A much smaller study (n=9 events) reported values for both pNGAL and uNGAL, corrected for urinary creatinine: AUC= 0.85[CI 0.73-0.97] and AUC= 0.96 [CI .90-1.0], respectively [44]. Haase-Fielitz compared the performance of conventional and novel markers for pNGAL in adult CPB patients, excluding patients with preoperative renal impairment NGAL: the results yielded AUC=0.80 [CI 0.58-0.99] [20]. In another large study (n=8790) for pNGAL measured immediately after CPB with 75 events, the AUC reported was 0.641 [0.58-0.71] [45]. Wagener et al. performed a study in adult CPB patients: for urine NGAL, the predictive performance was AUC=0.573 [CI 0.506-0.640] directly after the operation: the performance increased until 18 hours after ICU admission to a maximum of 0.611 [46]. In a study performed by Liangos et al. these results were similar in 103 CPB patients 2 hours after surgery: AUC= 0.50[CI 0.33-0.68] [47]. Among general adult ICU patients, 82 subjects developed AKI within 48 hours of admission, and the predictive performance for NGAL corrected for urinary creatinine concentration yielded AUC=0.55 [CI 0.48-0.63] [48]. Metzger et al. compared the classification performance of urinary proteome analysis with classical markers. For urine NGAL, the ROC analysis revealed low classification accuracy: AUC = 0.54 CI (not provided) [19]. The only meta-analysis published to date assessed pNGAL's ability to predict across different settings; when weighted for study sample size, this value yielded an overall AUC of 0.782 [CI 0.689-0.872]. [49].

Kidney Injury Molecule-1 (KIM-1)

Kidney Injury Molecule-1 (KIM-1) is a type I transmembrane glycoprotein with a cleavable ectodomain (90 kDa) which is localized in the apical membrane of dilated tubules in acute and chronic injury [50, 51]. Kim-1 is believed to play a role in regeneration processes

after epithelial injury and in the removal of dead cells in the tubular lumen through phagocytosis [50, 52]. A reduction in proteinuria with RAAS blockade is accompanied by a reduction in urinary KIM-1 excretion [53, 54]. Among general adult ICU patients, 82 subjects developed AKI within 48 hours of admission, and the predictive performance for KIM-1 corrected for urinary creatinine concentration yielded AUC=0.55 [CI 0.47-0.62] in the study of Endre et al [48]. Metzger et al. compared the classification performance of urinary proteome analysis with classical markers. For urine KIM-1, the ROC analysis revealed low classification accuracy (AUC = 0.71 CI, not provided) [19]. Several studies report its diagnostic properties in adult CPB patients [42, 47, 55-57] . Liang et al. reported an AUC for progressive AKI of 0.69 [CI 0.61-0.78] after 6 hours of inclusion. Notably, adding KIM-1 to IL-18 (AUC for IL-18 for progressive AKI 6 hrs after inclusion was 0.87 [CI 0.80-0.93]) in a predictive model improved the model's accuracy only minimally (AUC 0.88 [CI 0.82-0.93]). Liangos et al. reported an AUC 2 hours post-CPB surgery of 0.78 [CI 0.64-0.91]: however, in multivariate regression analysis, the association of KIM-1 was attenuated after adjustment. Koyner et al. found an AUC 0.56 [CI 0.45-0.67] as admission value for the entire cohort with an improvement when predicting AKIN stage 3 only (AUC=0.69 [CI 0.44-0.93]) [42].

Liver Fatty Acid Binding Protein (L-FABP)

Fatty Acid Binding Proteins are small (15 kDa) cytoplasmatic proteins abundantly expressed in tissues with active fatty acid metabolism. Their primary function is the facilitation of long-chain fatty acid transport, the regulation of gene expression and the reduction of oxidative stress. Urinary L-FABP is undetectable in healthy control urine, which is explained by efficient proximal tubular internalization via megalin-mediated endocytosis [58] [59]. Under ischemic conditions, tubular L-FABP gene expression is induced; in renal disease, the proximal tubular re-absorption of L-FABP is reduced [59, 60]. To date, there is one small study reporting on the early diagnostic performance of L-FABP in adult ICU patients. The reported ROC AUC value was 0.95, no CI provided. However, several uncertainties remain after disclosure of the study's methodology. First, patient selection (n=25 with 14 AKI and 11 non-AKI) seems to have been a result of convenient sampling. Second, the "true early diagnosis" remains very doubtful as peak SCr and L-FABP values are reported as having the same median value; no further clear information concerning timing is provided [61].

Interleukin -18

In animal models, Interleukin-18 (IL-18) has proven to be an important mediator in the process of AKI. Therefore, its urinary release has been anticipated as a possible early marker: several studies have explored the clinical application of this hypothesis.

Among general adult ICU patients, 82 subjects developed AKI within 48 hours of admission, and the predictive performance for IL-18 corrected for urinary creatinine concentration was AUC=0.55 [CI 0.47-0.62] [48]. Metzger et al. compared the classification performance of urinary proteome analysis with classical markers. For urine Il-18, the ROC analysis revealed low classification accuracy (AUC = 0.57 CI not provided) [19]. Nevertheless, in a large cohort of mixed patients (n=451) Siew et al. enrolled patients within 24 hours after ICU admission: 86 developed AKI. The overall predictive performance reported was AUC=0.62 [CI 0.54-0.69]; this value increased slightly in patients with an eGFR above 75 ml/min/1.73m^2 (AUC= 0.67 [CI 0.53-0.81]). There seemed to be a strong association with sepsis [62]. In patients with acute lung injury (ALI), uIL-18 predicted progression to AKI within 24 hours with an accuracy of AUC=0.731 (CI not provided) with substantial overlap between cases and controls in urine concentrations [63]. In CPB patients, 2 hr after CPB time, the optimal performance was reported to yield an AUC= 0.66 [CI 0.49-0.83][47].

Low molecular weight proteins

Urine Cystatin C

The urinary excretion of CyC (uCyC) specifically reflects tubular damage because systemically produced Cystatin C is normally not found in urine [64]. However, recent insights show that urinary CyC excretion is augmented by albuminuria [65] In patients without AKI on ICU entry, uCyC was not predictive of AKI occurring within 48 hours with AUC= 0.54 [CI 0.46-0.62] [66]. Liangos et al. used uCyC for this prediction, which resulted in very moderate performances 2 hours post-CPB surgery with ROC AUC= 0.50 [CI 0.27-0.72] in a cohort of 103 patients with 13 events of AKI [47]. In a study in patients undergoing CPB, Koyner et al. demonstrated that uCyC measured at ICU admission reached a maximum performance with an AUC of 0.693 [CI 0.567-0.818]. [21][48] Among general adult ICU patients, 82 subjects developed AKI within 48 hours of admission and the predictive performance for urine CyC corrected for urinary creatinine concentration yielded AUC=0.55 [CI 0.48-0.63]. Another study performed by Koyner et al. demonstrated the predictive value of uCyC at ICU admission for any stage of AKI with AUC=0.72 [CI 0.61-0.83]. For the prediction of AKIN stage 3 vs. the rest of the cohort, the predictive performance increased to AUC=0.84 [CI 0.68-0.99] [42]. Royakkers et al. regarded uCyC as a predictor for AKI 2 days prior to the first day of AKI and found no diagnostic value (AUC=0.49 no CI provided) [18].

Tubular Enzymes

Alpha-Glutathione s-transferase (α-GST) and Pi-Glutathione s-transferase (π-GST)

Alpha-Glutathione s-transferase (α-GST) and Pi-Glutathione s-transferase (π-GST) are both members of a multigene family of detoxification enzymes present in many organs including the kidney. Distribution across the entire nephron of structurally and functionally distinct isoforms has been demonstrated. In urine, these enzymes are normally not present. After injury, α-GST is primarily detected in the proximal cells, whereas π-GST is observed in the distal parts [67]. Westhuyzen et al. studied the predictive performance of tubular enzymes and their combination in adult critically ill patients. Four patients developed AKI defined as a 50% SCr increase or more. At the time of ICU admission, α-GST and π-GST measured and indexed to urine creatinine provided AUC's of 0.893 [CI 0.688-0.975] and 0.929 [0.740-0.990] respectively. [68]. However, the patients with AKI seemed to have established AKI at study inclusion with a median creatinine clearance of 38.1 ml/min. Walshe et al. reported that in patients with developing AKI and sepsis admitted to the general ICU, both enzymes were bad predictors. They suggested that sepsis might be the confounder triggering the production of these enzymes [69]. Finally, a study by Koyner et al. in 123 adult CPB patients reported AUC=0.59 [CI 0.47-0.71] and 0.54 [0.42-0.66] for the prediction of AKI stage 1 for α-GST and π-GST measured at ICU unadjusted for urine creatinine arrival, respectively, with similar test performances when using the harder endpoint of AKIN stage 3 AUC= 0.58 [0.31-0.85] and AUC= 0.70 [0.50-0.90] [42] .

Gammaglutanyl transpeptidase (GGT) and Alkaline Phosphatase (AP)

Gammaglutanyl transpeptidase and alkaline phosphatase both are tubular brush border enzymes that are released into urine when there has been significant damage to the brush border membrane with loss of the microvillus structures. Few clinical studies are available, but Westhuyzen et al. report data on 4 cases with developing AKI respectively of AUC= 0.950 [CI 0.789-0.999] and AUC= 0.863 [CI 0.676-0.973] [68]. However, these results should be interpreted with caution, because the cases must be considered as established AKI at study inclusion according to their reported creatinine clearance. In general adult ICU patients, 82 subjects developed AKI within 48 hours of admission and the predictive performance for urine GGT and urine AP corrected for urinary creatinine concentration AUC=0.57 [CI 0.50-0.64] and AUC 0.56 [CI 0.49-0.63], respectively [48].

Table 2: Therapeutic agents for the treatment of AKI

CATEGORY	AGENTS
Anti-inflammatory agents	β1-integrin-antagonist, adenosine receptor antagonist, mesenchymal stem cells, C5a receptor antagonist, Il-10, Il-6 antagonist, statins, erythropoietin, α Melanocyte stimulating hormone, heme oxygenase-1 inducers (rapamycin), Activated protein C (STM), TLR blockers (Eritoran), sphingosine 2A agonist, fibrates, statins, PPAR-γ agonist, minocylcine, iNOS inhibitor, insulin, ethyl pyruvate, C5-antagonists, Alkaline phosphatase
Anti-apoptotic agents	NGAL, adenosine receptor antagonist, mesenchymal stem cells, erythropoietin, α Melanocyte stimulating hormone, caspase inhibitors, minocycline, guanosine, pifithrin-α, PARP inhibitor
Iron-scavengers	NGAL, Apotransferirin, deferoxamine
Reactive oxygen species scavengers Anti-oxidants	Edavarone, Stobadine, deferoxamine
Vasodilators	Endothelin receptor antagonist, CO-releasing compounds , Fenoldopam, ANP
Growth factors	Erytropoetin, Hepatocyte growth factor

Table 3: Summary of studies reporting predictive performance on biomarkers for developing AKI in adult critically ill patients

BIOMARKER	STUDY [REF]	AUC [95% CI]	ENDPOINT	PATIENT POPULATION
Plasma CyC	Nejat [16]	0.65 [0.58-0.71]	Sustained AKI	General ICU
	Herget-Rosenthal [17]	0.82 [0.71-0.92]	ARF	General ICU
	Haase-Fielitz [20]	0.75 [0.59-0.90]	AKI	CPB
	Koyner [21]	0.62 [0.49-0.75]	AKI*	CPB
	Metzger [19]	0.67 [-]	AKI	General ICU
	Haase-Fielitz [20]	0.78 [0.58-0.99]	AKI	CPB
	Royakkers [18]	0.62 [-]	AKI	General ICU
Plasma NGAL	Cruz [38]	0.78 [0.65-0.90]	AKI	General ICU
	De Geus [71]	0.75 [± SE 0.103]	RIFLE I and F	General ICU
	Martensson [40]	0.85 [0.67-1.0]	AKI	Septic general ICU
	Tuludhar [44]	0.85[0.73-0.97]	AKI	CPB
	Haase [49]	0.782 [0.689-0.872]	AKI	Meta-analysis CPB and general ICU
	Haase-Fielitz [20]	0.80 [0.58-0.99]	AKI	CPB
	Perry [45]	0.641 [0.58-0.71]	AKI	CPB
Urine NGAL	Siew [37]	0.77 [0.64-0.90]	AKI	General ICU
	De Geus [39]	0.79 [± SE 0.085]	RIFLE I and F	General ICU
	Endre [48]	0.55 [0.48-0.63]	AKI	General ICU
	Liangos [47]	0.50[CI 0.33-0.68]	AKI	CPB
	Koyner [21]	0.705 [0.581-0.829]	AKI	CPB
	Koyner [42]	0.69 [0.57-0.80]	AKI	CPB
	Koyner [42]	0.79 [0.65-0.94]	AKIN stage 3	CPB
	McIlroy [43]	0.68 [0.54-0.81]	AKI	CPB
	Metzger [19]	0.54 [-]	AKI	General ICU
	Martensson [40]	0.86 [0.68-1.0]	AKI	Septic general ICU

Table 3 (Continued)

BIOMARKER	STUDY [REF]	AUC [95% CI]	ENDPOINT	PATIENT POPULATION
	[44]	0.96 [0.90-1.0]	AKI	CPB
	Wagener [46]	0.573 0.506-0.640]	AKI	CPB
Urine KIM-1	Liang [57]	0.69 [0.61-0.78]	AKI	CPB
	Liangos [47]	0.78 [0.64-0.91]	AKI	CPB
	Koyner [42]	0.56 [0.45-0.67]	AKI	CPB
	Koyner [42]	0.69[0.44-0.93]	AKIN stage 3	CPB
	Endre [48]	0.55 [0.47-0.62]	AKI	General ICU
	Metzger [19]	0.71 [-]	AKI	General ICU
Urine L-FABP	Matsui [61]	0.95 [-]	AKI	General ICU
Urine CyC	Liangos [47]	0.50 [0.27-0.72]	AKI	CPB
	Koyner [42]	0. 72 [0.61-0.83]	AKI	CPB
	Koyner [42]	0. 84 [0.68-0.99]	AKIN stage 3	CPB
	Koyner [21]	0.693 [CI 0.567-0.818].	AKI*	CPB
	Nejat [66]	0.54 [CI 0.46-0.62]	AKI	General ICU
	Endre [48]	0.57 [0.50-0.64]	AKI	General ICU
	Endre [72]	0.57 [0.50-0.64]	AKI	General ICU
	Royakkers [18]	0.49 [-]	AKI	General ICU
Urine IL-18	Endre [48]	0.55 [0.47-0.62]	AKI	General ICU
	Liangos [47]	0.66 [0.49-0.83]	AKI	CPB
	Metzger [19]	0.57 [-]	AKI	General ICU
	Siew [62]	0.62 [CI 0.54-0.69]	AKI	General ICU
	Parikh [63]	0.731 [-]	AKI	ALI
Urine α-GST	Walshe [69]	-	AKI	General ICU with sepsis
	Koyner [42]	0.59 [0.47-0.71]	AKI	CPB

Table 3 (Continued)

BIOMARKER	STUDY [REF]	AUC [95% CI]	ENDPOINT	PATIENT POPULATION
	Koyner [42]	0.58 [0.31-0.85]	AKIN stage 3	CPB
	Westhuyzen [68]	0.893 [0.688-0.975]	AKI	General ICU
Urine π-GST	Koyner [42]	0.54 [0.42-0.66]	AKI	CPB
	Koyner [42]	0.70 [0.50-0.90]	AKIN stage 3	CPB
	Westhuyzen [68]	0.929 [0.740-0.990]	AKI	General ICU
Urine NAG	Liangos [47]	0.62 [0.41-0.83]	AKI	CPB
	Westhuyzen [68]	0.845 [0.639-0.955]	AKI	General ICU
Urine GGT	Westhuyzen [68]	0.95 [CI 0.789-0.999]	AKI	General ICU
	Endre [48]	0.57 [CI 0.50-0.64]	AKI	General ICU
Urine AP	Westhuyzen [68]	0.863 [0.676-0.973]	AKI	General ICU
	Endre [48]	0.56 [0.49-0.63]	AKI	General ICU

N-acetyl-β-D-glucosaminidase (NAG)

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme (> 130 kDa) that is localized in the renal tubules. Due to its large molecular weight, it precludes glomerular filtration, implying that urinary elevations have a tubular origin. Increased activity suggests injury to its cells but may also reflect increased lysosomal activity without cell disruption. NAG catalyzes the hydrolysis of terminal glucose residues in glycoproteins. Westhuyzen reported on the ability to predict developing AKI in 4 cases in general ICU patients with AUC=0.845 [CI 0.639-0.955]: however, these patients seem to have established AKI with reduced creatinine clearance at the time of study inclusion [68]. In adult CPB patients, 13 cases of developing AKI were reported: and the 2-hr postoperative prediction for NAG was very moderate: AUC=0.62 [CI 0.41-0.83] [47].

Treatment of AKI

The pathogenesis of AKI is very complex with multiple mechanisms underlying its course. Furthermore, critically ill patients do not generally die from AKI as such but more from the multiple organ dysfunction syndrome (MODS) associated with it. Given the multiple interactive pathways underlying AKI, it might be a mistake to concentrate therapeutic effects on one single part of the interrelated cascades. Therapies may need to target multiple sites in the pathophysiological pathways of AKI and MODS in order to be of any benefit for patients. Such combination therapies must involve agents with potential beneficial effects on vascular tone, tubular obstruction, and inflammation. Furthermore, it is unlikely that targeting events that occur late in AKI will be effective. Pharmacological therapy in the prevention and treatment of AKI has been largely unsuccessful despite proven benefits as seen in preclinical studies. A number of drugs and investigational compounds seem promising in preclinical studies. There are six major categories of treatment strategies: anti-inflammatory agents, anti-apoptotic agents, iron scavengers, anti-oxidants, vasodilators and growth factors (Table 2).

Conclusions

In the quest for earlier markers for the recognition of AKI several biomarkers have been investigated. The reported AUC's are disappointing ranging from 0.50-0.84, with one or two exceptions which can be explained by statistical or methodological differences in study design. The discriminatory function in heterogeneous populations is poor and influenced by pre-existing renal function and time of sample collection with respect to the renal insult [48]. Clinical appraisal of a patient and using standard parameters such

as SCr and diuresis remains the cornerstone for now [70]. Therefore it seems reasonable to perhaps shift our views and using biomarkers together with other parameters such as traditional clinical characteristics to optimize the accuracy of prediction of developing AKI might be an interesting option. Ultimately, the potential of new therapeutic agents can be tested and their use evaluated.

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Chapter 2

Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients

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At a glance commentary:

Acute kidney injury is an independent risk factor for ICU mortality. The current gold standard for detection of AKI, serum creatinine, is not a perfect biomarker. Measuring other biomarkers, such as NGAL, may have the potential to aid in improved outcomes in patients with AKI because of earlier and more accurate prediction.
This study shows that NGAL, an injury biomarker, can accurately predict the development of AKI in adult ICU patients, that combining NGAL with other clinical predictors improves this prediction, that serial NGAL measurements do not improve the prediction for severe AKI and that sepsis is a trigger for urine NGAL production.

Abstract

Rationale: Measured at ICU admission, the predictive value of neutrophil-gelatinase-associated-lipocalin (NGAL) for severe acute kidney injury (AKI) is unclear.
Objective: To assess the ability of plasma and urine NGAL to predict severe AKI in adult critically ill patients.
Design: Prospective-cohort-study.
Patients: 632 consecutive patients
Measurements: Samples were analysed by Triage® immunoassay for NGAL expression. The primary outcome measure was occurrence of AKI based on RIFLE classification (Risk-Injury-Failure) during the first week of ICU-stay.
Results: A total of 171 (27%) patients developed AKI. Of these 67, 48 and 56 were classified as RIFLE R, I and F respectively. Plasma and urine NGAL values at ICU admission were significantly related to AKI severity. The areas under the ROC curves for plasma and urine NGAL were for RIFLE R (0.77±0.05 and 0.80±0.04 respectively), RIFLE I (0.80±0.06 and 0.85±0.04 respectively) and RIFLE F (0.86±0.06 and 0.88±0.04 respectively) and comparable to those of admission estimated-glomerular-filtration-rate (eGFR) (0.84±0.04, 0.87±0.04 and 0.92±0.04 respectively). Plasma and urine NGAL significantly contributed to the accuracy of the "most efficient clinical model" with the best 4 variables including eGFR, improving the AUC's for RIFLE F prediction to 0.96±0.02 and 0.95±0.01. Serial NGAL measurements did not provide additional information for the prediction of RIFLE F.
Conclusion: NGAL measured at ICU admission predicts the development of severe AKI similarly to serum-creatinine-derived eGFR. However NGAL adds significant accuracy to this prediction in combination with eGFR alone or to other clinical parameters and has an interesting predictive value in patients with normal serum creatinine.

Introduction

In critically ill patients, acute kidney injury (AKI) is independently associated with increased costs of medical care, as well as increased risk of morbidity and mortality [1-4]. In addition, when AKI develops during hospital admission it results in accelerated progression towards end-stage renal disease (ESRD), especially in elderly patients [5]. Recent observational studies have shown a 14% incidence of dialysis dependency at the time of hospital discharge among survivors of critical illness [3]. Therefore, early recognition of renal injury is important and may help prevent further renal damage and functional impairment.

Recent experimental [6-8] and clinical [9-11] studies have identified biomarkers that may serve as early indicators of AKI. Of these, neutrophil gelatinase-associated lipocalin (NGAL) seems to be the most promising. NGAL is a 25-kDa protein that is covalently bound to gelatinase and is secreted from human neutrophils [12]. It is generally expressed at low concentrations in various organs containing epithelial tissues, including the kidney. When acute tubular damage occurs it is rapidly expressed at high concentrations in both plasma and urine. [7-9, 13].

The first clinical validation was carried out in pediatric cardiac surgery patients [9]. In this study NGAL measured two hours after surgery was an excellent predictor of AKI whereas serum creatinine (SCr) did not start to rise until 24 to 72 hours following surgery. However, in settings in which the initiation of renal injury is unclear, such as in cases of sepsis, trauma, acute and critical illness, the predictive value of plasma and urine NGAL is less certain [13-19]. Furthermore, whether NGAL on its own or in combination with clinical parameters can be of additional value for the prediction of severe AKI has yet to be determined.

We therefore, conducted a prospective study in a large cohort of adult ICU patients to assess the predictive value of plasma and urine NGAL levels at the time of admission with regard to the development of severe AKI during the early days of ICU treatment and their extended contribution in early diagnosis beyond eGFR. None of the results of this current study have been previously reported in abstract form.

Patients and Methods

A detailed method session is given in the online data supplement (ODS)

Patients

The EMC institutional review board of the approved the study. All consecutive admitted patients between September 2007 and April 2008 were eligible for enrollment. Exclusion

criteria included age under 18 years, refusal of consent, nephrectomy, chronic kidney disease (CKD), end stage renal disease (ESRD) and renal transplantation. Deferred consent was used, and written informed consent was obtained from all participants or their heath care proxy [20].

Procedures

Following admission, plasma and urine samples were collected (T=0) and thereafter at 4, 8, 24, 36, 48, 60 and 72 hours. Missing admission (T=0) samples were replaced by first collection values at either 4 or 8 hours after admission. Plasma and urine NGAL were measured on the Triage® NGAL Test point-of-care fluorescence immunoassay in a laboratory, blinded to patient clinical data (Biosite Inc, San Diego USA). The Triage NGAL test has been validated against an NGAL ELISA assay [21] (Detailed assay description ODS-1&2). Serum creatinine (SCr) was measured at admission and thereafter daily at 6:00 am. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study Equation (MDRD) (ODS 3) [22]. Baseline SCr was defined as the steady state level four weeks prior to admission. If not available, the admission value was used as a surrogate baseline. Other variables included age, gender, body mass index (BMI), temperature, pH, bicarbonate, potassium, blood urea nitrogen content (BUN), white blood cell count, C-reactive protein and lactate. For disease severity assessment, the Acute Physiology and Chronic Health Evaluation score (APACHE II) and the sequential organ failure assessment score (SOFA) were used. Furthermore, the cumulative urine output, initiation of renal replacement therapy (RRT), ICU-days, ICU mortality and hospital mortality were recorded. The primary outcome variable was AKI occurring within seven days after ICU admission according to the RIFLE (Risk-Injury-Failure) classification [23]. The RIFLE classification is based on the rise in SCr compared to a baseline value. Risk represents a 1.5-2 times increase, Injury a 2-3 times increase and Failure a more than 3 times increase.

Statistical analysis

MATLAB version 7.5.0 and SPSS version 16.0 were used. The relationships between AKI and NGAL levels were assessed using the Mann-Whitney U test and the chi-square test. Continuous variables were described by medians and interquartile ranges. Receiver operating characteristic (ROC) curves with their area under the curve (AUC) with two times its standard error (2SE) was calculated. Uni-and multivariable logistic regression analyses were used to assess the predictive value of NGAL in combination with clinical parameters. Statistical significance was assessed by estimating the standard error of its coefficient and conducting a Wald-test of the null hypothesis. Stepwise forward likelihood ratio regression was used to determine the model's most efficient predictors. Goodness of fit was assessed using the Hosmer-Lemeshow test. The net reclassification

improvement (NRI) was calculated. All reported p–values are two-tailed, and p-values < 0.05 were considered statistically significant.

Role of funding source

Biosite Incorporated (San Diego, CA, USA) provided biomarker measurements and statistical support. They had no role in study design, data collection or writing of the manuscript. The first author had full access to all data and had final responsibility to submit for publication.

Results

Patient characteristics

Of the 700 consecutive patients who were screened for inclusion in the study, 68 (9.8%) were excluded because of refusal of consent (n=6), nephrectomy (n=6), CKD, ESRD and kidney transplantation (n=25) or missing admission data (n=31). Thus, 632 (90.2%) patients were included in the analysis. Patient characteristics are shown in Table 1. AKI occurred in 171 patients (27%). Of those patients, 67 developed RIFLE R, 48 patients developed RIFLE I and 56 patients developed RIFLE F. The time to reach a SCr increase of more than 50% compared to baseline for the first time (=RIFLE R) was T=0 in 58.5% of the patients, T=24 in 24.0%, T=48 in 6.4% and T=72 in 5.8% of the patients. Thus 94.7% of the patients reached "first AKI" within 72 hours after ICU admission. Twenty-eight (50%) of the AKI patients in the RIFLE F class received RRT (4.4% of the overall patient cohort). Baseline characteristics in all RIFLE classes were compared with subjects who did not develop AKI. There were no differences with respect to age, gender or BMI. Patients with AKI had higher APACHE II and SOFA scores than patients without AKI (Table 1). Furthermore, there were positive correlations between the severity of kidney injury and length of stay, ICU mortality and hospital mortality (Table 1). The incidence of AKI was higher in patients admitted after cardiopulmonary resuscitation (CPR) was performed, as well as in patients with sepsis or multi-organ failure syndrome ($p < 0.0001$) (Table 1).

Association between NGAL and AKI development

Patients' plasma and urine NGAL concentrations at the time of ICU admission were significantly related to their RIFLE scores ($p< 0.0001$, Table 1, Fig.1). The plasma NGAL test performance for predicting the severity of AKI in the entire cohort showed an AUC of 0.77±0.05 for RIFLE R and above, 0.80±0.06 for RIFLE I and above and 0.86±0.06 for RIFLE F. Similar analysis for urine NGAL revealed AUC's of 0.80±0.04 (RIFLE R), 0.85±0.04 (RIFLE I) and 0.88±0.04 (RIFLE F) (Fig.2A and B). The differences between the plasma and urine AUCs were not significant. The AUC's and ROC curves

Table 1: Patients' characteristics and clinical outcome.

	Non-AKI (N=461)	RIFLE R (N=67)	RIFLE I (N=48)	RIFLE F (N=56)	P
Age (years)	58 (43,68)	59 (45,70)	61.5 (53,75)	62 (50,68)	NS
Male, n (%)	264 (57)	46 (69)	29 (60)	30 (54)	NS
BMI (kg/m^2)	24.5 (22.5,27.2)	25.5 (22.5,27.4)	36.6 (35.8,37.7)	36.9 (36.3,37.8)	NS
SCr (mg/dL)	0.75 (0.61,0.91)	1.10 (0.82,1.39)	1.30 (0.82,1.64)	2.09 (1.31,2.86)	<0.0001
eGFR (mL/min/1.73 m^2)	104 (84,129)	70 (50,97)	54 (41,92)	32 (21,50)	<0.0001
Plasma NGAL (ng/ml)	153 (85,233)	268 (145,397)	353 (169,531)	680 (332,1195)	<0.0001
Urine NGAL (ng/ml)	75 (37,206)	323 (74,963)	523 (199,2640)	2013 (564,4124)	<0.0001
pH	7.39 (7.34,7.44)	7.35 (7.29,7.42)	7.33 (7.27,7.41)	7.31 (7.26,7.40)	<0.0001
HCO_3^- (mmol/l)	22.0 (20.1,24.3)	21.0 (18.2,23.7)	19.9 (16.0,23.7)	18.0 (13.4,20.9)	<0.0001
K (mmol/l)	3.9 (3.6,4.3)	4.1 (3.7,4.6)	4.3 (3.6,4.5)	4.3 (3.9,4.9)	<0.0001
BUN (mmol/l)	5.5 (4.2,7.3)	8.6 (5.1,12.1)	8.8 (5.8,17.1)	14.1 (8.4,26.6)	<0.0001
White cell count (*10^9/ml)	11.4 (8.4,15.1)	10.0 (6.9,14.8)	11.8 (6.9,16.2)	11.0 (6.3,17.6)	NS
CRP (mmol/l)	12 (3,68)	72 (8,158)	25 (6,134)	118 (36,198)	<0.0001
Lactate (mmol/l)	1.5 (1.0,2.4)	2.2 (1.4,3.2)	2.3 (1.3,4.6)	2.3 (1.2, 4.2)	<0.0001
Apache II score	16 (13,22)	19 (15,28)	24 (20,29)	25 (22,28)	<0.0001
SOFA score	4 (2,6)	7 (4,9)	8 (6,11)	11 (8,13)	<0.0001
UP (ml/kg/h)	1.1 (0.8, 1.7)	1.0 (0.7, 1.4)	0.8 (0.6, 1.3)	0.5 (0.2, 0.9)	<0.0001
RRT, n (%)	0 (0)	0 (0)	0 (0)	28 (50)	<0.0001
ICU mortality, n (%)	49 (8)	10 (15)	9 (19)	26 (46)	<0.0001
Hospital mortality, n (%)	71 (11)	20 (30)	16 (33)	30 (54)	<0.0001
Diagnostic group, n (%)					
Postoperative	166 (36)	15 (22)	6 (13)	5 (9)	<0.0001
Medical	99 (22)	13 (19)	15 (31)	11 (20)	NS
Neurological	88 (19)	5 (8)	1 (2)	1 (2)	<0.0001
Neurotrauma	27(6)	2(3)	0(0)	1(2)	NS

Table 1: (Continued)

	Non-AKI (N=461)	RIFLE R (N=67)	RIFLE I (N=48)	RIFLE F (N=56)	P
Multitrauma	26(6)	6(9)	4(8)	1(2)	NS
LTX	19(4)	8(12)	1(2)	1(2)	NS
Sepsis	14(3)	6(9)	8(17)	15(27)	<0.0001
CPR	11(2)	6(9)	7(15)	3(5)	<0.0001
Hemorr.shock	9(2)	4(6)	3(6)	3(5)	NS
MOF	1(0)	2(3)	3(6)	15(27)	<0.0001

BMI, body mass index; SCr, serum creatinine; eGFR, estimated glomerular filtration rate according to the Modification of Diet in Renal Disease Study Equation; Plasma and Urine NGAL values, HCO_3^-, plasma bicarbonate concentration; K, serum potassium concentration; BUN, blood urea nitrogen concentration; CRP, C-reactive protein; APACHE II, acute physiology and chronic health evaluation score at T=24; SOFA, sequential organ failure assessment score at T=24; UP, urine production first 24 hours after admission; RRT, renal replacement therapy; ICU, intensive care unit; LTX, liver transplant surgery; CPR, cardiopulmonary resuscitation; Hemorr.shock, hemorrhagic shock; MOF, multi organ failure; NS, non significant.

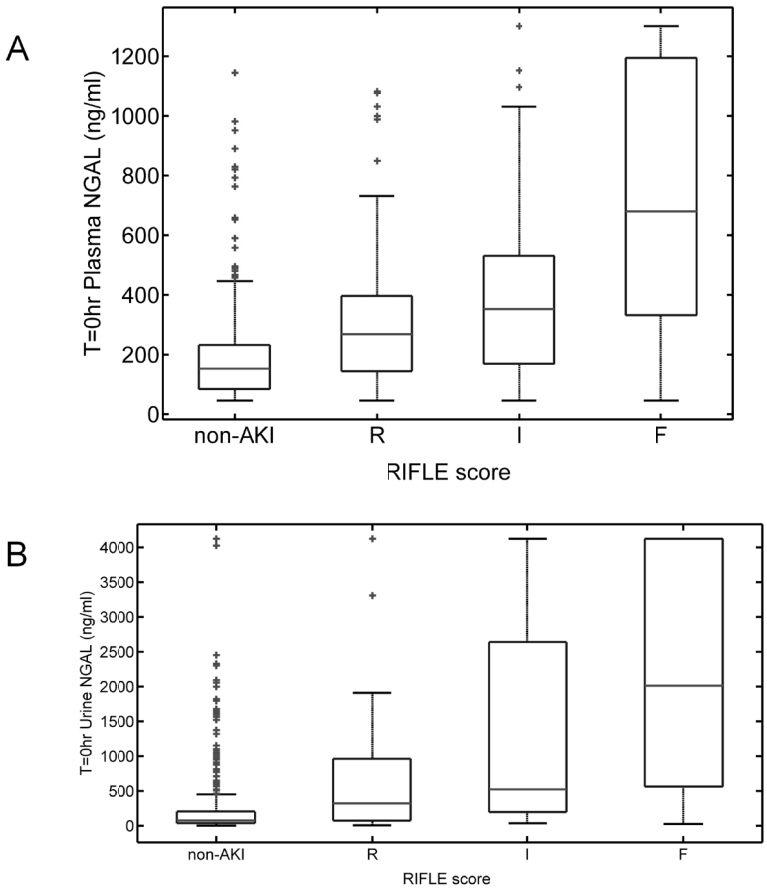

Figure 1: Admission plasma (A) and urine (B) NGAL concentrations stratified by RIFLE classification. An exploratory Mann-Whitney U test of adjacent categories, including non-AKI vs. R, R vs. I, and I vs. F, resulted in p-values of <0.0001, 0.10, and 0.0005, respectively, for plasma NGAL and <0.0001, 0.028 and 0.001, respectively, for urine NGAL.

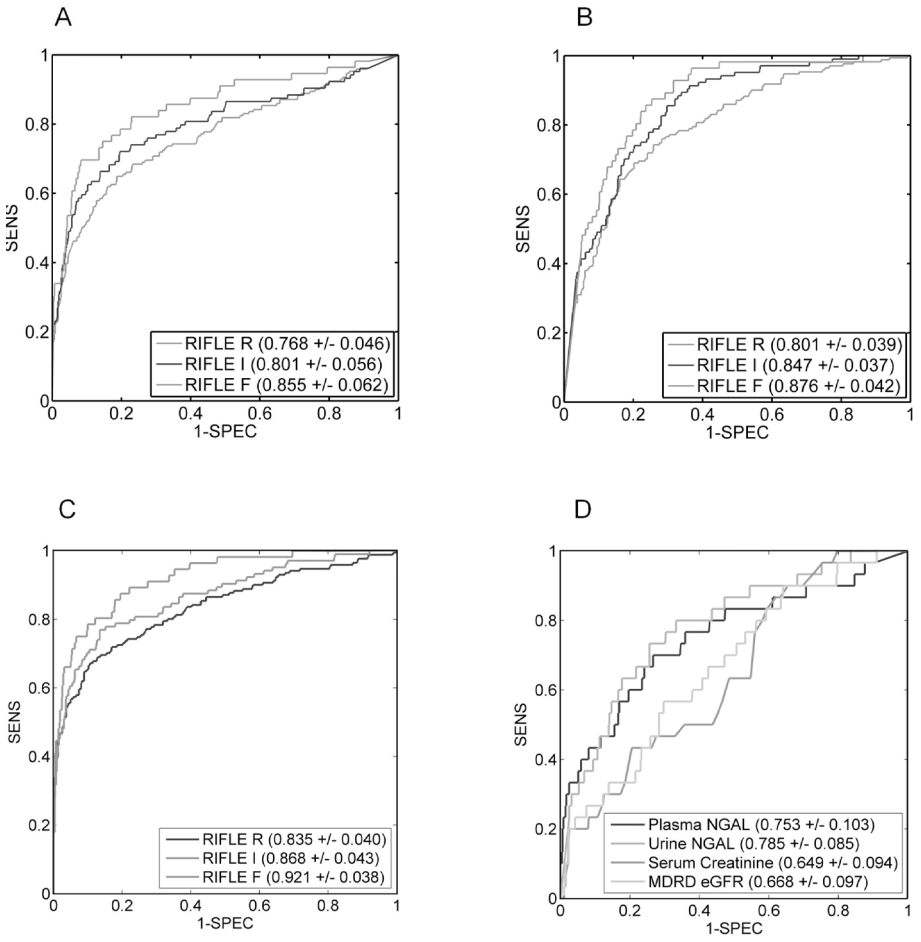

Figure 2: ROC curve analysis for the ability of admission plasma (A) and urine (B) NGAL, eGFR (C) to predict AKI, stratified by RIFLE classification. With panel (D) showing both plasma and urine NGAL's predictive properties for RIFLE I or worse in patients with eGFR above 60 mL/min/1.73 m^2. AUC values ± 2SE are presented parenthetically after the RIFLE classification.

Table 3 Multivariable logistic regression for the prediction of RIFLE F combining NGAL with eGFR and other clinical predictors.

	Plasma NGAL				Urine NGAL			
		RC				RC		
Variable	OR	(B)	(SE)	P	OR	(B)	(SE)	P
NGAL (ng/ml)	1.83	0.6	(0.25)	0.017	1.49	0.40	(0.23)	0.088
eGFR (ml/min/1.73m^2)	0.95	-0.05	(0.01)	0.000	0.94	-0.06	(0.01)	0.000
Age(years)	0.98	-0.02	(0.02)	0.240	0.97	-0.03	(0.02)	0.131
BMI (kg/m^2)	0.93	-0.07	(0.06)	0.240	0.91	-0.09	(0.06)	0.144
Temp (°C)	0.68	-0.39	(0.16)	0.016	0.68	-0.39	(0.16)	0.015
Sepsis	10.52	2.35	(0.70)	0.001	14.16	2.65	(0.66)	0.000
PH	2.07	0.73	(3.35)	0.828	3.79	1.33	(3.57)	0.709
HCO_3^- (mmol/l)	1.01	0.01	(0.06)	0.822	1.01	0.01	(0.06)	0.819
K (mmol/l)	2.10	0.74	(0.34)	0.028	1.93	0.66	(0.35)	0.057
BUN (mmol/l)	0.99	-0.01	(0.04)	0.816	1.00	0.00	(0.04)	0.972
WBC (*10^9/ml)	0.93	-0.07	(0.03)	0.025	0.94	-0.06	(0.03)	0.025
CRP (mmol/l)	1.00	0.00	(0.00)	0.259	1.00	0.00	(0.00)	0.326
Lactate (mmol/l)	0.87	-0.14	(0.11)	0.224	0.88	-0.13	(0.11)	0.237
Total				0.014				0.092

RC, regression coefficient; B, eGFR, estimated glomerular filtration rate according to the Modification of Diet in Renal Disease Study Equation (MDRD); BMI, body mass index; Temp, temperature; HCO_3^- , bicarbonate; K, potassium; BUN, blood urea nitrogen; WBC, white blood cell count; CRP, C-reactive protein; SE, standard error; OR, Odds Ratio; RC, regression coefficient.

Table 4 Stepwise forward likelihood ratio logistic regression for determination of most efficient clinical model for the prediction of RIFLE F.

	Plasma NGAL				Urine NGAL			
	RC				RC			
Variable	OR	(B)	(SE)	P	OR	(B)	(SE)	P
NGAL (ng/ml)	1.71	0.54	(0.21)	0.010	1.42	0.36	(0.17)	0.039
eGFR (mL/min/1.73 m^2)	0.95	-0.05	(0.01)	0.000	0.95	-0.06	(0.01)	0.000
Sepsis	9.94	2.30	(0.59)	0.000	9.15	2.21	(0.53)	0.000
WBC (*10^9/ml)	0.95	-0.06	(0.03)	0.057	0.95	-0.05	(0.02)	0.051
Temp (°C)	0.78	-0.25	(0.13)	0.061				
Total				0.000				0.000

eGFR, estimated glomerular filtration rate according to the Modification of Diet in Renal Disease Study Equation (MDRD); WBC, white blood cell count; Temp, temperature; SE, standard error; OR, Odds Ratio; RC, regression coefficient.

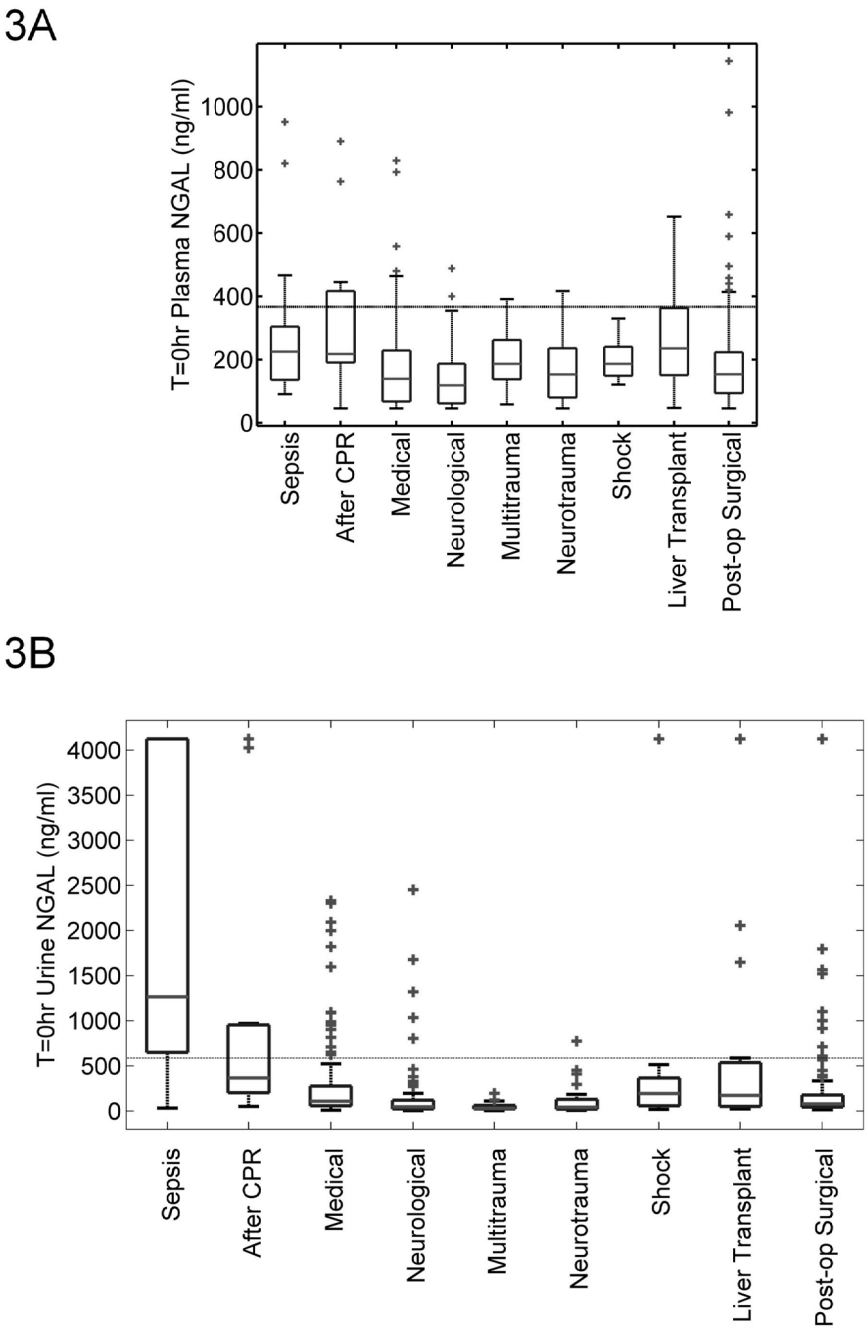

Figure 3: Admission plasma (A) and urine (B) NGAL values for patients without AKI stratified by diagnostic groups.

for eGFR predicting AKI stratified for RIFLE stage are shown in Fig. 2C. Comparing the performance of eGFR to plasma and urine NGAL showed that only plasma NGAL predicting R and above, or I and above were significantly different compared to the corresponding AUC's of eGFR (p=0.015 and p= 0.039). Table 2 lists the calculated sensitivities at fixed specificities of 50, 70 and 90% (derived by visual inspection of the ROC curves) with the corresponding cut-off concentrations of plasma and urine NGAL for the prediction of RIFLE F.

Association between NGAL and AKI development in patients with eGFR > 60 mL/min/1.73 m²

In order to determine the potential additional contribution of NGAL as a biomarker predicting AKI before SCr has started to rise and consequently eGFR has started to decline a subset analyses was performed in patients with apparently normal renal function (n=498) at the time of ICU admission (i.e. excluding patients with an eGFR< 60 mL/min/1.73 m^2). ROC analysis demonstrated that in patients who did not show any increase in SCr yet at ICU admission, plasma and urine NGAL had diagnostic superiority over SCr and eGFR for predicting severe AKI (RIFLE I and F). The AUC's for plasma and urine NGAL were respectively 0.75±0.10, 0.79±0.10 compared to 0.65±0.10 and 0.67±0.10 for SCr and eGFR respectively (Fig. 2D)

Relative contribution of NGAL to the most efficient clinical prediction model at admission for prediction of RIFLE F

Adding pNGAL and uNGAL to eGFR in a multivariable logistic regression model improved the prediction significantly (p<0.001). To determine the added contribution of NGAL to eGFR and other available clinical variables at ICU admission for predicting the occurrence of RIFLE F within the first week of patients' ICU stay, additional logistic regression analysis was performed (Table 3). The available clinical predictors included age, BMI, temperature, diagnosis of sepsis, pH, bicarbonate, potassium, BUN, white blood cell count, CRP and lactate. Adding NGAL to eGFR and clinical variables improved the prediction significantly for pNGAL (p=0.014) and almost significantly for uNGAL (p= 0.092). Using a stepwise forward likelihood ratio logistic regression NGAL, eGFR, diagnosis of sepsis, white blood cell count (WBC) and temperature on admission made the most efficient clinical model for the prediction of RIFLE F for plasma out of the available variables in this study. For urine the most efficient model comprised NGAL, eGFR, diagnosis of sepsis and WBC (Table 4). Adding NGAL changed the model's AUC's from 0.95±0.02 to 0.96±0.02 for plasma NGAL and 0.94±0.02 to 0.95±0.01 for urine NGAL. Furthermore, we assessed the ability of plasma and urine NGAL to "reclassify" the degree of risk for RIFLE F within 7 days as assessed by the model. Subjects were categorized into prespecified "low", "medium" and "high risk" groups using cut-offs of

<30%, 30-60% and >60% respectively. We compared the proportions of reclassified subjects across these three risk groups when NGAL was added to the clinical model for plasma and urine (ODS-4 for detailed reclassification table). For 5 patients with RIFLE F reclassification was more accurate when the model with all 4 variables for pNGAL was used and for 2 patients it became less accurate. Among the subjects without RIFLE F, 9 were correctly reclassified in a lower risk category while 3 were incorrectly reclassified to be at higher risk. The same analysis was performed for uNGAL (ODS-4). The generated net reclassification improvement (NRI) for plasma and urine NGAL added to the clinical prediction model was 8.5% (p=0.087) and 2.3% (p=0.370) respectively.

Relative contribution of serial NGAL measurements to the most efficient clinical prediction model for prediction of RIFLE F

The progression of mean plasma and urine NGAL concentrations stratified by RIFLE classification over time is shown in the ODS-5 (Fig. E1 and E2). To determine if serial sampling could be of additional value for the prediction of RIFLE F plasma and urine NGAL values and those of the other predictors at T=0 and T=24 (temperature, pH, bicarbonate, potassium, BUN, WBC, CRP, lactate) were used for multivariable logistic regression analysis. In addition, age, BMI, diagnosis of sepsis, eGFR MDRD T=0, the 24-hour urine production, the 24-hour cumulative fluid balance, APACHE II and SOFA score were added. All subjects with established RIFLE F or missing data in first 24 hours were excluded, leaving 429 patients for the plasma and 411 for the urine analysis. With stepwise forward likelihood ratio logistic regression the most efficient predictors were pNGAL T=24 (p=0.000) and CRP T=0 (p=0.024) for the plasma NGAL model. Adding pNGAL T=24 changed the model's AUC from 0.63±0.04 to 0.91±0.03, underlining that pNGAL T=24 is a very strong predictor for RIFLE F. For urine, the model showed NGAL T=24 (p=0.001), Temp T=0 (p=0.02), APACHE T=24 (p=0.011), UP T=24 (p=0.009), pH T=24 (p=0.005) and potassium T=0 (p=0.055) as most efficient predictors. Adding uNGAL T=24 changed the model's AUC from 0.84±0.06 to 0.93±0.04. Assessment of both plasma and urine NGAL values' difference scores in the logistic regression analysis showed that the temporal changes were not relevant; pointing out that the NGAL value measured closer to the endpoint "RIFLE F" was the strongest predictor of all. Analyzing further contribution of serial measurements over the succeeding time points was not possible due to the significant reduction in sample size with the diminished availability of equal measurements. Furthermore since the difference in serial measurements in the first 24 hours did not add to the prediction of RIFLE F, it is not expected that the results will be different when analyzing subsequent time points.

Association of NGAL and sepsis in patients without AKI

In patients with sepsis (n=14) who did not develop AKI, urine NGAL levels were significantly higher than those of patients in the other diagnostic groups. The median NGAL value was 1264.1 ng/ml (650.3, 4124) (Fig.3). In the group of 14 septic non-AKI patients, one received renal drainage because of obstructive hydronephrosis, one had a positive urine culture with Acinetobacter species, one had a positive white cell and nitrite count in the urine sediment without a positive culture already under antibiotic treatment and one patient had a proven renal abscess with E. Coli. After we adjusted the urine NGAL analysis removing those patients and patients who died within 48 hours after admission to the ICU, urine NGAL levels were still significantly higher among patients with a diagnosis of sepsis than among patients in the other diagnostic groups (p=0.0005).

Association between NGAL and RRT or mortality

In the entire cohort both NGAL plasma and urine values were predictive of RRT initiation within the first week of ICU admission (respectively AUC 0.88±0.06 and AUC 0.89±0.04). However, SCr and eGFR reached similar performances (respectively AUC 0.90±0.05 and 0.91±0.05). Both plasma and urine NGAL have a minor role in predicting hospital mortality with very modest performances (AUC 0.63±0.06 and AUC 0.64±0.06).

Discussion

The present study shows that plasma and urine NGAL levels at time of ICU admission predict the development of severe AKI and the initiation of RRT in critically ill patients within the first 7 days of their ICU stay. Furthermore, adding NGAL values to a model with eGFR alone or to the most efficient clinical model with available parameters improves the prediction significantly. Using serial NGAL measurements did not provide additional accuracy in the prediction of RIFLE F. Finally, non-AKI patients with sepsis have significantly higher urinary NGAL values compared to other non-AKI patients.

NGAL fulfills a central role in regulating epithelial neogenesis, as well as in iron chelation and delivery after ischemic or toxic insults to the renal tubular epithelium [24, 25]. Following kidney injury, NGAL is rapidly expressed on the apical epithelial membranes of the distal nephron. NGAL is excreted in the urine through exocytosis and has local bacteriostatic and pro-apoptotic effects [26, 27]. Plasma NGAL is easily filtered by the glomerulus and reabsorbed in the apical membranes of the proximal tubules. Reabsorbtion is mediated by megalin/cubulin dependent endocytosis with a very high affinity. The delivered iron is needed in processes activating and repressing iron-responsive genes that are vital to the regeneration processes that occur after damage

is inflicted to these cells. Under normal circumstances the estimated half life of plasma NGAL is approximately 10 minutes, with urinary loss <0.2% [28, 29]. Plasma and urine NGAL concentrations increase by 10- to 100- fold during the 2 hours that follow tubular injury[7-9], whereas serum creatinine does not start to rise 24 to 72 after the initial renal insult [9, 16, 30].

Because we are interested in the possible prevention of (further) kidney injury in critically ill patients, AKI was assessed only during the first week of each patient's ICU stay in order to link the condition of the patient at the time of admission and the initial resuscitation efforts to the development of AKI.

In this study, we found that plasma and urine NGAL measured at the time of admission were good predictors of AKI. The test performance of both plasma and urine NGAL increased as the severity of the functional damage to the kidney's increased; the AUC's ranged from 0.77 (RIFLE R) to 0.86 (RIFLE F) for plasma NGAL and from 0.80 (RIFLE R) to 0.88 (RIFLE F) for urine NGAL.

Previous studies in pediatric ICU patients with sepsis and septic shock [14] and in a group of adult critically ill patients [17] have studied the predictive accuracy of plasma and urine NGAL reporting AUC's of 0.68 and 0.64 for sustained AKI. Both Zappitelli et al. (pediatric population) and Cruz et al. (adult population) observed AUC's for prediction of RIFLE R or worse AKI by NGAL that were comparable to those observed in the present study[16, 19]. Constantin and Nickolas et al. reported very high AUC's for the ability of plasma and urine NGAL to predict AKI in critically ill adult and emergency department patients (0.92 and 0.95 respectively) [13, 18]. Several explanations exist for the observed variability of NGAL's test performance in these studies, in which the timing of renal insult was not strictly identified.

First, in the current study NGAL measurement was performed immediately following ICU admission and patients were monitored for the occurrence of AKI for the next 7 days. The timing of NGAL measurement in the previous studies mentioned above ranged from 48 hours after the initiation of mechanical ventilation (up to 3 days after admission) to within 24 hours of ICU admission to the first possible moment upon ICU admission. With the rapid changes in plasma and urine NGAL concentrations, the slow changes in SCr concentrations, the reversibility of the early phases in the continuum of AKI and the effects of intensive resuscitation in the golden hours after ICU admittance, timing of measurement will have effects on the NGAL concentrations measured in relation to the changes in SCr [31]. Therefore the time at which NGAL levels are measured clearly influences their test performance.

Second, the number of AKI patients in a given study as well as their RIFLE class distribution also influences test results [32]. Due to the large sample size in this study and the fairly equal patient distribution between RIFLE categories, we were able to analyse the ability of NGAL to predict more severe AKI endpoints, such as RIFLE F.

In contrast, Wheeler et al. used very unusual criteria for AKI, making it impossible to compare their results with those of other studies. The AKI cohort in the study performed by Siew et al. comprised of patients with less severe stages of AKI (median urine NGAL 127 ng/ml IQR: 32- 623 and median SCr 1.5 mg/dL IQR: 1.0-2.2 at enrollment) resulting in low performance characteristics of NGAL (AUC= 0.71 95% CI 0.63-0.78). Nickolas et al. reported that NGAL was an excellent predictor of AKI (AUC= 0.95 95% CI: 0.88-1.0) in an emergency department setting. However, the mean SCr and fractional sodium excretion of this entire AKI subgroup at the time of study inclusion were 5.6 mg/dL (SD=5.5) and 6.9% (SD 9.1) respectively, indicating that severe loss of renal function had already occurred in the majority of these patients. Accordingly, test results generated in patients with established AKI should not be used for the comparison with those in a cohort of developing AKI.

Third, AKI and its severity defined by RIFLE are dependent upon how baseline SCr values are determined and will contribute to different outcomes between studies. In our study the first available SCr value was used as a surrogate baseline when a patient's historical data were not available. This undoubtedly has resulted in an underestimation of attained RIFLE stage in some of these patients. Furthermore, with the artificial definition of AKI using three set severity stages the issue of timing may simply be definitional.

This study adds to the current literature as it showed that NGAL significantly improves the diagnostic accuracy for severe AKI adding it to MDRD eGFR calculated at ICU admission, even in patients having an apparently normal eGFR at admission. Especially in these patients this could be of value as their AKI is not yet reflected in an increase in SCr. ICU patients are typically diagnosed with AKI several days after the onset of their illness or injury, resulting in a delay in the discontinuation or dose adjustment of nephrotoxic medications or continued use of procedures that could cause further renal damage. Whether NGAL levels have the potential to influence clinical decision making in the ICU should be the topic for further randomized studies that should be performed before using NGAL measurements in clinical practice.

These studies may include applying more intensive resuscitation, avoiding nephrotoxic drugs or implementation of a more timely initiation of RRT in patients with elevated NGAL levels [33]. Additionally, recent animal studies examining interventions to reverse AKI have been promising, implying that it may be possible to reverse AKI in humans if it is treated early [29, 34-39]. Secondly, this study adds to current knowledge since we defined a most efficient clinical model in the prediction of AKI using available data at the time of ICU admission improving the predictive accuracy for RIFLE F significantly with NGAL above eGFR and clinical predictors. The predictive accuracy of eGFR on its own was roughly comparable to that of plasma or urine NGAL. However, we should take into account that SCr is used to define the endpoint RIFLE F and is likewise used to calculate eGFR which is incorporation bias. Therefore it is somewhat biased to compare NGAL's

performance with the ability of SCr to predict itself. Furthermore, in this study, the point of first AKI was satisfied in many patients at the time of ICU admission (58.5%). As such, it is to be expected that in many of the AKI cases, SCr would already be elevated at the time of admission. AKI that was present at the time of ICU admission was determined by retrospective collection of baseline SCr values from the patient records prior to admission. However in clinical practice, a prior baseline SCr is more often not available at ICU admission and as such it is not possible to correctly determine the endpoint of AKI as compared to CKD. Furthermore, we should also take into account that NGAL is a direct injury marker that is unfortunately compared to a "gold standard" AKI diagnosis that is based on a functional marker (SCr) which has major imperfections on its own [40]. In this context it is indispensable to emphasize the importance of (injury) biomarker combinations to achieve more accurate predictions irrespective of SCr.

Thirdly, we showed that temporal changes in NGAL measurements do not provide additional information for the prediction of RIFLE F. And finally, we found that septic patients without AKI had markedly increased urine NGAL concentrations, whereas there were no significant differences between groups with regard to the plasma NGAL values. A possible explanation for our results lies in the two-compartment model theory of NGAL (which applies to an animal model under relatively normal conditions) [27] as well as the fact that AKI is an inflammatory disease [41]. In patients with AKI, Human Toll-like receptor 2 (TLR2) stimulates tubular epithelial apoptosis [42] and NGAL expression [43]. Bacterial pathogens produce lipoproteins and activate cytokine networks by inducing the expression of multiple proinflammatory genes. Lipoproteins also have strong affinity for TLRs that trigger an innate immune response. Therefore it could be postulated that these circulating ligands that are linked to tubular epithelial TLR activation are responsible for the increased urine NGAL concentrations that we observed in patients who had sepsis but showed no increases in their SCr levels [44]. However, a very recent study in patients with sepsis, septic shock and systemic inflammatory response syndrome has reported contradictory findings [45]. A possible explanation for this difference is the variability of the subject inclusion time (up to 48 hours after ICU admission). Intensive resuscitation and the administration of antibiotics may have already occurred before study inclusion, therefore most likely inducing rapid changes of urine NGAL values.

In conclusion, the present study shows that both plasma and urine NGAL levels at ICU admission are good predictors of severe AKI and significantly add to the prediction of AKI using eGFR and to a model with clinical parameters. Because the study population reflects a mixed group of diagnoses that are present in most ICU's these findings could have major clinical implications regarding optimization of therapy in patients at risk for

AKI. Our findings could also facilitate studies of the effectiveness of early therapeutic and supportive interventions in patients with established AKI.

Conflict of Interest Statement

We declare that we have no conflicts of interest.

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Online data supplement

Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients

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ODS-1 Patients and Methods

Laboratory sample processing

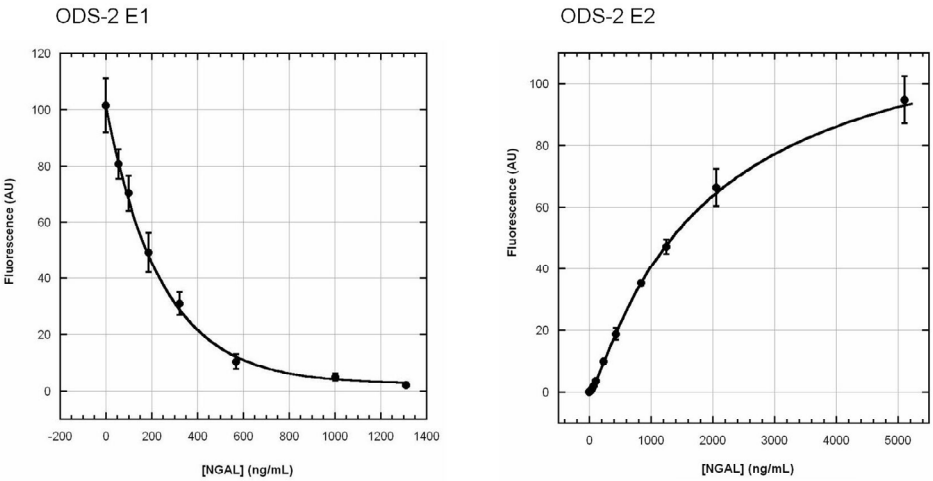
Following admission, plasma and urine samples were collected (T=0) and thereafter at 4, 8, 24, 36, 48, 60 and 72 hours. Missing admission (T=0) samples were replaced by first draw values at either 4 or 8 hours after admission. This occurred for 40 plasma samples and 61 urine samples.

Arterial blood was sampled in EDTA tubes from an arterial line and freshly voided urine was collected from an indwelling catheter. Both blood and urine samples were sent immediately to the hospital's general laboratory and centrifuged at 3,000 rpm at 4°C for 10 minutes with a relative centrifugal force of 1700-g. The supernatants were aliquotted equally into 3 cryovials and stored at -80°C. After study completion all samples were shipped by air transportation on dry ice to San Diego.

NGAL quantification with Triage® point-of-care immunoassay

Plasma NGAL levels were measured with the Triage® NGAL Test point-of-care fluorescence immunoassay on a Triage MeterPlus (Biosite Inc, San Diego USA) using a commercially available NGAL cartridge. The assay device is a single-use plastic cartridge that contains an NGAL-specific monoclonal antibody conjugated to a fluorescent nanoparticle, NGAL antigen immobilized on a solid phase, and stabilizers. In addition, the device was engineered with integrated control features including positive and negative control immunoassays. After the device containing the sample is inserted into the Triage® MeterPlus, quantitative measurements of NGAL concentration are displayed on the meter's screen within 15 minutes. The devices used in this investigation reported plasma NGAL concentrations between 46 ng/ml and 1300 ng/ml. The device was able to measure the NGAL concentrations of almost all (89.7%) samples. However, 7.9% of the T=0 samples had NGAL concentrations that were below the lower limit of the detection range and 2.4% had concentrations above the upper limit of the detection range. The assay variability for plasma NGAL was 15.8%. Urinary NGAL levels were measured using a non-commercial Triage® immunoassay cartridge with a sandwich format immunoassay in order to provide an appropriate detection range for urine samples. Before application to the cartridge, the urine samples that had NGAL concentrations exceeding the assay's upper limit of detection were diluted with an equal volume of 200 mM BES buffer, pH 7.2. The effective concentration range of the urine NGAL assay was 2.6 ng/ml to 4100 ng/ml; none of the T=0 urine samples had NGAL concentrations that were below the lower limit of detection and 8.5% of the T=0 samples had NGAL concentrations that were above the upper limit of detection. The assay variability for urine NGAL was 13.9%.

ODS-2 Triage® NGAL Test point-of-care standard curves



ODS-2: The Triage® NGAL Test point-of-care fluorescence immunoassay standard curves for plasma (Fig. E1) and urine (Fig. E2) samples. The curves plot NGAL concentrations vs. the absorbance response (AU). The dots represent the integrated fluorescence signal averaged over 10 individual devices for a series of known NGAL calibration samples.

ODS-3 MDRD formula

The estimated glomerular filtration rate (eGFR) was calculated for each patient at ICU admission using the Modification of Diet in Renal Disease Study Equation (MDRD) [1]. MDRD Formula: eGFR= 186 * (sCr in µg/L * 0.0113) ^ (-1.154) * (Age in years) ^ (-0.203). The result is multiplied by 0.742 if the patient is female, assuming ethnicity as non-Black.

Table E1: Risk reclassification using plasma NGAL and clinical predictors compared with the best clinical model alone for RIFLE F.

	Plasma NGAL added to best clinical model			
Best clinical model	<30%	30-60%	>60%	Total no.
Subjects with RIFLE F (n=43)				
<30%	8	1	0	9
30-60%	0	9	4	13
>60%	0	2	19	21
Total no.	8	12	23	43
Subjects without RIFLE F (n=408)				
<30%	389	2	0	391
30-60%	8	4	1	13
>60%	0	1	3	4
Total no.	397	7	4	408

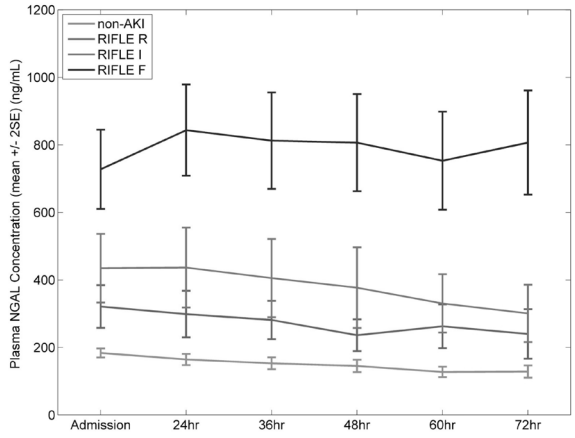
Table E2: Risk reclassification using urine NGAL and clinical predictors compared with the best clinical model alone for RIFLE F.

	Urine NGAL added to best clinical model			
Best clinical model	<30%	30-60%	>60%	Total no.
Subjects with RIFLE F (n=53)				
<30%	12	1	0	13
30-60%	5	5	6	16
>60%	0	1	23	24
Total no.	17	7	29	53
Subjects without RIFLE F (n=530)				
<30%	502	4	0	506
30-60%	7	9	3	19
>60%	0	2	3	5
Total no.	509	15	6	530

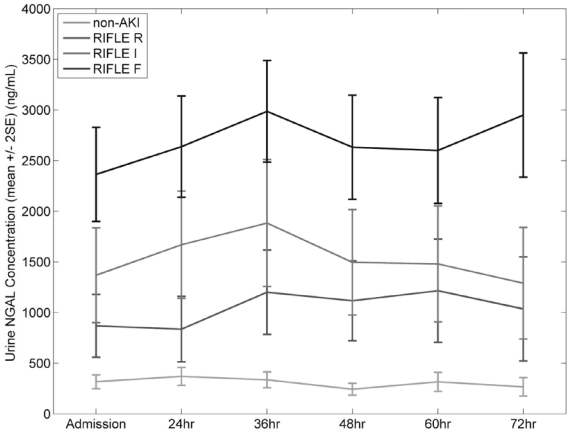
Clinical predictors making the most efficient model with plasma NGAL (n=451; 181 missing): eGFR, diagnosis of sepsis, white blood cell count and admission temperature; Net reclassification improvement for plasma NGAL = 8.4% (p =0.087). Clinical predictors used in the urine NGAL model (n=583; 49 missing): eGFR, diagnosis of sepsis and white blood cell count; Net reclassification improvement for urine NGAL = 2.3% (p=0.370).

ODS-5 Progression of NGAL concentrations over time after ICU admission

ODS-5 E1



ODS-5 E2



ODS-5: The progression of plasma (E1) and urine (E2) NGAL concentrations (ng/ml) over time are stratified by RIFLE classification and plotted against time.

Comment

Limits of Neutrophil gelatinase associated Lipocalin at intensive care admission for prediction of acute kidney injury.

Hilde RH de Geus and Jan Bakker

Am J Respir Crit Care Med. 2011 July:1: 184(1):143-143

We would like to thank both Dr. Glassford et al and Dr. Darmon et al for their insightful and complimentary letters on our article [1]. We agree with Dr. Glassford's comment that the use of the current insights in different forms of urinary Neutrophil Gelatinase associated Lipocalin (NGAL) could have contributed to the interpretation of the increased NGAL levels we observed in non-Acute Kidney Injury (AKI) patients with sepsis. Unfortunately, at the time of this study, this differentiation was not available. Furthermore, we agree that measuring NGAL routinely in every ICU patient at ICU admission might not be cost effective. However, in a recent study [2] we demonstrated that the average daily costs for patients requiring renal replacement therapy (RRT) is 36% higher compared to those of an "average" ICU patient without RRT. Therefore, early detection and prevention of progressive renal failure requiring RRT in ICU patients could save a significant amount of resources. In this context, the cost-effectiveness of routinely measuring NGAL in all patients at the time of ICU admission (single NGAL test costs: $10) is therefore highly dependent on the availability of successful therapies to prevent deterioration of renal function to RRT dependency. We believe at this moment the challenge remains to further improve the diagnostic tools for AKI using multivariable models combining clinical and biomarker data to reach more predictive precision. This may include an accurate definition of patients with increased a priori likelihood of developing AKI. The described multivariable clinical model in our study was a first attempt.

Regarding Dr. Damon's comments, we would like to point out that the mentioned more timely initiation of RRT in the discussion section guided by biomarker levels should be read in a hypothetical framework and is certainly not a conclusion based on the presented data. However, as Lameire et al [3] state in their review: "The best timing for dialysis also remains unclear. Early initiation might be associated with improved survival, but the discussion is blurred by the lack of a robust marker of renal function." Our study explored whether NGAL would be a sufficient biomarker to solve this problem. We agree that in that sense further stratification irrespective of the RIFLE (Risk-Injury-Failure) classification should be further explored, such as the suggested transient vs. sustained AKI. We acknowledge that plasma NGAL's performance might be influenced by several confounders in an adult ICU population. In fact that was exactly the rationale to initiate this study. Accordingly, we emphasized that NGAL is comparable to estimated Glomerular Filtration Rate (eGFR), but has important significant additional value when used in a model including eGFR and other clinical parameters. Furthermore, in downsizing the patient population to patients with a seemingly normal eGFR, we clearly showed that in these patients, when serum creatinine (SCr) is not useful, NGAL does have important predictive value.

Finally, the suggestion of Dr. Darmon et al to use the serum creatinine value after recovery of the acute disease as the presumed steady state baseline value is incorrect in our opinion for several reasons. First, patients treated for severe illness in the intensive

care undoubtedly loose a large percentage of their muscle mass due to the catabolic state and will therefore have a different new absolute baseline value when compared to the state before admission. Because the RIFLE/AKIN scoring systems are based on relative changes in absolute SCr values, this will result in inaccurate stratification. Secondly, complete renal recovery after an episode of severe AKI is never a guarantee. Furthermore, it is commonly known that many of these patients will reach a new lower steady state renal function; therefore, it is inappropriate to use this value as the pre ICU baseline value for RIFLE/AKIN stage determination.

References

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2. Tan SS, Hakkaart-van Roijen L, Al MJ, et al.: A microcosting study of intensive care unit stay in the Netherlands. J Intensive Care Med 23:250-257, 2008
3. Lameire N, Van Biesen W, Vanholder R: Acute kidney injury. Lancet 372:1863-1865, 2008

Chapter 3

Urinary NGAL measured at ICU admission accurately discriminates between sustained and transient AKI in adult critically ill patients

Hilde RH de Geus, Jessica G Woo, Yu Wang, Prasad Devarajan Michiel G Betjes, Jos LML le Noble, Jan Bakker

Nephron extra. 2011 Jan;1(1):9-23

Abstract

Background: First to evaluate the ability of NGAL and Cystatin-C (CyC) in plasma and urine to discriminate between sustained, transient and no-AKI. And second, to evaluate their predictive performance for sustained AKI in adult ICU patients.

Methods: Prospective cohort study of 700 patients. Sample collection was performed over 8 time points starting at admission.

Results: After exclusion 510 patients remained for the analysis. All biomarkers showed significant differentiation between sustained and no-AKI at all time points ($p \leq 0.0002$) except for urine CyC (uCyC) at admission ($p=0.06$). Urine NGAL was the only biomarker significantly differentiating sustained from transient AKI at ICU admission ($p=0.02$). Individually, uNGAL performed better than the other biomarkers (AUC=0.80, 95%CI=0.72-0.88) for the prediction of sustained AKI. The combination with plasma NGAL (pNGAL) showed a non-significant improvement (AUC=0.83, 95%CI=0.75-0.91). The combination of individual markers with a model of clinical characteristics (MDRD eGFR, HCO_3^- and Sepsis) didn't improve its performance significantly. However the integrated discrimination improvement (IDI) showed significant improvement when uNGAL was added ($p=0.04$).

Conclusions: Urine NGAL measured at ICU admission differentiates patients with sustained AKI from transient or no-AKI patients. Combining biomarkers such as pNGAL, uNGAL and pCyC with clinical characteristics adds some value to the predictive model.

Introduction

Acute kidney injury (AKI) is common in adult hospitalized patients and associated with significant morbidity and mortality (up to 50%) [1-3]. AKI in critically ill patients is rarely an isolated event but usually more often part of a more multisystemic immunological condition contributing to remote organ dysfunction.

However, several large cohort studies have established a strong independent relationship between the severity of AKI and its associated mortality. Moreover, even small changes in kidney function and periods of transient azotemia have considerable impact on outcome [4]. In order to initiate timely therapies, it seems essential to discriminate patients with high risk for sustained AKI from patients with lower risk. Unfortunately, at the moment, specific therapies for AKI are lacking even though experimental animal data show promising possibilities. One major setback is that these new agents show promise only in the early reversible stages of AKI [5]. Therefore it is essential to possess early and accurate detection tools for sustained AKI.

The rise in levels of serum creatinine (SCr) is still used for the diagnosis of AKI; consequently it is the basis of the AKI definitions such as the RIFLE criteria. However, it has a poor predictive value for AKI in the early stage of renal injury. In recent years, a number of novel biomarkers have been studied for their potential to detect AKI in its early stage [6].

Neutrophil Gelatinase-associated Lipocalin (NGAL) is a 25-kDa protein combined with a siderophore, produced by epithelial tissues throughout the human body. It is involved in nephrogenesis, induction of nephritic structures from mesenchymal stroma cells, protection against ischemic tubular damage and in iron transportation [7-10]. Plasma NGAL (pNGAL) is under normal conditions excreted by glomerular filtration and undergoes complete proximal tubular reabsorption. Urine NGAL (uNGAL) is expressed in the distal tubular segments where it is part of bacterial defense mechanisms.

Cystatin-C (CyC) is a 13 kDa nonglycosylated cysteine protease inhibitor produced by all nucleated human cells in a constant rate unaffected by muscle mass. Plasma CyC (pCyC) is also excreted by glomerular filtration and has a similar catabolic pathway as pNGAL; therefore it is not normally found in urine in significant amounts. Some evidence suggests that pCyC is a superior functional biomarker compared to SCr especially to detect acute changes in estimated glomerular filtration rate (eGFR) [11, 12].

Several recent clinical studies have reported encouraging predictive properties of NGAL and CyC for AKI development [12-20]. Despite the reasonable individual biomarker performances it is recognized that perhaps biomarker combinations or combined models with clinical parameters might provide additional accuracy to successfully guide clinical decision making [16].

The purpose of this study was to evaluate the discriminative properties of pNGAL, uNGAL, pCyC and uCyC between various AKI disease states and the biomarkers' performance on the prediction of developing sustained AKI.

Materials and methods

Study population

Between September 1, 2007, and April 1, 2008, all consecutive admitted patients to the adult ICU of the Erasmus MC University Medical Center In Rotterdam, the Netherlands, (which treats general surgical, trauma, medical, neurological and neurosurgical patients, but not cardiac surgery patients) were considered for participation (n=700). Exclusion criteria were age under 18, re-admission during the study period (only the first admission records were included), refusal of informed consent, nephrectomy, chronic kidney disease (CKD stages 3, 4 and 5 based on eGFR criteria calculated with the baseline SCr values) and renal transplantation. Written informed consent was obtained from all participants or their legal representative with a deferred consent policy [21]. The study was approved by the institutional review board.

Study protocol

At ICU admission (T=0) and at 7 time points thereafter (T=4, 8, 24, 36, 48, 60, 72 hrs) plasma and urine sampling was performed using an intra-arterial line and a urine catheter for spot urine. The samples were immediately processed in the hospital's laboratory and the supernatants were stored at -80°C. NGAL and CyC concentrations were measured by Biosite Inc in San Diego using the Triage® immunoassay. SCr values were measured in the hospital's clinical chemical laboratory (using the enzymatic kit produced by Roche which is traceable to a reference method based on isotope dilution-mass spectrometry) at the time of admission and thereafter daily at 6:00 am. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study Equation (MDRD) [22] and baseline SCr was defined as the patient's steady state level four weeks prior to ICU admission. If not available, the admission value was used as the baseline. AKI was defined according to the RIFLE (Risk-Injury-Failure) classification [23]. The RIFLE classification is based on the rise in SCr compared to a baseline value. Risk represents a 1.5-2 times increase, Injury a 2-3 times increase and Failure a more than 3 times increase. Sustained AKI was defined as any AKI occurring and persisting for more than 24 hours after admission. Transient AKI was defined as reaching AKI only once with normalizing SCr levels within 24 hours. Other recorded variables included age, gender, body mass index (BMI), bicarbonate levels (HCO_3^-), blood urea nitrogen content (BUN), white blood cell count (WBC), Acute Physiology and

Chronic Health Evaluation score (APACHE II), sequential organ failure assessment score (SOFA), cumulative urine output (UP), initiation of RRT, patient discharge diagnosis, length of ICU stay, ICU mortality and hospital mortality.

NGAL measurements

The Triage® point-of-care immunoassay was used to determine plasma and urine NGAL concentrations. The detection limit for the pNGAL assay was 46 ng/ml to 1300 ng/ml and 2.6 ng/ml to 4100 ng/ml for uNGAL. Urine samples that had concentrations exceeding the assay's upper limit were diluted with an equal volume of 0.2 M BES buffer before application to the cartridge. The pNGAL assay on this platform has been shown to correlate strongly with other methods of pNGAL detection [24].

Cystatin-C measurements

The Triage® Test point-of-care immunoassay was used to measure CYC in both plasma and urine on a research based cartridge. Plasma samples were measured neat and calibrated with a cystatin C-spiked plasma pool. The urine assay was calibrated with a urine pool diluted 1:1 with a buffer (0.2M BES), which was necessary to dilute the urine samples for appropriate detection. The plasma CyC assay's performance was validated against the commercially available Dade method.

Data analysis

Patients were classified into three groups: no-AKI, transient AKI and sustained AKI. Demographics and clinical outcomes were summarized and compared among groups. For continuous variables, median and inter-quartile ranges (IQR) were reported and Kruskal-Wallis test was used to test for group differences. For categorical variables, frequencies and proportions were reported and Fisher's exact test was used to test for group differences. Considering the highly skewed values in some biomarker measurements, medians and IQRs were presented for biomarker levels measured at each time point to show the temporal trend of biomarker levels over time. At each time point, the medians were compared between groups (no AKI, transient AKI and sustained AKI) for all patients and between RIFLE categories for sustained AKI patients using the rank transformation approach. Tukey-Kramer multiple comparison adjustment was utilized to adjust for the three pairs of comparisons at each time point, and adjusted p-values were reported where multiple comparisons were involved.To compare the discrimination of sustained AKI vs. no-AKI and sustained AKI vs. transient AKI using the four biomarkers at admission to ICU, receiver operating characteristic (ROC) curves were generated and the areas under the curves (AUC) were compared to select the best performing biomarker. The performance of the combination uNGAL and the others was

also investigated to show if there was any improvement in the discrimination of AKI patients.

Multivariable logistic regression analyses were conducted to assess predictors of sustained AKI and the performance of the predictive models while combining biomarkers with clinical factors. The clinical factors considered included MDRD eGFR at admission, diagnosis of sepsis and the following laboratory measures measured at admission: HCO_3Ð, BUN, white blood cell count (WBC). A parsimonious clinical model was first determined based on stepwise backward elimination. Each of the four biomarkers was added individually to the parsimonious clinical model and the performance of the predictive models was evaluated using Akaike Information Criterion (AIC), Hosmer-Lemeshow test of goodness of fit, AUC, Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI). Statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC) and R package Hmisc (http://CRAN.R-project.org/package=Hmisc) was used to calculate NRI and IDI. A significance level of 0.05 was used for all analyses.

Role of the Funding Source

Biosite incorporated provided biomarker measurements. They had no role in study design, conduct, data-analysis and reporting. Besides biomarker measurements no additional funding was provided.

Results

Patients

Seven hundred patients were initially consented to be enrolled in the study. After adopting the exclusion criteria, 98 patients were excluded. Another 92 patients were excluded because of established AKI before or at ICU admission leaving 510 patients included in the analysis. Patients' baseline and clinical characteristics are presented in Table 1. Patients with sustained AKI had a significant higher baseline SCr, with correspondingly lower estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation (MDRD eGFR), lower serum bicarbonate levels ($HCO3^-$), higher blood urea nitrogen content (BUN), higher pNGAL, uNGAL and pCyC values at admission, higher APACHE II and SOFA scores, lower 24-hour urine production (UP), higher 24-hour cumulative fluid balance (FB-24) and significantly increased ICU and hospital mortality.

Table 1: Baseline characteristics and clinical outcome

	No-AKI	Transient AKI	Sustained AKI	P
	(N=444)	(N=19)	(N=47)	
Age (years)	57(43,68)	60 (45,69)	62(54,73)	0.07
Male, n(%)	250(56)	16(84)	30(64)	0.03
BMI (kg/m^2)	24.6(22.7,27.5)	24.1 (21.9, 27.1)	26.0 (23.9, 27.8)	0.07
SCr baseline (mg/dL)	0.74 (0.62, 0.90)	0.74 (0.68, 0.86)	0.90 (0.68, 1.02)	<0.0001
MDRD eGFR (mL/min)	100.0 (86.4, 100.0)	100.0 (92.4, 100.0)	70.4 (52.2, 93.6)	<0.0001
SCr (mg/dL)	0.75 (0.62, 0.89)	0.83 (0.72, 0.98)	1.01 (0.74, 1.20)	<0.0001
$HCO3^-$ (mmol/l)	22.0 (20.1, 24.1)	21.3 (19.9, 23.8)	19.7 (17.0, 21.8)	<0.0001
BUN (mmol/l)	5.4 (4.2, 7.0)	6.0 (4.2, 8.5)	7.2 (4.9, 9.4)	0.006
WBC (*10^9/ l)	11.2 (8.4, 14.9)	9.0 (5.5, 13.4)	10.6 (6.0, 12.8)	0.02
pNGAL (ng/ml)	148.8 (83.2, 223.0)	243.4 (154.4, 294.7)	286.3 (197.8, 412.0)	<0.0001
uNGAL (ng/ml)	73.1 (34.5, 190.8)	89.8 (52.5, 276.6)	389.8 (106.3, 1833.2)	<0.0001
pCyC (mg/L)	0.9 (0.8, 1.2)	1.1 (1.0, 1.3)	1.3 (1.0, 1.8)	<0.0001
uCyC (mg/L)	2.6 (2.6, 2.7)	2.6 (2.6, 3.5)	2.6 (2.6, 3.9)	0.05
APACHE II-24 score	16(13,22)	22(15,26)	23(17,28)	<0.0001
SOFA-24 score	4(2,6)	7.5(6.0,10.0)	9(7,12)	<0.0001
UP-24 (ml/kg/h)	1.1(0.8-1.7)	0.9(0.6-1.3)	0.8(0.5,1.3)	0.004
FB-24 (ml)	1949 (693, 3815)	4714 (1792, 5334)	4241 (1774, 6948)	<0.0001
RRT, n (%)	0(0)	0(0)	7(15)	<0.0001
ICU length of stay (days)	4(2,8)	6(4,17)	10(4,17)	<0.0001
ICU mortality, n (%)	44(10)	2(11)	14(30)	0.001
Hospital mortality, n (%)	61(14)	5(26)	18(38)	0.0001
Diagnostic group, n (%)				
Postoperative	163(37)	2(11)	8(17)	-

Table 1: (Continued)

	No-AKI	Transient AKI	Sustained AKI	P
	(N=444)	(N=19)	(N=47)	
Medical	82(18)	2(11)	7(15)	-
Neurological	92(21)	3(16)	4(9)	-
Neurotrauma	27(6)	2(11)	1(2)	-
Multitrauma	30(7)	4(21)	4(9)	-
LTX	19(4)	1(5)	7(15)	-
Sepsis	12(3)	1(5)	5(11)	-
CPR	9(2)	1(5)	4(9)	-
Hemorrhagic shock	9(2)	3(16)	1(2)	-
MOF	1(0)	0(0)	6(13)	-

BMI: Body Mass Index; SCr: serum creatinine; $HCO3^{-}$: plasma bicarbonate concentration; BUN: Blood Urea Nitrogen content; WBC: white blood cell count; MDRD eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation measured at ICU admission ; pNGAL: plasma neutrophil gelatinase associated lipocalin; uNGAL: urine NGAL; pCyC: plasma cystatin-C; uCyC: urine CyC; APACHE II-24: Acute Physiology and Chronic Health Evaluation score over first 24 hours; SOFA-24: sequential organ failure assessment score over first 24 hours; UP-24: urine production over first 24 hours; FB-24: fluid balance over first 24 hours; RRT: renal replacement therapy; LTX: liver tra--plantation; CPR: cardio pulmonary resuscitation and MOF: multi organ failure. Median (IQR) was reported for continuous variables. Frequency (proportion) was reported for categorical variables.

Biomarker performance for differentiation between no-AKI, transient AKI and sustained AKI

The temporal trend of each biomarker and SCr for patients grouped into no AKI, transient AKI and sustained AKI is shown in Fig.1A-E. All four biomarkers as well as SCr showed significant differentiation between sustained AKI and no AKI at all time points (all $p \leq 0.0002$) except for uCyC at the time of ICU admission ($p=0.06$) (Table 2). The timing of first significant differentiation between sustained AKI and transient AKI differed substantially by biomarker. Urine NGAL was the only biomarker to show significant differentiation of sustained from transient AKI at admission and at all time points thereafter (all $p \leq 0.02$); uCyC significantly differed at T=4 ($p=0.03$) and T=8 hours ($p=0.04$); pCyC first showed significant differences at 24 hours ($p=0.005$); pNGAL did not differ until 36 hours ($p=0.0002$) (Table 2). Differentiation between transient and no AKI was more variable, with pCyC differing only at 4 hours ($p=0.02$); pNGAL differentiating only at 4 and 8 hours ($p \leq 0.009$); SCr differing at 24, 48 and 72 hours ($p \leq 0.0008$) which is as expected by definition; and uNGAL and uCyC unable to distinguish no AKI from transient AKI at any time point at or after ICU admission (Table 2).

Biomarker performance for differentiation of AKI severity

Patients with sustained AKI were divided into three groups based on their RIFLE categories (R=Risk 1.5-2.0 times increase in baseline SCr, I=Injury 2.0-3.0 times and F=Failure more than 3 times) (n=47). The temporal trends of each biomarker and SCr are shown in Fig. 2A-E. Neither pCyC nor uCyC showed significant differentiation among the RIFLE categories at any time. Plasma NGAL did not significantly differentiate between F and I or between I and R; but started to show differentiation between F and R at 8 hours after ICU admission ($p=0.01$). The differentiation of RIFLE categories using uNGAL was similar to pNGAL. Urine NGAL only showed significant differentiation between F and R at 4 hours ($p=0.04$), 24 hours ($p=0.02$) and 72 hours ($p=0.049$), and between I and R at 24 hours ($p=0.03$). At ICU admission and 24 hours after ICU admission, SCr did not show significant differentiation among RIFLE categories, and only started to differentiate F vs. R at 48 hours ($p=0.0004$) and F vs. I at 72 hours ($p=0.03$) (Table 2).

Performance of biomarkers measured at admission in prediction of sustained AKI

To compare the performance of each individual biomarker on the prediction of AKI and to investigate the performance of combinations of biomarkers, subjects who had missing values in one or more biomarkers at the time of admission were excluded for ROC curve analysis. Four hundred and seventeen patients were included to investigate the discrimination of sustained AKI (RIFLE R and above for more then 24 hours,

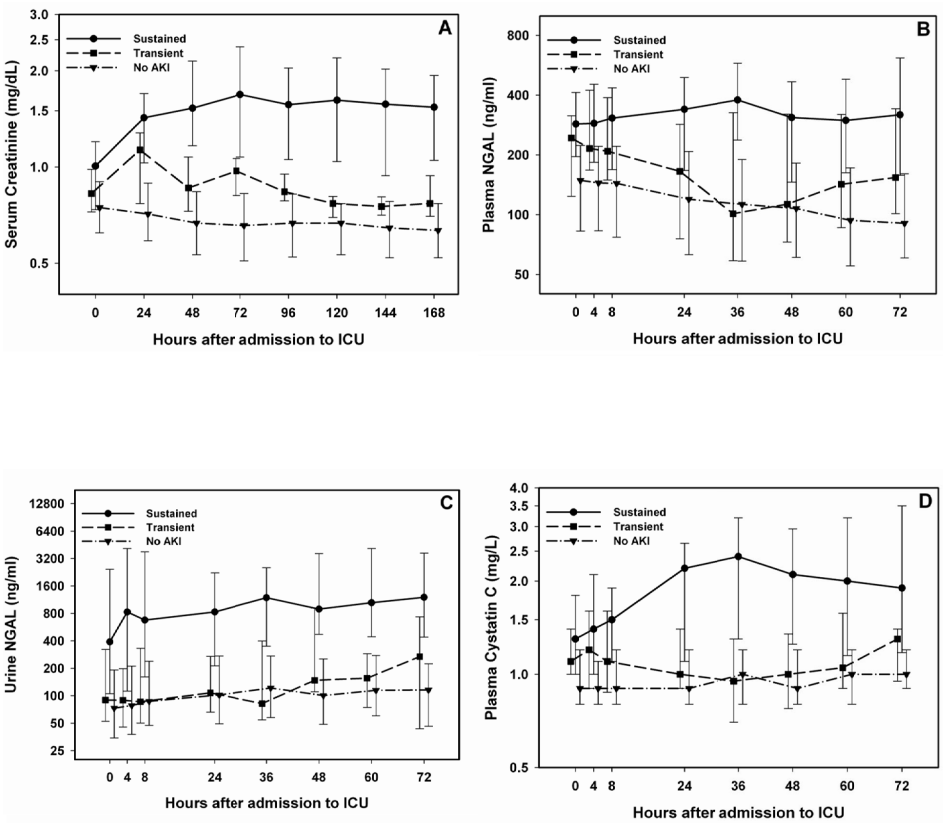


Figure 1: The changes of the individual biomarker concentrations over time after ICU admission summarized by Sustained, Transient and no AKI; for SCr (A) and the four biomarkers pNGAL (B), uNGAL (C), pCyC (D) and uCyC(E). Median and interquartile range (IQR) presented on a Log_{10} scale.

Table 2: Biomarker performance for distinguishing different disease states. --: non significant and hr: hours

		Sustained, Transient and No AKI			AKI RIFLE Categories		
Biomarker	Time since admission (hrs)	Sustained vs. No AKI	Sustained vs. Transient	Transient vs. No AKI	Failure vs. Injury	Failure vs. Risk	Injury vs. Risk
pNGAL	0	< 0.0001	--	--	--	--	--
	4	< 0.0001	--	0.001	--	--	--
	8	< 0.0001	--	0.009	--	0.01	--
	24	< 0.0001	--	--	--	0.02	--
	36	< 0.0001	0.0002	--	--	0.03	--
	48	< 0.0001	0.02	--	--	0.03	--
	60	< 0.0001	--	--	--	--	--
	72	< 0.0001	--	--	--	0.03	--
uNGAL	0	< 0.0001	0.02	--	--	--	--
	4	< 0.0001	0.01	--	--	0.04	--
	8	< 0.0001	0.002	--	--	--	--
	24	< 0.0001	0.004	--	--	0.02	0.03
	36	< 0.0001	< 0.0001	--	--	--	--
	48	< 0.0001	0.002	--	--	--	--
	60	< 0.0001	0.0008	--	--	--	--
	72	< 0.0001	0.01	--	--	0.049	--

Table 2: (Continued)

		Sustained, Transient and No AKI			AKI RIFLE Categories		
Biomarker	Time since admission (hrs)	Sustained vs. No AKI	Sustained vs. Transient	Transient vs. No AKI	Failure vs. Injury	Failure vs. Risk	Injury vs. Risk
pCyC	0	< 0.0001	--	--	--	--	--
	4	< 0.0001	--	0.02	--	--	--
	8	< 0.0001	--	--	--	--	--
	24	< 0.0001	0.005	--	--	--	--
	36	< 0.0001	< 0.0001	--	--	--	--
	48	< 0.0001	0.0001	--	--	--	--
	60	< 0.0001	0.01	--	--	--	--
	72	< 0.0001	--	--	--	--	--
uCyC	0	--	--	--	--	--	--
	4	< 0.0001	0.03	--	--	--	--
	8	< 0.0001	0.04	--	--	--	--
	24	< 0.0001	--	--	--	--	--
	36	0.0002	--	--	--	--	--
	48	< 0.0001	--	--	--	--	--
	60	< 0.0001	--	--	--	--	--
	72	< 0.0001	--	--	--	--	--

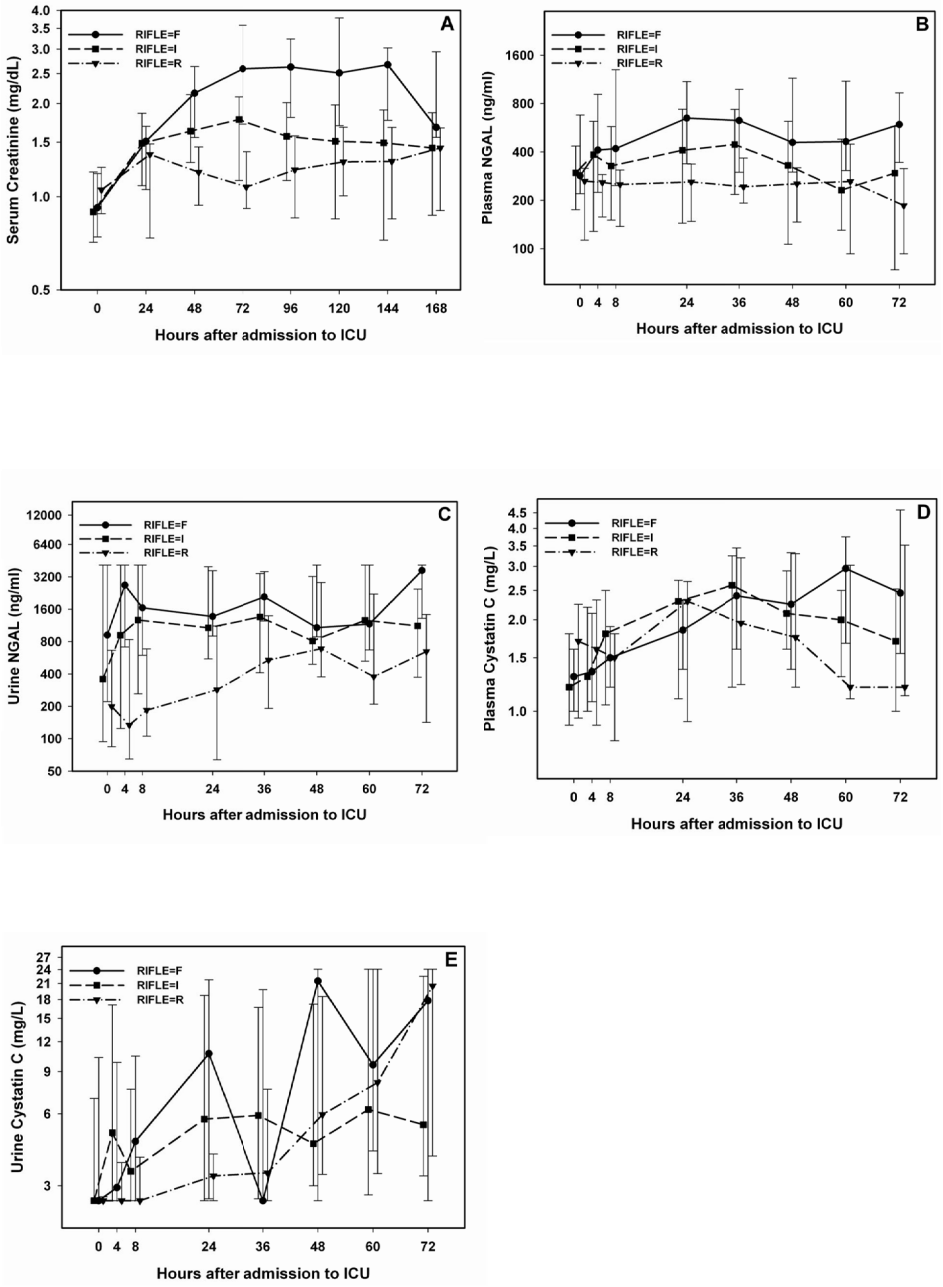
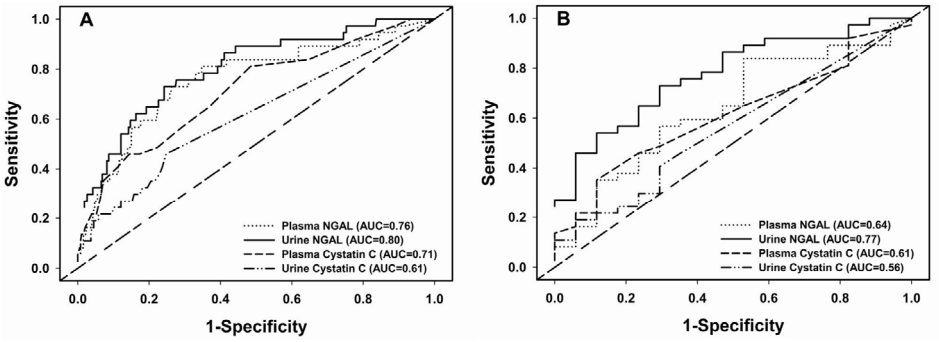


Figure 2: The changes of the individual biomarker concentrations over time after ICU admission summarized by RIFLE stages Risk (=R), Injury (=I) and Failure (=F); for SCr (A), and the four bioamarkers pNGAL (B), uNGAL (C), pCyC (D) and uCyC (E). Median and interquartile range (IQR) presented on a Log_{10} scale.



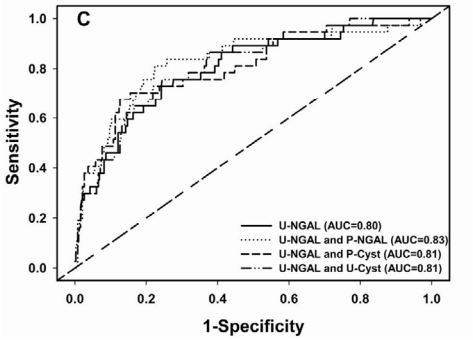


Figure 3 A: ROC curves and corresponding AUC's for the prediction of sustained AKI (n=37) vs. no-AKI (n=375) at ICU admission pNGAL, uNGAL, pCyC and uCyC. B: ROC curves and corresponding AUC's for the prediction of sustained (n=37) vs. transient AKI (17) at ICU admission pNGAL, uNGAL, pCyC and uCyC. C: ROC curves and corresponding AUC's for the prediction of Sustained (n=37) vs. no-AKI (n=375) at ICU admission for uNGAL in combination with SCr, pNGAL, pCyC and uCyC

Table 3: Comparison of the performance of the predictive models after adding the four biomarkers

Model	Biomarker P-value	AIC	H-L test P-value	AUC (95% CI)	NRI (P-value)	IDI (P-value)
Clinical model	---	197.2	0.01	0.79 (0.70-0.89)	---	---
Clinical + uNGAL	0.0008	188.7	0.21	0.82(0.73-0.91)	0.38 (0.04)	0.06 (0.04)
Clinical + pNGAL	0.052	195.5	0.01	0.81 (0.72-0.90)*	0.42 (0.02)	0.02 (0.28)
Clinical + uCyC	0.76	199.1	0.01	0.79 (0.70-0.89)	-0.25 (0.17)	0.0004 (0.88)
Clinical + pCyC	0.006	191.3	0.02	0.81 (0.72-0.90)	0.08 (0.67)	0.04 (0.09)

pNGAL: plasma neutrophil gelatinase associated lipocalin; uNGAL: urine NGAL; pCyC: plasma cystatin-C; uCyC: urine CyC. * $p<0.05$ for improvement in AUC vs. clinical model alone.

n=37) vs. no AKI (n=380) using different biomarkers. All studied biomarkers (pNGAL, uNGAL, pCyC and uCyC) were significant predictors of sustained AKI ($p< 0.005$). Urine NGAL showed the best discrimination of sustained AKI vs. no-AKI with an AUC of 0.80 (95%CI=0.72-0.88) compared to pNGAL (AUC=0.76, 95% CI=0.67-0.85), pCyC (AUC=0.71, 95%CI=0.62-0.80) and uCyC (AUC=0.61, 95%CI=0.52-0.70) (Fig. 3A). There was no significant difference in AUC between uNGAL and pNGAL, or between uNGAL and pCyC. But uNGAL had significantly higher AUC compared to uCyC ($p< 0.0001$). As to the discrimination of sustained AKI (n=37) vs. transient AKI (n=17), uNGAL was the only marker which showed significance with an AUC of 0.77 (95% CI =0.64-0.90) (Fig. 3B).

Biomarker combinations for prediction of sustained AKI measured at admission

Combining uNGAL with one of the other three biomarkers did improve the AUC's for the discrimination of sustained vs no-AKI; however the improvements were not statistically significant. The best combination was uNGAL and pNGAL, improving the AUC to 0.83 (95% CI = 0.75-0.91) from 0.80 with uNGAL alone (Fig. 3C). There is no improvement in combining biomarkers for the discrimination between sustained and transient AKI.

Multivariable logistic regression analysis for prediction of sustained AKI

To have a fair comparison between the biomarkers patients with missing values in any of the five clinical variables (MDRD eGFR, $HCO3^-$, BUN, WBC, Sepsis) and the four biomarkers at the time of ICU admission were excluded from the multivariable logistic regression analysis. This resulted in a total of 389 patients (33 sustained AKI vs 356 others). After a stepwise backward elimination, MDRD eGFR, HCO_3^- and diagnosis of sepsis remained in the parsimonious clinical model as independent predictors of sustained AKI ($p=0.002$, $p=0.045$ and $p=0.0001$ respectively). The additional contribution of biomarkers to the prediction of sustained AKI was evaluated by adding each biomarker individually to the parsimonious clinical model (Table 3). Urinary NGAL and pCyC showed to be significant predictors of sustained AKI even with the existence of the three clinical factors. Plasma NGAL was marginally significant ($p=0.052$) and uCyC was not significant ($p=0.76$) after they were added to the parsimonious clinical model. The model with uNGAL showed the best goodness of fit based on the comparison of AIC (>7 decrease in the magnitude of AIC from the clinical model) and Hosmer-Lemeshow test ($p=0.21$). There was minor improvement in AUC (0.018 – 0.024) when combining a biomarker with the parsimonious clinical model, but the improvement was not statistically significant except for pNGAL ($p=0.03$). Additional evaluation of the performance of the combined models was conducted by calculating NRI and IDI for each combined model vs the parsimonious clinical model. Since no standard scale is available for the choice of classification risk

levels, NRI was calculated based on the increase or decrease of predicted individual probability of developing sustained AKI. Significance was shown in NRI for models containing uNGAL (NRI=0.38, p=0.04) or pNGAL (NRI=0.42, p=0.02). Another index for the evaluation of the performance of a predictive model, IDI, showed significance improvement for the model containing uNGAL (IDI=0.06, p=0.04). Considering the components of IDI, the combined model with uNGAL showed significant improvement in classification specificity (p= 0.04).

Discussion

This study is the first to our knowledge to demonstrate the utility of urinary NGAL as a biomarker that could be used upon admission to the ICU to distinguish critically ill adults who are likely to develop sustained versus transient AKI from those at minimal risk of AKI. Such an early clinical determination may allow for the alteration of clinical care toward prevention and early treatment of AKI, prior to the rise in serum creatinine typically used to diagnose AKI. However, this study also suggests that NGAL and cystatin-C, either alone or in combination, are not able to accurately or persistently discriminate AKI RIFLE severity among critically ill patients with developing AKI. Furthermore, although based upon small event sample size the results suggest modest additional value of the markers when added to a model with clinical variables. These negative findings are also important, as they suggest important limitations of these biomarkers in this critical care setting.

The cellular and molecular aspects of ischemic and septic AKI are currently being increasingly unraveled. Major progress has been made in the knowledge of the underlying pathophysiological mechanisms and possible targets for therapeutic interventions. However the translation of these findings to clinical patient related practice has been disappointing. Different pathophysiological stages of AKI have been identified, including a prerenal, initiation, extension and maintenance phase. Therapy must be considered in the context of these different stages, because applying them in the maintenance phase has proven to be unsuccessful. Therefore early recognition and discrimination of the first stages of AKI, not yet reflected by functional impairment, is very important [5, 25]. The presented data show to our knowledge for the first time that that urine NGAL is able to provide information on the discrimination between sustained and transient AKI in the early clinical course of AKI development, upon admission to the ICU. Such information may prove useful in the early initiation of several emerging strategies, such as innate immune system modulators, growth factors, regulatory T-cells, cytoprotective agents such as activated protein- C, statins and erythropoietin. In addition, there are issues we would potentially address differently in current practice in patients destined for

sustained AKI if we could discriminate them from patients with self limiting renal injury. For example, we would attempt a more intense regime of "golden hour resuscitation" with intensive hemodynamic monitoring of macro- and micro circulation, we would avoid nephrotoxic agents and most importantly we would attempt more timely initiation of RRT to at least control cumulative fluid balances and toxic uremic conditions that are clearly correlated with survival and ventilator free days [26-31].

Several studies report promising test results for NGAL (AUC's ranging from 0.71-0.92) [13-16, 32] and CyC (AUC's ranging from 0.50- 0.78) [18, 19, 33-35] for AKI prediction in adult critically ill patients. However, the AUC results are not very consistent and highly depended on the selected patient cohort (general ICU patients vs. cardio pulmonary bypass surgery patients vs. septic patients etc), and on the inclusion or exclusion of pre-existing CKD or pre-existing AKI. Therefore newer trends in biomarker research have been directed towards use of biomarker models that include clinical parameters to improve the test accuracy for early detection of AKI in well defined clean cohorts [36-38].

The current study excluded patients with known CKD or AKI, improving the homogeneity of our cohort and allowing for an assessment of incident AKI only. The current study also describes the individual and combined performance of four different biomarkers (pNGAL, uNGAL, pCyC and uCyC) allowing for their direct comparison. In this analysis the prediction of sustained AKI was not significantly different when considering the ROC AUC's between pNGAL, uNGAL, pCyC and uCyC. This may be partly explained by the reduction in sample size using patients with developing AKI only. Adding the individual biomarkers to a model with clinical characteristics the improvement of the combined model with uNGAL, pNGAL and pCyC was comparable and therefore any potential superiority of a biomarker could not be demonstrated, except for uCyC which was not a significant predictor in the multivariable model.

Several issues have to be considered concerning potential limitations of this current work: First, defining a patient's baseline SCr is a problem in emergency medicine. Not uncommonly these variables are not available due to the absence of a patient's medical history in the hospital charts. Using the first available SCr value, such as we did, might therefore be a reason for some underestimation of AKI severity and might contribute to biomarker test non-specificity. However, the procedures we employed in this study are consistent with the clinical determination of AKI. Furthermore, these described results have to be considered in a research based setting and may not reflect entirely common clinical practice. For the purpose of the analysis this cohort was reduced to patients with developing AKI only and thus patients having reached a 50% or more increase in SCr before or at admission were excluded. This is only possible with the availability of the historical SCr baseline values. It is very common however that in clinical practice the information on a patient's true baseline SCr value is not immediately available and

thus the clinical applicability of the differentiation between transient and sustained AKI remains to be proven as having additional value.

Second, we have to take into account that new biomarkers such as NGAL (injury marker) and CyC (functional marker) are tested against a gold standard that has huge imperfections on its own. Therefore we should keep in mind that the lack of a biomarker's sensitivity and specificity in the present approach may actually reflect SCr's imperfections [6]. Third, the biomarker urine values are not corrected for urine creatinine concentrations; however this is defendable since there is evidence that it is not likely to have a considerable impact on overall test performance [39]. Fourth, in this study all patients with chronic kidney disease (CKD) were excluded making the results possibly less applicable to the general adult intensive care population where we observe increasing numbers of patients entering the ICU with CKD. Finally, plasma and urine NGAL were the only inducible damage markers used in this panel. The additional contribution of uCyC in this model was disappointing. Our findings are in contrast with the conclusions of the work of Koyner et al. [34] and several others stating that CyC may be an improved estimator of GFR particularly in patients with very subtle changes in kidney function and injury [18, 19, 38, 40]. Recent data demonstrate that in the absence of CKD, serum and urine CyC may be associated with inflammatory biomarkers in an elderly ambulatory population and sepsis in general ICU patients [41, 42]. A possible hypothesis is that CyC itself may be a regulator of inflammation, which might be of influence in adult critically ill patients being known for their pro-inflammatory state. Accordingly, this population receives frequent corticosteroid treatment in subgroups such as sepsis and liver transplant patients and suffers from thyroid dysfunction syndromes. Using other particular damage markers such as Kidney Injury Molecule-1 (KIM-1) [43], Interleukin-18 (IL-18) [44], Netrin-1 [45, 46] or liver fatty acid binding proteins (L-FABP) [47] might potentially have a significant additional predictive contribution to the biomarker panel model, which should be a subject of exploration in the near future.

In conclusion, we showed that urine NGAL accurately discriminates adult patients likely to develop sustained AKI from transient AKI at the time of ICU admission. In addition, we showed that NGAL and cystatin-C, either alone or in combination, are not able to accurately or persistently discriminate AKI RIFLE severity among critically ill patients with developing AKI. However, adding uNGAL, pNGAL and pCyC to a model with clinical characteristics slightly improved the discrimination of the predictive model. And especially, adding uNGAL to the clinical model significantly improved the performance of the prediction in terms of both NRI and IDI.

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Chapter 4

Plasma neutrophil gelatinase associated lipocalin similarly predicts acute kidney injury in sepsis and non-sepsis

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Abstract

Plasma neutrophil gelatinase-associated lipocalin (NGAL) is released in sepsis irrespective of acute kidney injury (AKI). We investigated the effect of sepsis on its diagnostic value for AKI. In 700 ICU admissions, at 4 time-points (<24 hours) following admission, NGAL was measured. Six-hundred-sixty-three admissions were included in the final analysis, of which 80 patients had sepsis (12%). AKI occurred in 22% of the patients without and 66% with sepsis. NGAL levels were higher in no-AKI patients with sepsis compared to no-AKI patients without sepsis at all time points (p=0.03 or lower). In patients with AKI a similar difference was observed (p<0.001). The area under the curve (AUC) for AKI was unaffected by the presence of sepsis (0.76 in sepsis vs. 0.78 in non-sepsis [p=0.72]), but the optimal test cut-off values were higher in the former.

Introduction

Acute kidney injury (AKI) is common in adult hospitalized patients and is associated with high morbidity and mortality (up to 50%) [1-3]. Biomarker-guided risk stratification for occurrence of AKI might allow timely provision of supportive and interventional care which in turn might influence outcome. Several biomarkers have been proposed as potential candidates such as neutrophil gelatinase-associated lipocalin (NGAL).

AKI is defined using a relative increase of serum creatinine (SCr) compared to a predefined steady state baseline value [4]. However, in clinical practice the baseline value is difficult to obtain or simply not available. In clinical studies, therefore, the baseline SCr values are frequently estimated using age, gender and bodyweight or the first available SCr value is applied as a surrogate "steady sate" baseline value. The applied definition of baseline SCr value evidently determines the occurrence of AKI, especially in the milder severity stages, and therefore might change the value of biomarkers to diagnose AKI.

NGAL is an early predictor for AKI in specific populations such as pediatric patients after cardiopulmonary bypass surgery, in which onset of AKI can be specified [5]. Sepsis is prevalent in critical illness and the leading cause of AKI [3], and septic AKI in particular is characterized by distinct pathophysiology [6]. Plasma NGAL also differentiates between bacterial and viral infections irrespective of the presence of AKI [7-10]. Therefore, the distinction between septic and non-septic AKI in a heterogeneous patient cohort may have clinical relevance for the biomarker test characteristics.

Two studies describe the behavior of plasma NGAL in patients with sepsis and show that sepsis elevates NGAL levels in plasma, but it is still unclear what the precise impact is on the diagnostic test characteristics [11, 12]. To clarify this issue and to asses the effect of different SCr baseline values we evaluated the role of sepsis on the diagnostic test properties for AKI of plasma NGAL in a large cohort of adult critically ill patients. The hypothesis is that sepsis alters optimal cut off values but not the diagnostic value of NGAL for AKI, as compared to non-sepsis.

Methods

Design and setting

This is a prospective single center cohort study in a 30-bed closed format university hospital intensive care unit (ICU) on which general surgical, trauma, medical, neurological and neurosurgical patients are treated (no cardiac surgery). All consecutively admitted adult critically ill patients between September 1 2007 and April 1 2008 were considered eligible. Exclusion criteria were age under 18, refusal of informed consent, a history

Table 1: Patient characteristics and clinical outcome.

	Non-sepsis (N=583)			Sepsis (N=80)		
	No-AKI (N=455,78%)	AKI (N=128,22%)	P	No-AKI (N=27, 34%)	AKI (N=53, 66%)	P
Age, yr	57(25)	61(24)	0.04	53(26)	60(21)	0.03
Male, n(%)	261(57)	88(69)	0.02	10(37)	28(53)	0.18
APACHE II	16(9)	23(11)	<0.001	20(9)	24(10)	0.03
Non-renal SOFA	4(4)	8(6)	<0.001	7(3)	8(6)	0.46
-Diagnostic group, n(%)-						
Medical	117(26)	70(55)	<0.001	20(74)	49(92)	0.04
Surgical	218(48)	42(33)		5(19)	4(8)	
Neurological	119(26)	15(12)		2(7)	0	
-Renal characteristics-						
Baseline SCr, µmol/l	65(23)	80(26)	<0.001	60(30)	62(30)	0.05
UP, ml/kg/h	1.1(1)	0.8(1)	<0.001	1.2(1)	0.8(1)	0.002
FB, l/day	1.9(3)	3.4(4)	<0.001	3.1(2)	3.7(4)	0.36
AKI stage 1	-	67(12)	-	-	10(13)	-
AKI stage 2	-	32(6)	-	-	12(15)	-
AKI stage 3	-	29(5)	-	-	31(39)	-
-Inflammation parameters –						
WBC, 10^9/l	11.1(6)	10.9(7)	0.44	10.9(12)	14.9(12)	0.05
CRP, mg/l	77(109)	113(133)	0.001	218(137)	225(125)	0.97
-Outcome –						
CVVH, n(%)	0	14(11)	-	0	15(28)	-
Mortality, n(%)	65(14)	44(34)	<0.001	7(26)	29(55)	0.01

AKI is classified applying a baseline serum creatinine value until 6 months prior to ICU admission.Values are presented as median with interquartile range or absolute numbers and percentages. SOFA-24: sequential organ failure assessment score over first 24 hours; APACHE II-24: Acute Physiology and Chronic Health Evaluation score over first 24 hours; SCr: serum creatinine; UP: urine production over first 24 hours per kg ideal bodyweight; FB: cumulative fluid balance in first 24 hours; AKI: acute kidney injury network criteria; WBC: white blood cell count; CRP: C-reactive protein and CVVH: continuous veno venous hemofiltration. Values are presented as median with interquartile range or absolute numbers and percentages.

of nephrectomy, documented chronic kidney disease (CKD) (> stage 3) and renal transplantation. The study was approved by the institutional review board of the Erasmus MC University Medical Center in Rotterdam, the Netherlands. Deferred consent was used with written informed consent obtained from the participants or their heath care proxy within 48 hours after ICU admission [13]. In the case of consent refusal (n=6 0.9%) the collected plasma specimens were appropriately destroyed. The current study is an extended analysis of one previously reported [14], now focusing on the effects of sepsis on biomarker behavior. Demographic data were recorded including severity of illness scores, renal characteristics and outcome parameters (Table 1).

Sample collection

At ICU admission (T=0) and at 4 time points thereafter (T=4 8 24), plasma samples were collected using an intra-arterial catheter. The samples were processed in the hospital's laboratory and the supernatants stored at -80°C. NGAL concentrations were measured after study closure by Biosite Inc (Alere), San Diego, USA using the Triage® immunoassay. The detection limit for the plasma assay was 46 ng/ml - 1300 ng/ml. The assays coefficient of variation was 15.8%. SCr values were measured in the hospital's clinical chemical laboratory at time of admission and daily thereafter at 6:00 am. SCr values were measured with the Roche enzymatic kit which is traceable to a reference method based on isotope dilution mass spectrometry.

Definitions

Two definitions of baseline SCr were applied. First, the steady state level at four weeks to six months prior to ICU admission, when available, and second, the first available SCr 24 hours prior to ICU admission or on admission (available in the electronic supplementary material, ESM). AKI was defined using the acute kidney injury network (AKI) classification for changes in SCr values (AKI 0= no-AKI AKI 1= SCr increase >50% or an absolute SCr rise of 0.3 mg/dL [=26.5 µmol/L], AKI 2= SCr increase > 100% and AKI 3= SCr increase >200% compared to baseline) [4]. Sepsis on admission was defined according to ACCP/SCCM consensus criteria. Patients with a proven or suspected infection, two or more systemic inflammatory response syndromes (SIRS) criteria and an infection-induced organ dysfunction were classified as having severe sepsis. The admission and peak biomarker levels were used for diagnosis of AKI. The primary study endpoint was AKI (AKI stage 1 or more) within 48 hours after ICU admission. The Acute Physiology And Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) score (without renal parameters) were calculated from documented physiological and chronic disease variables. Other collected parameters were demographic data (age, gender, diagnosis), renal parameters (urine production (UP), cumulative fluid balance (FB), application of continuous venovenous

renal replacement therapy (CVVH)), inflammatory parameters (white blood cell count (WBC), C-reactive protein (CRP), sepsis source and cultured micro organisms) and in hospital mortality.

Statistical analysis

Patients were grouped according to the presence of sepsis. Most data were non-normally distributed and reported as number (percentage) and median (interquartile range), where appropriate. Univariate analysis for continuous variables was with Mann-Whitney U test and for categorical variables with the χ^2 test or Fisher's exact test. Receiver operating characteristic curves (ROC) were generated for the occurrence of AKI using the admission and peak biomarker level within first 24 hours after admission. The area under the curve (AUC) was expressed with the 95% confidence interval (95% CI) and the optimal cut off level (Youden index) was calculated with corresponding sensitivity and specificity. The significance level (P-value) was two tailed and 0.05 was used for all analyses. Exact P values are given unless <0.001. Statistical analyses were performed with the statistical software package SPSS version 20.0 (IBM SPSS Chicago IL USA) for windows and MedCalc for Windows version 9.5.0.0 (MedCalc Software Mariakerke Belgium).

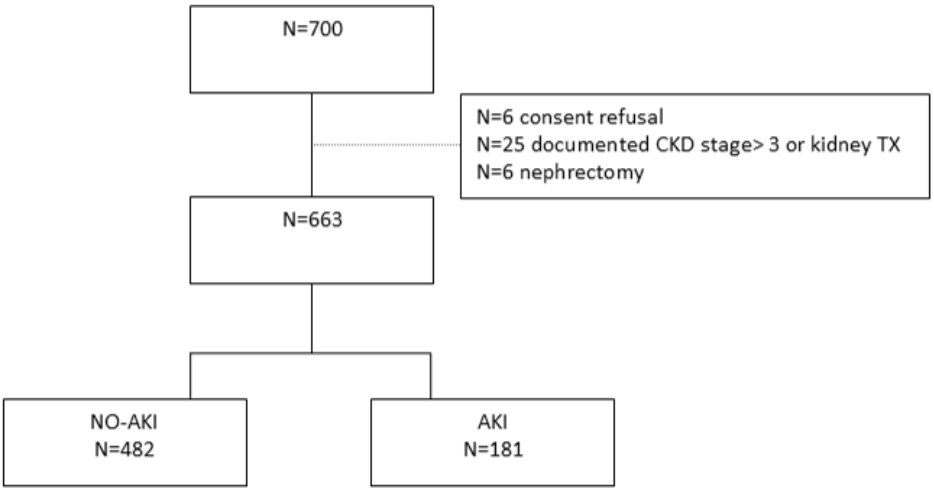

Figure 1: Patient cohort. AKI is classified applying a baseline serum creatinine value until 6 months prior to ICU admission. CKD: chronic kidney disease; AKI: acute kidney injury; TX: transplantation

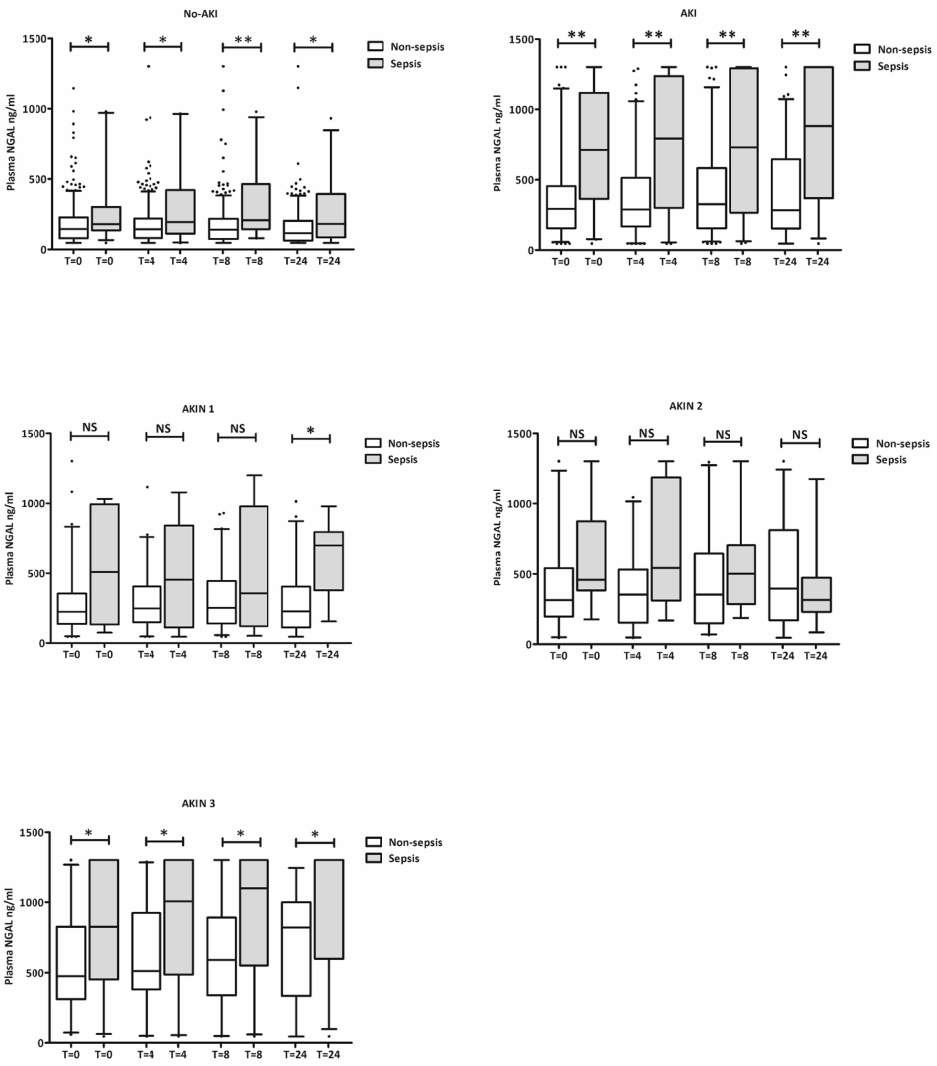

Figure 2: Plasma NGAL concentrations in patients stratified for sepsis and (severity of) AKI. AKI is classified applying a baseline serum creatinine value until 6 months prior to ICU admission. NGAL: neutrophil gelatinase-associated lipocalin; AKI: acute kidney injury. Groups are compared using Mann-Whitney-U-test: * P<0.05, ** P<0.001 and NS= non-significant.

Table 2: Median biomarker values (inter quartile range, IQR) compared between (septic) patients with and without acute kidney injury.

Time	Plasma NGAL (ng/ml)(IQR)	No-AKI	AKI	P
T=0	Non-sepsis	146(148)	295(297)	<0.001
	Sepsis	180(168)	713(755)	<0.001
T=4	Non-sepsis	144(139)	290(345)	<0.001
	Sepsis	195(309)	792(755)	<0.001
T=8	Non-sepsis	141(141)	326(430)	<0.001
	Sepsis	208(320)	731(1028)	<0.001
T=24	Non-sepsis	115(141)	285(493)	<0.001
	Sepsis	181(307)	883(933)	<0.001

AKI is classified applying a baseline serum creatinine value recorded until 6 months prior to ICU admission. Groups are compared using Mann-Whitney-U-test: * P<0.05, ** P<0.001 and NS= non-significant.

Table 3: Receiver operating characteristics curve analysis for the diagnosis of AKI stratified by sepsis using admission (A) and peak (B) plasma NGAL values

Plasma NGAL (ng/ml)	AUC (95% CI)	P	Cut-off	Sens	spec
Non-sepsis (N=542, N=115 AKI)	0.74(0.70-0.78)	<0.0001	266	59	83
Sepsis (N=75, N=50 AKI)	0.80(0.69-0.88)	<0.0001	304	80	80
Plasma NGAL (ng/ml)	AUC (95% CI)	P	Cut-off	Sens	spec
Non-sepsis (N=582, N=127 AKI)	0.76(0.72-0.79)	<0.0001	338	58	84
Sepsis (N=80, N=53 AKI)	0.78(0.67-0.86)	<0.0001	979	51	100

AKI is classified applying a baseline serum creatinine value until 6 months prior to ICU admission.
SCr: serum creatine, NGAL: plasma Neutrophil Gelatinase-associated Lipocalin AUC: area under receiver operating curve; CI: confidence interval sens: sensitivity and spec: specificity. P=0.63 for difference in AUC between non-sepsis and sepsis patients.

Results

Patients

The initial cohort comprised of 700 consecutive admissions. Six patients refused permission for participation (0.9%), 6 patients had undergone a nephrectomy (0.9%) and 25 patients had chronic kidney disease (CKD) stage 3 or more or with a kidney transplant (4%) leaving 663 admissions for the overall analysis. All readmissions during the study period were used in the analysis (N=56). When applying the first definition of AKI, using the baseline SCr value 4 weeks to 6 months prior to admission, 39 cases (6%) lacked a steady state baseline SCr of whom 2 developed AKI. Baseline clinical characteristics are depicted in Table 1. One hundred eighty one (27%) patients had AKI (=AKI stage 1 2 or 3) within the first 48 hours of admission. In patients without sepsis (N=583), 128 had AKI (22%) and in patients with sepsis 53 out of 80 (66%) (Figure 1). Non-septic patients with AKI were more severely ill according to the APACHE II and non-renal SOFA scores, had a higher baseline SCr, a lower averaged urine production and a higher cumulative fluid balance during the first 24 hours of admission compared to non-septic patients without AKI. In septic patients the SOFA score was comparable between patients with and without AKI. The source of sepsis and the cultured micro–organisms is described in ESM-1.

Plasma NGAL concentrations in patients with and without sepsis.

Median peak plasma NGAL concentrations were higher at all time points in patients with sepsis compared to patients without sepsis irrespective of the presence of AKI ($P=0.03$ in non-AKI and $P<0.001$ in AKI) (Figure 2). This trend persisted when AKI patients were sub classified into the different severity stages (AKI 1, AKI 2 and AKI 3) (Figure 2). Median peak NGAL concentrations were different at all time points after ICU admission between patients with and without AKI, both in septic and non-septic patients ($P<0.001$) (Table 2).

Diagnosis of AKI

ROC curve analysis was performed for admission and peak plasma NGAL levels in patients with and without sepsis. The area under de curve (AUC) for peak values was unaffected by the presence of sepsis (0.78 [0.67-0.86] for sepsis [$P=0.72$] and AUC=0.76 [0.72-0.79] for non-sepsis) ($P=0.72$). Similar AUC's were obtained with admission NGAL values. The optimal cut-off level of NGAL was higher in sepsis than in non-sepsis (979 vs 338 ng/ml) (Table 3A and B).

Alteration of baseline SCr definition

The above described analysis was also performed applying the alternate baseline SCr value (SCr available at ICU admission or 24 hours prior to admission) (ESM 2-7). Less patients classified as having AKI (N=120 patients instead of N=181) (ESM-2). Plasma NGAL concentrations were similarly higher in septic and non-septic patients with and without AKI (P<0.001) (ESM-4 and 6). Furthermore, the ROC AUC's for diagnosis of AKI with admission and peak NGAL levels in septic and non-septic patients were similar to those generated with peak NGAL levels (ESM-5).

Discussion

The present study confirms that sepsis enhances the production of plasma NGAL in adult critically ill patients irrespective of the presence of AKI. However, the diagnostic test accuracy for AKI is unaffected by sepsis, but optimal cut-off values are elevated.

Two other studies report on the possible confounding role of sepsis on NGAL as a diagnostic tool for AKI. Martensson et al. found higher plasma NGAL levels with increasing severity of sepsis but found no differences between AKI and non-AKI patients (p=0.06). Our analysis resulted in the opposite: plasma NGAL levels were higher in non-AKI patients with sepsis compared to non-sepsis patients without AKI and this pattern persisted in patients with (increasing severity of) AKI. A possible explanation might be the more severe AKI in our patients as compared to those of Martensson et al. [12].

Bagshaw et al. described patients with AKI with and without sepsis. Higher NGAL levels were detected in case of septic AKI (381 [253-585] ng/ml vs. 176 [92-269]). Remarkably, the NGAL levels are lower than in our cohort which again might be explained by a greater severity of illness in our patients [11].

One of the concerns in AKI related biomarker research is the primary study endpoint used: the change of serum creatinine over a 48 hour timeframe. The disability of SCr to detect minimal tubular damage is well recognized which generated the quest for earlier and more specific signals in plasma or urine, including NGAL. The determination of a baseline SCr used for the classification of AKI impacts on the study endpoint. Therefore we chose to apply two definitions of the baseline SCr level in order to address this matter. Our results show that the application of a baseline SCr closely prior to or at ICU admission reduces the number of cases in the less severe stage of AKI (AKI 1) without having an affect on the AUC of plasma NGAL for AKI diagnosis.

A possible limitation of this study is that patients with sepsis had more severe AKI compared to patients with non-septic related AKI. The higher biomarker levels in patients with sepsis than non-sepsis might therefore partly be explained by this

difference. However, stratification into the different AKI stages showed a consistent difference in marker values between sepsis and non-sepsis, especially in patients with severe AKI (stage 3).

Conclusion

The diagnostic accuracy of plasma NGAL for AKI is not affected by the presence of sepsis. Absolute biomarker values are increased in case of sepsis irrespective of AKI which, as a consequence, increases optimal cut-off values in these patients. Varying baseline SCr to define AKI does not impact on NGAL test accuracy.

Future perspective

Our findings suggest that levels of plasma NGAL differ dependent upon the underlying etiology of AKI. This should be taken into account when NGAL is used into diagnostic models or in clinical decision making.

Acknowledgements

We thank the clinical and laboratory staff of the Erasmus University Medical Center Rotterdam the Netherlands. We thank Wil Mol our nurse coordinator for her contribution in patient inclusion and study logistics as well as the patients and their families for their participation. We thank Gillian Parker from Alere (former Biosite San Diego CA USA) for the provided NGAL measurements

Statement of competing interests

Hilde de Geus has received speaker fees from Alere.

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Online data supplement

Plasma neutrophil gelatinase associated lipocalin similarly predicts acute kidney injury in sepsis and non-sepsis

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Biomarkers Med. 2013 June: 7(3):1-7

Electronic Supplementary Material (ESM)-1

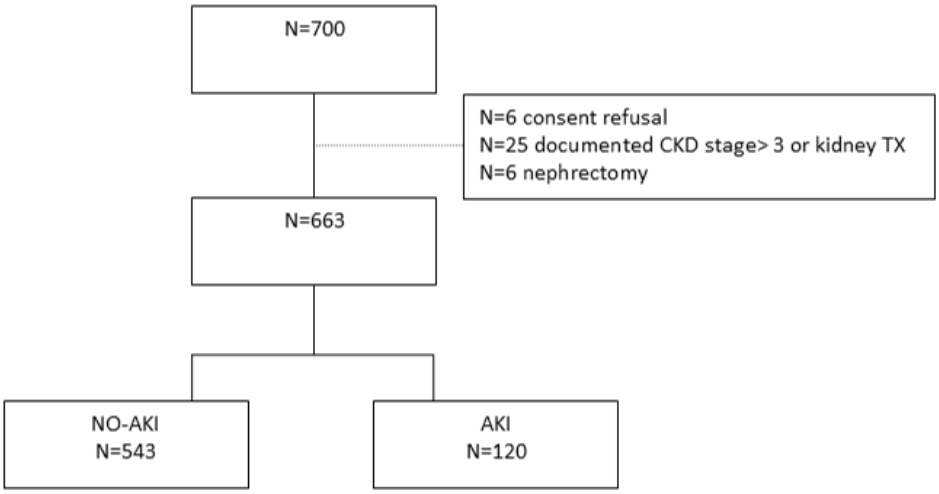
Source of sepsis and cultured micro-organism

ESM-1: Source of sepsis and cultured micro-organism

Sepsis source, n (%)	Gram negative, n (%)	Gram positive, (%)	Mixted, n (%)	Fungi, n (%)	Culture negative, n (%)	Viral, n (%)
Intra-abdominal 40(50)	16(40)	5(12.5)	12(30)	3(7.5)	4(10)	-
Respiratory 14(18)	3(21)	1(7)	3(21)	1(7)	5(36)	1(7)
Immuno-suppressed 6(7)	2(33)	2(33)	-	-	2(33)	-
Mediastinitis 6(7)	2(33)	1(17)	2(33)	-	1(17)	-
Urological 6(7)	3(50)	-	2(33)	-	1(17)	-
Skin and soft tissue 5(6)	2(40)	3(60)	-	-	-	-
CVC related 1(1)	-	-	1(100)	-	-	-
Central nervous system 2(3)	-	-	1(50)	1(50)	-	-

CVC: central venous catheter

Electronic Supplementary Material (ESM)-2



ESM-2: Patient cohort with baseline SCr 24 hrs prior to or at ICU admission

ESM-3: Patient characteristics, using baseline SCr 24 hrs prior to or at ICU admission

	Non-sepsis (N=583)			Sepsis (N=80)		
	No-AKI (N=502, 86%)	AKI N=81, 14%)	P	No-AKI (N=41, 51%)	AKI (N=39, 49%)	P
Age, yr	58(24)	62(24)	0.02	56(24)	62(22)	0.13
Male, n(%)	298(59)	51(63)	0.54	20(49)	18(46)	0.81
APACHE II score	16(10)	24(10)	<0.001	21(9)	25(10)	0.01
Non-renal SOFA score	4(4)	8(6)	<0.001	6.5(4)	8.5(6)	0.03
Diagnostic group, n(%)						
Medical	142(28)	45(56)	<0.001	32(78)	37(95)	0.08
Surgical	236(47)	24(30)		7(17)	2(5)	
Neurological	122(24)	12(15)		2(5)	0	
Renal characteristics						
Baseline SCr, µmol/l	68(28)	92(65)	<0.001	90(92)	116(130)	0.10
UP, ml/kg/h	1.1(1)	0.8(1)	<0.001	1.1(1)	0.6(1)	<0.001
FB, l/day	1.9(3)	3.6(4)	<0.001	2.8(4)	3.7(5)	0.36
AKI stage 1	-	38(8)	-	-	11(14)	-
AKI stage 2	-	16(3)	-	-	7(9)	-
AKI stage 3	-	17(3)	-	-	21(26)	-
Inflammation parameters						
WBC, 10^9/l	11.1(6)	11.1(7)	0.52	11.5(11)	15.3(11)	0.53
CRP, mg/l	79(108)	101(171)	0.03	225(128)	223(132)	0.89
Outcome						
CVVH, n (%)	0	14(17)	-	0	15(39)	-
Mortality, n (%)	75(15)	34(42)	<0.001	12(29)	24(62)	0.004

SCr: serum creatine, SOFA-24: sequential organ failure assessment score over first 24 hours; APACHE II-24: Acute Physiology and Chronic Health Evaluation score over first 24 hours; SCr: serum creatinine; UP: urine production over first 24 hours per kg ideal bodyweight; FB: cumulative fluid balance in first 24 hours; AKI: acute kidney injury network criteria; WBC: white blood cell count; CRP: C-reactive protein and CVVH: continuous veno venous hemofiltration. Values are presented as median with interquartile range or absolute numbers and percentages, where appropriate.

Electronic Supplementary Material (ESM)-4

Median (IQR) plasma NGAL values in patients with and without AKI stratified by sepsis, using baseline SCr 24 hrs prior to or at ICU admission

ESM-4: Median (IQR) plasma NGAL values in patients with and without AKI stratified by sepsis, using baseline SCr 24 hrs prior to or at ICU admission

Time	Plasma NGAL (ng/ml)(IQR)	No-AKI	AKI	P
T=0	Non-sepsis	154(161)	295(282)	<0.001
	Sepsis	296(641)	695(870)	0.01
T=4	Non-sepsis	151(146)	362(374)	<0.001
	Sepsis	320(594)	883(844)	0.002
T=8	Non-sepsis	145(159)	345(457)	<0.001
	Sepsis	310(504)	789(994)	0.003
T=24	Non-sepsis	120(143)	362(619)	<0.001
	Sepsis	303(314)	1069(827)	<0.001

SCr: serum creatine, NGAL: plasma neutrophil gelatinase associated lipocalin AKI: acute kidney injury.

Electronic Supplementary Material (ESM)-5

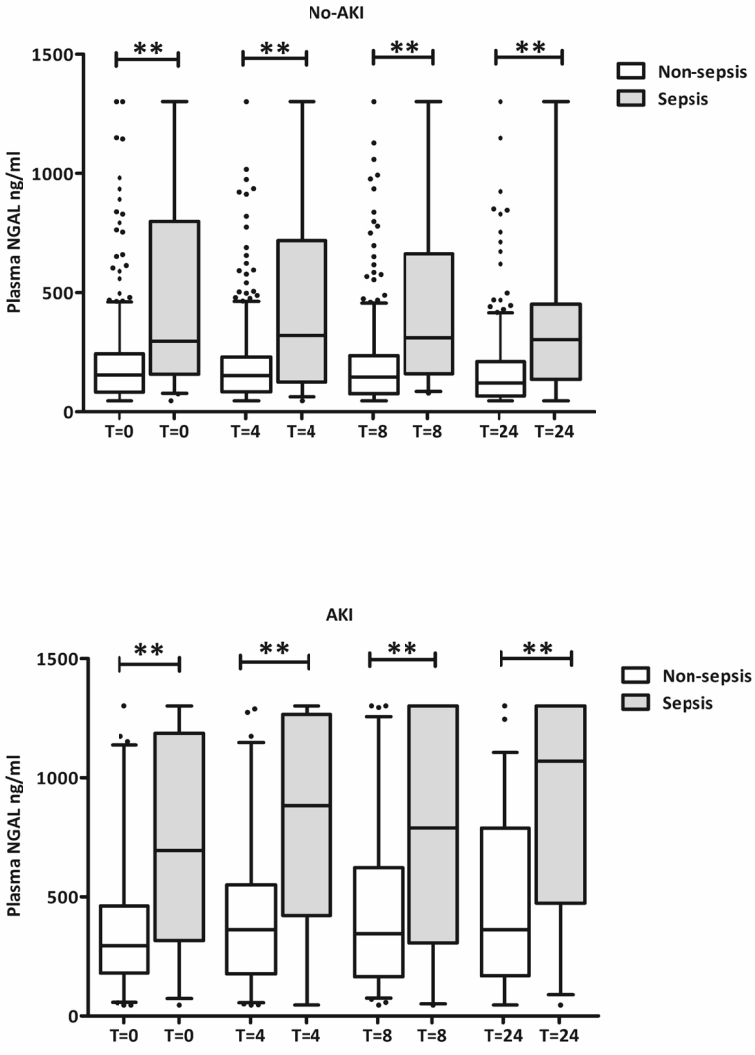
ESM-5: Receiver operating characteristics curve analysis for the diagnosis of AKI stratified by sepsis using admission (A) and peak (B) plasma NGAL values

ESM-5: Receiver operating characteristics curve analysis for the diagnosis of AKI stratified by sepsis using admission (A) and peak (B) plasma NGAL values

Plasma NGAL (ng/ml)	AUC (95% CI)	P	Cut-off	Sens	spec
Non-sepsis (N=542, N=73 AKI)	0.73(0.70-0.77)	<0.0001	266	63	80
Sepsis (N=80, N=39 AKI)	0.67(0.55-0.78)	0.0073	304	77	55
Plasma NGAL (ng/ml)	AUC (95% CI)	P	Cut-off	Sens	spec
Non-sepsis (N=582, N=80 AKI)	0.76(0.73-0.80)	<0.0001	324	68	80
Sepsis (N=80, N=39 AKI)	0.73 (0.62-0.82)	0.0001	979	56	88

AKI is classified applying a baseline serum creatinine value SCr 24 hrs prior to or at ICU admission
SCr: serum creatine, NGAL: plasma Neutrophil Gelatinase-associated Lipocalin AUC: area under receiver operating curve; CI: confidence interval sens: sensitivity and spec: specificity. P=0.63 for difference in AUC between non-sepsis and sepsis patients.

Electronic Supplementary Material (ESM)-6



ESM-6: Plasma NGAL concentrations in patients stratified for sepsis and (severity of) AKI

Electronic Supplementary Material (ESM)-7

SCr baseline definitions

ESM-7: SCr baseline definitions

	New definition		
	No-AKI	AKI	Total
Old definition			
No-AKI	473	9	482
AKI	70	111	181
Total	543	120	663

SCr: serum creatine, Fisher exact test P<0.001

	New baseline SCr definition				
	No-AKI	AKI 1	AKI 2	AKI 3	Total
Old definition					
No-AKI	473	7	1	1	482
AKI 1	44	29	4	0	77
AKI 2	17	14	13	0	44
AKI 3	9	9	5	37	60
Total	543	59	23	38	663

SCr: serum creatine, Pearson Chi-Square P<0.001

Chapter 5

Time and site of injury affect the predictive value of urinary biomarkers for acute kidney injury in non-septic, critically ill patients

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Abstract

Background: The predictive value of urinary biomarkers for acute kidney injury (AKI) may depend on the time interval after tubular injury and its predominant site, thereby explaining in part their heterogeneous performance reported in the literature. We studied the influence of these factors on the predictive values of tubular proteins, measured before the rise of serum creatinine (SCr) in critically ill, non-septic patients.

Methods: Seven hundred adult critically ill patients were prospectively included for urine measurements at four time-points prior to rise in serum creatinine (T=0, -16, -20 and -24 h). Patients with sepsis and or AKI at ICU entry were excluded. The urinary excretion of the proteins neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) up-regulated in distal and proximal tubules, respectively, were measured as well as the constitutive cytoplasmatic enzymes π- and α-glutathione-S-transferase (GST), released by distal and proximal tubules, respectively.

Results: 543 subjects were eligible for further analysis; 49 developed AKI in de first 48 h. Both NGAL (P=0.001 at T=-24 vs no-AKI) and KIM-1 (P<0.0001 at T=0 vs. no-AKI) concentrations gradually increased until AKI diagnosis, whereas π- and α-GST peaked at T=-24 before AKI (P=0.006 and P=0.002 respectively vs no-AKI) and showed a rapid decline afterwards. The predictive values at T=-24 prior to AKI were modest for π- and α-GST, whereas NGAL predicted AKI already at T=-24 and even better when the time interval to AKI decreased (area under the receiver operating characteristic curve AUC 0.79, P<0.0001). KIM-1 was a good discriminator at T=0 only (AUC=0.73, P<0.0001).

Conclusions: Our data suggest that the predictive value for developing AKI depends upon time and predominant site of tubular injury, with decreasing predictive values in time of constitutive enzymes and increasing values for upregulated proteins, and higher predictive values of markers of distal than of proximal injury.

Key words: urinary biomarkers, AKI, injury site, NGAL, KIM-1, Pi-GST and alpha GST.

Background

There is ongoing search for biomarkers for AKI prediction, which may help, in the future, to guide preventive and therapeutic measures to benefit patients [1-13]. The AKI-induced up regulation of low molecular weight proteins, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), and their subsequent release and excretion into urine have been studied in patients with or at risk for AKI [6-9, 11, 13-18]. Currently, NGAL, presumably from distal tubular origin at least in experimental AKI [19], is the most frequently described, albeit imperfect, biomarker of human AKI and is considered as a reference standard [3, 5, 6, 8-13, 20, 21]. Still, there is a markedly heterogeneity of its reported predictive power for AKI. The clinical value of KIM-1, a predominantly ischemic proximal tubular injury marker [1, 4, 5], remains uncertain, with reports suggesting superiority [1, 4] or inferiority [3, 5, 13] compared to other markers. The constitutive cytoplasmatic enzymes π- and α-glutathione-S-transferase (GST) are detectable in urine when the integrity of the cell wall of distal and proximal tubules is damaged, respectively. Therefore, urinary release of GSTs reflects the site of tubular injury [22]. The few clinical studies on the predictive value of these enzymatic markers for AKI are conflicting [2, 3, 9, 23] and a comparison with urinary NGAL is limited to post cardiac surgery only [9]. The available literature thus reports heterogeneous predictive performance of biomarkers for AKI in different patient populations and conditions, such as adult vs pediatric patients, sepsis vs non-sepsis states, surgical vs non-surgical conditions, developing vs established AKI, fixed vs non-fixed intervals between injury and sampling.

In this study we wished to evaluate predictive performance of AKI biomarkers as a function of time (to sampling) and site of tubular injury in non-septic patients, since predictive values and optimal cutoff levels of AKI markers may differ between septic and non-septic AKI [24] . In contrast to our previous study including AKI at entry [11], the primary endpoint for the current study was the development of AKI within 48 h after ICU admission. Indeed, predicting is more useful than confirming established AKI and many studies lumped developing and established AKI together, thereby potentially leading to overestimation of predictive values [1-6, 8-10, 12, 13, 21].

Methods

Setting

This is a prospective single center cohort study in a 30-bed closed format university hospital intensive care unit (ICU) on which general surgical, trauma, medical, neurological and neurosurgical, but not cardiac surgery, patients are treated. All consecutively

admitted adult critically ill patients, between September 1, 2007, and April 1, 2008, were considered eligible. Exclusion criteria were age under 18 years, refusal of informed consent, a history of nephrectomy, documented chronic kidney disease (CKD) (>stage 3) or kidney transplantation and a diagnosis of sepsis at the time of ICU entry. The study was approved by the institutional review board of the Erasmus MC University Medical Center in Rotterdam, the Netherlands. Deferred consent was used with written informed consent obtained from the participants or their health care proxy within 48 h after ICU admission. In the case of consent refusal (n=6, 0.9%) the collected urine specimens were appropriately destroyed. This study is a sub-study of one previously reported [11], with other biomarkers and focusing on developing AKI.

Protocol, sample collection and processing. Demographic data were recorded, including severity of illness scores, several renal and outcome parameters such as the serum creatinine at hospital discharge, the duration of stay on the ICU in days, the mortality rate at 28 days after ICU admission and in the hospital. Serum creatinine values were available at the time of admission and daily thereafter at 6:00 am until 72 h after entry. The serum creatinine was measured in the hospital's clinical chemical laboratory with the Roche enzymatic kit which is traceable to a reference method based on isotope dilution mass spectrometry. Urinary output and fluid balances were recorded. At ICU admission (T=0) and at T=4, 8, 24 hrs thereafter urine samples were collected using an urine catheter. The samples were processed in the hospital's laboratory and the supernatants stored at -80 °C. NGAL (Biosite Inc Alere, San Diego, Clf, USA, using the Triage® immunoassay), KIM-1, π- GST, and α-GST (Argutus Medical, Dublin, Ireland) concentrations were measured using research-based immunoassays. The detection limits for the urine NGAL assays was 2.6 - 4100 ng/ml, respectively. The assays' coefficient of variation was 13.9%. The π- GST assay detection limits are 3.12-100 ng/ml, for α-GST 6.25-200 ng/ml and for KIM-1 0-10 ng/ml. The average assays' coefficient of variation in this study were 4%, 3% and 1%, respectively.

Definitions

Baseline serum creatinine was defined as the steady state level four weeks to six months prior to ICU admission. If not available, the admission value was applied as baseline. Criteria for sepsis were clinically suspected or confirmed infection, a temperature above 38.5 ℃ or below 36.0 ℃, tachycardia (>90 beats/min) and tachypnea (>20/min) or necessity for mechanical ventilation, and leukocytosis >12 x10^9/L or >10% bands, or leucopenia <4 x10^9/L. AKI was defined using the acute kidney injury network (AKIN) classification for changes in serum creatinine relative to a steady state baseline value (AKIN 0= no-AKI, AKIN 1= serum creatinine increase >50% or an absolute serum creatinine rise of 0.3 mg/dL (=26.5 μmol/L) compared to baseline, AKIN 2= serum creatinine increase >100% and AKIN 3= serum creatinine increase >200%) without using the urine output (UP) criteria. In order to plot the

expression of biomarkers preceding AKI, the time-points after ICU admission were recoded into time-points preceding AKI. AKI occurred either at T=24 or T=48 after ICU admission. The time-point of AKI was recoded to T=0 and the available measurements recoded relative to T=0 (T= -48, T= -44, T= -40, T= -24, T= -20 and T= -16 hrs). Fifty-six patients had more than one ICU admission and only the data of the first admission were used.

Statistical analysis

Patients were grouped according to no-AKI or developing AKI within the first 48 h of admission. Most continuous data were distributed non-normally (Kolmogorov-Smirnov test $P<0.05$). We compared developing AKI with no-AKI and univariate analysis for continuous variables was with help of the Mann-Whitney U test and for categorical variables using the χ^2 or Fisher exact test. Tests were two-tailed throughout. Receiver operating characteristics curve (ROC) analysis was used in patients with developing AKI to assess the predictive value of the biomarkers. The area under de curve (AUC) with 95% confidence intervals [95% CI] was calculated and compared. The optimal cut off level (Youden index) was calculated with the corresponding sensitivity, specificity, positive and negative likelihood ratios. Statistical analyses were performed with the statistical software package SPSS, version 16.0 (SPSS, Chicago, IL, USA) for windows and MedCalc for Windows, version 9.5.0.0 (MedCalc Software, Mariakerke, Belgium). Data are reported as number (percentage) or median (interquartile range), where appropriate. However, means and standard errors of the mean (SEM) were used in the time course graphs for the sake of clarity. A $P\leq0.05$ was considered statistically significant and exact values are given.

Results

Patients' characteristics

Seven hundred consecutive ICU admissions were included. Six patients refused consent (0.9%), 6 patients had undergone a nephrectomy (0.9%) and 25 patients had chronic kidney disease (CKD) stage 3 or kidney transplant (4%). Of the remaining 607 patients, 156 subjects had AKI within the first 48 h of admission (25%). Of those, 99 cases had AKI at entry (established AKI=63.5%) and 57 developed AKI (developing AKI=36.5%) within 48 h. Eight patients with developing AKI were diagnosed with sepsis at ICU admission (Figure 1). Patients with developing AKI were older, more severely ill and more often male (Table 1). Furthermore, developing AKI patients had a higher pre-admission baseline serum creatinine and a higher cumulative fluid balance within the first 24 h of ICU admission. At hospital discharge SCr values were higher in patients who had an episode of AKI compared to patients without, 28-day and hospital mortality rates were higher as well.

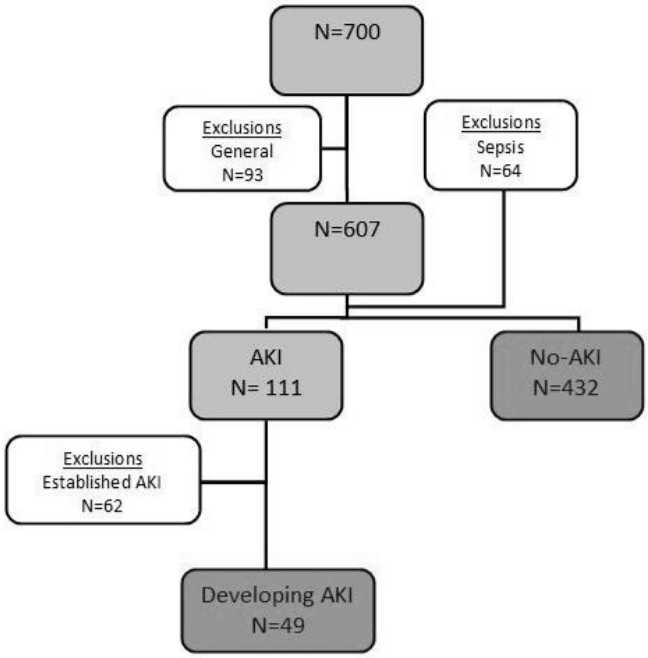

Figure 1: Study cohort flow chart. N: number; AKI: acute kidney injury; established AKI: AKI at the time of ICU admission and developing AKI: AKI developing at or after 24 hours of admission.

Biomarker patterns following ICU admission

The biomarker levels following ICU admission for patients with developing AKI and those without AKI are shown in Figure 2A. This panel represents the not-recoded data. The concentrations of the upregulated proteins NGAL and KIM-1 increase over time following ICU admission, with NGAL showing an increasing pattern right from the time of admission ($P<0.0001$) and KIM-1 differentiating between no-AKI and AKI T=24 hours for the first time ($P=0.008$). The concentrations of KIM-1 in patients without AKI increased over time after ICU admission. The constitutive enzymes π- and α-GST both display decreasing concentrations and remain higher up until 8 hours after admission compared to no-AKI ($P\leq0.048$ and $P\leq0.017$ respectively).

Biomarker patterns preceding AKI

Figure 2B shows the biomarker patterns before the time of AKI. All available no-AKI biomarker values were pooled to represent the no-AKI concentration in the graph represented at T=-72. The upregulated proteins KIM-1 and NGAL displayed a gradual increase in concentrations prior to SCr increase. KIM-1 being different compared to patients without AKI right at the time of AKI (T=0, $P<0.0001$) in contrast to NGAL showing a quicker response with different concentrations compared to no-AKI starting at 24 hours prior to the time of AKI ($P=0.0005$). The constitutive enzymes, π- and

Table 1: Patient characteristics

		No-AKI (N=432)	Developing AKI (N=49)	P
Age, years		57(25)	61(25)	0.04
Male, n(%)		243(56)	38(77)	0.004
BMI (kg/m^2)		24.6(4.8)	25.1(4.4)	0.67
APACHE II		16(9)	23(11)	<0.001
SOFA		4(4)	9(6)	<0.001
Admission diagnosis, n(%)				
	Mecdical	100(23)	18(36)	0.11
	Surgical	215(49)	20(40)	
	Neurological	117(27)	11(22)	
Renal characteristics				
Baseline SCr (mg/dl)		0.74(0.3)	0.85(0.3)	0.002
UP (ml/kg/h)		1.1(0.8)	0.9(0.9)	0.32
FB (l)		1.9(3.0)	4.3(4.4)	<0.001
AKIN stages, n(%)				
	AKIN-1	-	34	-
	AKIN-2	-	11	-
	AKIN-3	-	4	-
Patients with CVVH, n(%)		-	3(6)	-
Outcome				
SCr at hospital discharge (mg/dl)		0.68(0.2)	0.77(0.6)	0.002
ICU days		3(5)	7(11)	<0.001
28-day mortality (%)		53(12)	15(30)	0.002
Hospital mortality (%)		59(13)	17(34)	0.001

AKI: acute kidney injury; BMI: body mass index: APACHE II: Acute physiology and chronic health evaluation score; SOFA score: sequential organ failure assessment score; UP: urine production in 24 h after admission per kg ideal body weight; FB: fluid balance in 24 h; CVVH: continuous veno-venous hemofiltration; ICU: intensive care unit. Median (IQR) or number of patients (percentage) where appropriate

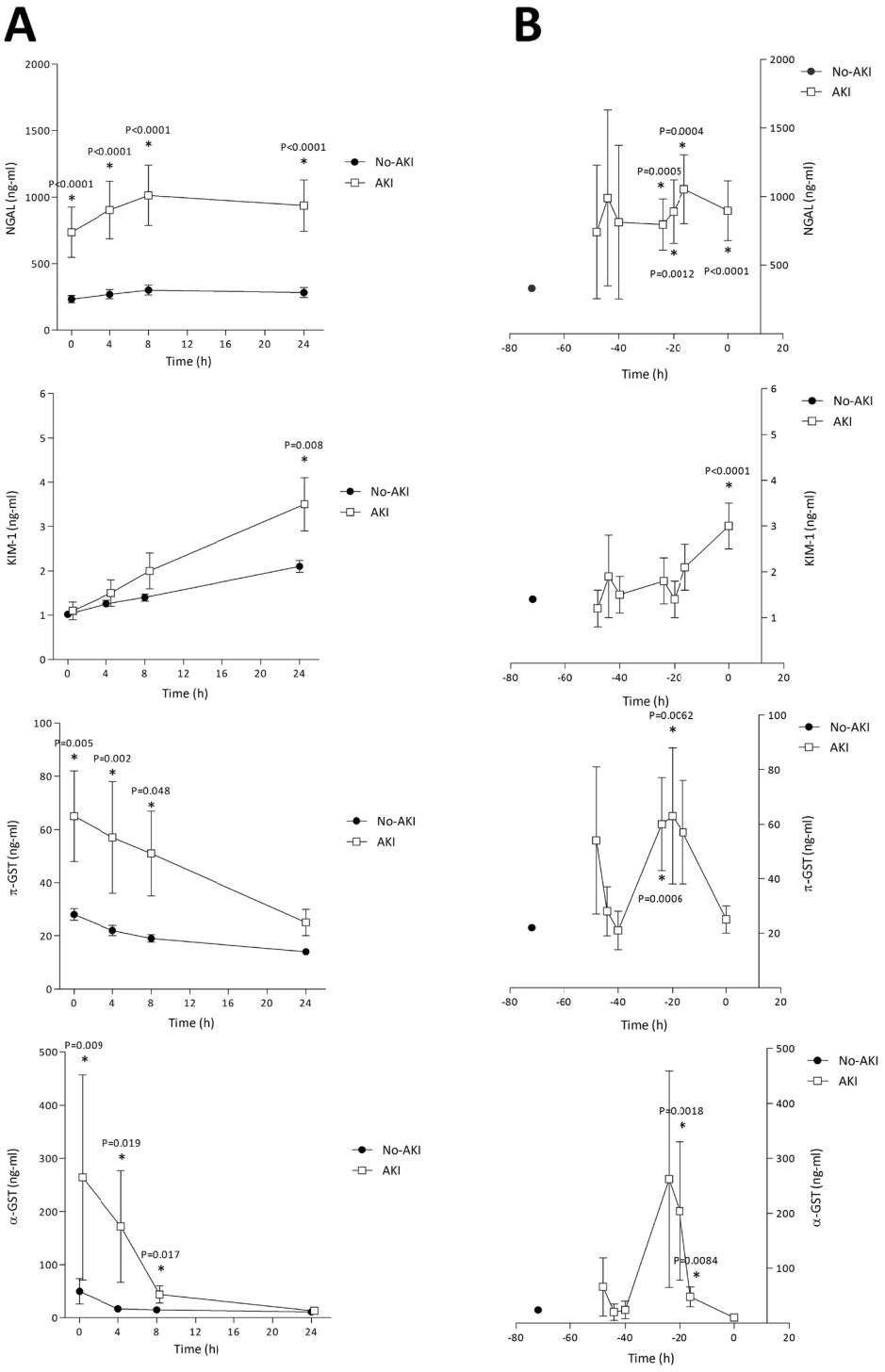
A
B
NGAL (ng-ml)
KIM-1 (ng-ml)
π-GST (ng-ml)
α-GST (ng-ml)
Time (h)
No-AKI
AKI
P<0.0001
P=0.008
P=0.005
P=0.002
P=0.048
P=0.009
P=0.019
P=0.017
P=0.0004
P=0.0005
P=0.0012
P=0.062
P=0.0006
P=0.0018
P=0.0084

Figure 2: Biomarker patterns after ICU admission (A) and preceding AKI (B). Biomarker concentrations are expressed in ng/ml and data represent mean (standard error of the mean; SEM). NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; GST: glutathione-S-transferase; AKI: acute kidney injury. Mean biomarker concentrations in patients with AKI vs patients without AKI at each time-point were compared using Mann-Whitney U test (A) and mean biomarker concentrations in patients with AKI were compared to the pooled mean value of all available no-AKI measurements using Mann-Whitney U test (B). Panel A represents the un-recoded data plotted against time after ICU admission, Panel B represents the recoded data prior to rise in SCr.

Table 2: ROC curves for the prediction of developing AKI vs no-AKI

Biomarker	Time	AUC (95% CI)	P
NGAL	T=-24	0.66(0.57-0.75)	0.0005
	T=-20	0.66(0.57-0.75)	0.001
	T=-16	0.68(0.57-0.78)	0.0004
	T=0	0.79(0.73-0.85)	<0.0001
KIM-1	T=0	0.73(0.64-0.83)	<0.0001
n-GST	T=-24	0.65(0.56-0.75)	0.0006
	T=-20	0.64(0.54-0.73)	0.006
α-GST	T=-20	0.65(0.56-0.75)	0.002

NGAL: neutrophil gelatinase associated lipocalin; KIM-1: kidney injury molecule 1; n-GST: pi glutathione-S-transferase; α-GST: alpha- glutathione-S-transferase; T: time; AUC: area under de curve, CI: confidence interval and P: level of statistical significance.

α-GST, had their peak concentrations 24 and 20 hours prior to the time of rise in SCr (T=0), respectively, as compared to patients without AKI (P=0.006 and P=0.0018). After a sudden peak, biomarker concentrations declined quickly prior to the time of AKI.

Prediction of AKI

Table 2 shows the area under de curves (AUC's) for the prediction of developing AKI for each individual biomarker at different time points. NGAL displays the most consistent predictive performance starting 24 hours before AKI (AUC=0.66, P=0.0005) and increases closer to the endpoint of AKI (AUC=0.79, P<0.0001). In contrast, KIM-1 "predicts" AKI only at the moment of rise in SCr for the first time (AUC=0.73 P<0.0001). For both π- and α-GST the predictive power is modest (AUC=0.65 for both) even at peak marker concentrations 24 and 20 hours prior to AKI.

Discussion

The present study suggests that the predictive performance of biomarkers for developing non-septic AKI depends on the time of sampling relative to the time of AKI and therefore on the, otherwise unknown, time interval between sampling after tubular injury. Furthermore, it suggests that the upregulated proteins show a consistent and gradually increasing signal over time whereas the constitutive enzymes display a narrow time window of expression. NGAL outperforms KIM-1 in early expression prior to AKI, suggesting greater distal than proximal injury.

The predictive value of NGAL for (non-septic) AKI in this study is in accordance with other work [3, 5, 6, 8-13, 20, 21, 25]. The expression pattern of NGAL prior to rise in SCr is early and consistent with the predictive power increasing closer to the time of AKI. This latter observation endorses the idea that the time-to-injury relationship matters and should be known for a correct interpretation of its predictive value for AKI. KIM-1's expression was less accurate and late (at the time of SCr increase) compared to NGAL in the current study and this confirms work by others in adult critically ill patients [1, 3, 4, 7, 9]. Moreover, the predictive value of KIM-1, only slowly increasing in time after renal injury, as our data suggest, is higher if AKI has already developed, as in cardiac surgery, rather than if AKI develops in the course of time, as in our study [1, 4, 5, 9]. The rise in KIM-1 over time even when AKI does not develop can perhaps be explained by subclinical injury, since KIM-1 is a transmembrane glycoprotein exclusively present in epithelial cells surviving after injury and facilitating phagocytosis of necrotic cell debris [1, 9].

π- and α-GST were only modest predictors for AKI in this population with slightly better or similar results compared to those reported in adult cardiac surgery patients

(AUC=0.54 [95% CI 0.42-0.66] [9, 26] but similar in another cohort of general critically ill patients [25]. In an older study, the markers were suggested to be superior to other enzymes AUC=0.93 [0.74-0.99] and 0.89 [0.69-0.98] respectively [23], these results were not reproduced in following studies. Several other studies described the diagnostic performances in established AKI, which results are incomparable to the present data [2, 26, 27]. Especially for these enzymatic markers, the time of sampling in relation to the injurious hit seems to be critical for their ability to predict a rise in SCr later on. This might make this category of biomarkers less well applicable in patients without a circumscribed time point of renal injury such as is the case in general ICU patients. However, due to the sudden changes in concentrations in urine their applicability might be more appropriate in a setting of monitoring renal toxic effects of drugs and contrast agents.

There are several limitations to the current results. Despite the large initial number of included patients the subset of patients with developing AKI was relatively small, since 64% of the AKI patients had AKI at ICU entry and they were excluded for the current analysis. The subset of patients with developing septic AKI was even smaller and did not allow sufficient data analysis, although it would have been interesting to study the possible differences in biomarker expression between both septic related and non-septic related AKI. In spite of the recognition of serum creatinine as a poor indicator of renal injury, confounded, among others by varying tubular secretion, it is still used in many studies [3, 12, 28]. Therefore, the usefulness of potentially sensitive markers might be underestimated. We believe this phenomenon is reflected by our data, which indicate the presence of subclinical tubular injury in no-AKI patients (increase in KIM-1 levels in patients without AKI according to AKIN classification). Urinary biomarkers can be used in non-anuric AKI only, narrowing their clinical applicability. Controversy exists on whether correction for urinary creatinine concentrations is necessary for the interpretation of the results. We believe that normalization to urine creatinine concentration poses a limitation by itself since patients with AKI are not in a steady state creatinine turnover. Furthermore, several authors have shown that this effort does not contribute much [1, 2, 5, 6, 9, 13, 23].

Conclusions

Our current data suggest that the expression pattern of different biomarkers such as upregulated proteins and constitutive enzymes and the time of sampling in respect to the time of actual cellular injury may partly explain the heterogeneity of predictive values. Furthermore, predictive values depend on the predominant site of injury. These factors should be taken into account in future studies.

Competing interests

Hilde de Geus has received speaker fees from Alere. Alere and Argutus medical kindly provided biomarker measurements. The other authors have noting to declare.

Author's contributions

HG conceived the study, participated in the design, created the database, performed statistical analysis and drafted the manuscript. GF assisted in additional data collection. MB participated in study design and helped drafting the manuscript. RS laboratory carried out sample processing and storage. JG participated in study design, statistical analysis and drafting of the manuscript. Alle authors read and approved the final manuscript.

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Part B

Biomarkers and RRT

Chapter 6

Biomarker Strategies to Predict Need for Renal Replacement Therapy in AKI

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Abstract

The early detection and diagnosis of acute kidney injury (AKI) with the standardization of novel kidney-injury specific biomarkers is one of the highest research priorities in nephrology. Accordingly, the majority of studies of novel AKI biomarkers have focused on the early diagnosis of AKI using serum creatinine-based definitions as the gold standard. However, another potential application of kidney-injury specific biomarkers is for guiding decisions on when to initiate renal replacement therapy (RRT). The purpose of this review is to summarize recent findings concerning some of the more promising AKI biomarkers on their capacity, either alone or integrated with traditional surrogate measures of kidney injury, for early prediction of whether patients will develop severe AKI requiring RRT.

Some studies which have examined NGAL, cystatin-C, NAG, KIM-1, and α_1-microglobulin, among others, have suggested these novel biomarkers have the potential to distinguish patients in whom RRT will be needed. This would imply that these biomarkers may be integrated into clinical decision algorithms, and could synergistically improve our current ability to predict worsening AKI and need for RRT. However, published studies have many recognized limitations , which preclude our ability to adopt their findings into clinical practice today. While currently available data are not sufficient to conclude that biomarkers should be used routinely for clinical decision making for RRT initiation, additional data may in the future significantly modify the clinical variability for initiation of RRT, and potentially translate into improved outcomes and cost-effectiveness. Finally, we propose a potential approach to future biomarker strategies for RRT initiation, integrating these biomarkers with "traditional" clinical factors.

Key words: acute renal failure, acute kidney injury, α_1-microglobulin, biomarkers, cystatin C, dialysis, Kidney Injury Molecule-1, N-acetyl-β-D-glucosaminidase, Neutrophil Gelatinase Associated Lipocalin (NGAL), renal replacement therapy

Introduction

It is well known that serum creatinine (sCr) is an unreliable indicator during acute changes in kidney function. Its value can vary widely with age, sex, muscle mass, medications and hydration status. Furthermore, its concentration may not change until a significant amount of kidney function is lost, therefore it carries the risk of missed therapeutic opportunity because of the time lag between the inciting insult and the diagnostic elevation. These limitations are felt to contribute to the still dismal clinical outcomes associated with acute kidney injury (AKI).

As a consequence, the American Society of Nephrology has assigned the highest research priority to the standardization and/or discovery of new biomarkers of AKI. In response there has been great scientific enthusiasm and abundant publications on these biomarkers. Most of the investigations have focused on the ability of these biomarkers to detect established AKI or the early diagnosis of AKI (almost universally defined as defined by sCr-based definitions). Several promising candidates have emerged, demonstrating reasonable diagnostic performance for AKI up to 48 hours prior to a significant change in sCr [2, 3]. With the advent of commercially available and standardized platforms for either point-of-care or rapid centralized central laboratory measurements for some of these, the medical community has shown great interest in incorporating such measurements into clinical practice.

At present, the actual clinical utility of these early biomarkers remains largely untested with a key question being whether they would add anything to management beyond the information provided by using a conventional, sCr-based diagnosis. Although a case could be made that early AKI detection could encourage "avoidance of harm", such as nephrotoxic medications or premature stepdown from the intensive care unit (ICU), the reality in 2010 is that we do not yet have much to offer in terms of therapeutics. One area of potential clinical use is that of guidance in the decision to initiate renal replacement therapy (RRT) in patients with AKI. Aside from predicting AKI, several biomarker studies have also examined prediction for "hard" outcomes such as need for RRT or death. In this next section we will briefly review selected studies which have looked at the use of novel biomarkers for the prediction of subsequent need for RRT.

Biomarker studies and need for RRT

Neutrophil gelatinase-associated lipocalin (NGAL)

High throughput functional genomic studies have identified NGAL as one of the most upregulated transcripts in the kidney very early after acute injury. Downstream proteomic studies have also revealed the 25 kD NGAL protein to be one of the earliest and most

Table 1: Neutrophil gelatinase-associated lipocalin for prediction of RRT.

Reference	Specimen	Population		RRT Endpoint	Results from Pooled analysis [9]
Cruz 2010 [8]	Plasma	ICU	Adults	15/301 (5%)	AUC 0.78 (95% CI, 0.65-0.92) Diagnostic Odds Ratio 12.9 (95% CI, 4.9-33.9) At cut-off 278 ng/ml, sensitivity 76%, specificity 80%
Constantin 2010 [11]	Plasma	ICU	Adults	7/88 (8%)	
Wheeler 2008 [10]	Plasma	ICU	Pediatric	227143 (15%)	
Nickolas 2008 [13]	Urine	ED patients	Adults	12/541 (2%)	
Koyner 2008 [14]	Plasma and urine	Cardiac surgery	Adults	7/72 (10%)	
Haase-Fielitz A 2009 [15]	Serum	Cardiac surgery	Adults	4/100 (4%)	
Wagener 2008 [16]	Urine	Cardiac surgery	Adults	5/81 (6%)	
Bennett 2008 [17]	Urine	Cardiac surgery	Adults	8/426 (2%)	
Nejat 2010 [18]	Urine	Cardiac surgery	Pediatric	4/196 (2%)	

AUC: area under the receiver operator characteristic curve; CI: confidence interval; ICU: intensive care unit; RRT: renal replacement therapy; ED: emergency department adapted from ref [9].

robustly produced proteins in the kidney after ischemic or nephrotoxic AKI in animal models [4]. It can be measured in the blood and the urine. Both have been shown to be an early predictor of AKI in several clinical settings, including cardiac surgery [5], radio contrast exposure [6], trauma [7] and in critical illness [8].

Several studies have examined the clinical endpoint of RRT initiation using NGAL, of which nine have been summarized in a recent meta-analysis [9]. Included studies for this endpoint represented a variety of clinical settings, including both children and adults (Table 1). Either urine or plasma/serum NGAL were measured. The overall incidence of RRT in the included studies was 4.3%, which was slightly higher in the ICU population, ranging from 5-15% [8, 10, 11]. The pooled analysis yielded an area under the Receiver Operator Characteristic curve (AUC) 0f 0.782 (95% CI, 0.648-0.917) for discriminating patients who would receive RRT associated with AKI. For a cut-off NGAL value of 278 ng/mL, the sensitivity was 76% and specificity was 80%; however, it is difficult to translate this to the patient's bedside because of the diverse patient populations, specimens and assays used in obtaining this estimate. However, this remains the largest pool of patients studied so far on NGAL, with 1948 patients from 9 studies included in this analysis for prediction of need to initiate RRT.

In a subsequent study, the median enrollment urine NGAL levels were also found to be significantly higher in the 17/490 (3.5%) patients who received acute RRT (548 ng/mg Cr [IQR 156-466), versus the 473/490 (96.5%) who did not (61 ng/mg Cr [IQR 17-232]) [12]. While no specific cut-off was evaluated, there was over a two-fold increase in the adjusted risk for RRT with increasing urine NGAL levels (adjusted Hazard Ratio [HR] for log-transformed urine NGAL (2.6 [95% CI, 1.6-4.4]). While these data require further confirmation given the relatively low event rate for patients initiated on RRT, these observations imply NGAL may have important interaction with conventional criteria to aid in the clinical decision to initiate RRT.

Cystatin C

Cystatin C is a 13 kDa nonglycosylated cysteine protease inhibitor produced by all nucleated cells in a constant rate unaffected by muscle mass. In healthy subjects, plasma CyC (pCyC) is excreted through glomerular filtration and metabolized completely by the proximal tubules. There is no evidence of tubular secretion and therefore it is normally not found in urine in significant amounts. Accordingly, urinary excretion of CyC (uCyC) reflects tubular damage [13]. Several studies and a subsequent meta-analysis demonstrated the superiority of pCyC compared with sCr for detection of minor reductions and acute changes in glomerular filtration rate [14]. More recently, it has been shown that pCyC levels are influenced by abnormal thyroid function [15, 16], immunosuppressive therapy [17] and the presence of inflammation or malignancies [18, 19].

Table 2: Cystatin C for prediction of RRT.

Reference	Specimen	Population		Endpoint	Results	Adjustments in multivariate analysis
Herget-Rosenthal 2004 [26]	Serum	ICU patients at high risk for AKI	Adults	RRT (17/85=38%)	With ≥50% increase in sCyC: Sensitivity 53%, specificity 82% on RIFLE-Risk Day -2 Sensitivity 76%, specificity 93% on RIFLE-Risk Day -1 Sensitivity 82%, specificity 93% on RIFLE-Risk Day 0	None
Perianayagam 2009 [30]	Serum	AKI patients on nephrology consult service	Adults	RRT or death (84/200=42%)	AUC 0.65 (95% CI, 0.58-0.73) for sCyC alone; unadjusted OR per 1-SD increase 1.87 (95% CI, 1.36-2.59); adjusted ORs range from 1.62 to 1.71	APACHE II score, liver disease, sepsis, mechanical ventilation
Nejat 2010 [18]	Plasma	Intensive care unit	Adults	RRT or death (71/442=16%)	AUC 0.61 (95% CI, 0.53-0,68) Performed similarly to plasma Cr	None
Haase-Fielitz A 2009 [15]	Serum	Cardiac surgery	Adults	RRT or death (5/100=5%)	AUC 0.99 (95% CI, 0.98-0.99)	None
Vaidya 2008 [27]	Urine	AKI patients on nephrology consult service (at least RIFLE-Risk)	Adults	RRT or death (47/102=46%)	Not a significant predictor	Age
Herget-Rosenthal 2004 [32]	Urine	Non-oliguric ATN patients who reported to nephrology department	Adults	RRT (26/73=36%)	AUC 0.92 (95% CI, 0.86-0.96); at cut-off 1 g/mol Cr, sensitivity 92%, specificity 83%	None
Koyner 2008 [14]	Urine	Cardiac surgery	Adults	RRT (7/73=10%)	After CPB, urine cystatin C concentrations were highest in AKI with RRT, followed by AKI without RRT, then No AKI ($p \leq 0.01$ for 3-group comparison)	None

AKI – acute kidney injury; APACHE - Acute Physiology And Chronic Health Evaluation; AUC – area under the receiver operator characteristic curve; CI – confidence interval; CPB – cardiopulmonary bypass; Cr – creatinine; ICU – intensive care unit; OR – odds ratio; RRT – renal replacement therapy

In human studies, both pCyC and uCyC have been shown to predict AKI [20, 21], although its superiority over sCr has not been a universal finding [22]. In terms of RRT requirement, plasma and serum CyC (sCyC) have been studied in different settings (Table 2). In a general ICU, sCyC was measured in 85 patients at high risk for AKI [20]. The capacity to predict need for RRT was tested at three time points: on Days -2, -1 and 0 of reaching RIFLE-Risk. The AUC for sCyC were 0.69, 0.75 and 0.76, respectively. Using a ≥50% increase in sCyC from Day-3 (considered as baseline), the sensitivity and specificity improved going from Day-2 to Day 0 (Table 2). Another study measured plasma Cr (pCr) and pCyC in 444 adults on ICU admission [23], of whom 124 already had AKI on entry. In the entire cohort, pCyC was moderately predictive of death or RRT (AUC 0.61, 95% CI, 0.53-0.68) and performed similarly as pCr (AUC 0.60, 95% 0.51-0.67). In an analysis excluding patients with AKI on admission, pCyC performed slightly better (AUC 0.84, 95% CI, 0.69-0.99) than pCr (AUC 0.77, 95%CI, 0.59- 0.94) for RRT prediction.

Serum CyC was measured in 200 AKI patients from the nephrology consult service (mean sCr 300 μmol/L at enrollment), of whom 83 died or underwent RRT [24]. Serum CyC had an AUC of 0.65 (95% CI, 0.58-0.73) for this composite endpoint. This was inferior to a basic prediction model using APACHE II, liver disease, sepsis and mechanical ventilation (AUC 0.82). When sCyC was added to this basic model, there was no significant improvement in performance (AUC 0.83). Furthermore, the diagnostic accuracy was quite similar whether sCr, serum urea nitrogen or urine output were used instead of sCyC (AUCs 0.83-0.84).

In cardiac surgery, two small studies demonstrate divergent results. Koyner et al included 72 adults following cardiac surgery; of these 34 developed AKI, of whom 7 subsequently needed RRT [25]. The authors concluded that pCyC was not a useful predictor for AKI development (AUCs for various time points after cardiopulmonary bypass [CPB] 0.617-0.631), and not further analyzed for RRT prediction. In contrast, a similar study on 100 adult patients, of whom 5 reached a composite endpoint of RRT or death, found excellent performance for prediction of this endpoint for pCyC (AUC 0.99, 95% CI, 0.98-0.99)[26]. In both studies, the absolute number of patients who reached the RRT or composite endpoint was quite small. The reason for discrepant results is not immediately clear. Unfortunately, the clinical utility of pCyC to predict RRT initiation from these data may be limited, due to many studies having relatively low event rates and using composite endpoints that may compete (i.e. death or RRT) and that no clear superiority to serum creatinine was observed.

Urine cystatin C has also been studied (Table 2). Several tubular proteins and enzymes were tested for their prognostic ability in 73 consecutive patients with established non-oliguric AKI (median sCrs 159-194 μmol/L), of whom 26 required RRT a median of 4 days after enrollment [27]. Good diagnostic performance was seen with uCyC (AUC

0.92, 95% CI, 0.86-0.96) and α_1-microglobulin (AUC 0.86, 95% CI, 0.78-0.92). At a cut-off of 1g/mol Cr, uCyC appeared to be highly sensitive (92%) and reasonably specific (83%) for predicting RRT. No comparison was made with sCr. Results are, however, not consistent. In another study (discussed below), uCyC was not predictive of RRT in 102 patients recruited from a nephrology consult service [21]. Unfortunately, this study did not report the sCr of the patients, precluding meaningful comparison with the previous study. In one of the cardiac surgery studies mentioned above, uCyC performed better for AKI prediction than did pCyC [25]. At various timepoints after CPB, uCyC concentrations were highest in AKI with RRT, followed by AKI without RRT, then No AKI ($p \leq 0.01$ for 3-group comparison). While no specific analysis was performed on the operative characteristics of uCyC to predict RRT initiation, these observations are encouraging, yet also appear inconsistent. Additional confirmatory evidence is needed from studies enrolling a population of patients with AKI with higher risk features for RRT initiation.

N-acetyl-β-D-glucosaminidase (NAG)

NAG is a lysosomal enzyme (> 130 kDa) that is localized in several human cells including the renal tubules. Due to its large molecular weight it precludes glomerular filtration, implying that urinary elevations have a merely tubular origin. Increased activity suggests injury to its cells but also may reflect increased lysosomal activity without cell disruption. NAG catalyzes hydrolysis of terminal glucose residues in glycoproteins and is the most active glycosidase found in proximal tubular epithelial cell lysosomes. Urinary NAG activity remains elevated during different kinds of active renal disease [28].

The diagnostic and prognostic ability of 9 urinary biomarkers, including NAG, was evaluated in a cross sectional study with 102 patients with established AKI compared to 102 subjects without AKI [21]. The non-AKI subjects included healthy controls, ICU patients and subjects who underwent coronary angiography, while AKI patients were recruited at the time of nephrology consultation. In age-adjusted analysis using log-transformed biomarker values, NAG was found to be a significant predictor for RRT, mortality and their composite endpoint among AKI patients (Table 3). The median normalized NAG level in AKI patients who underwent RRT was 0.06 U/mg Cr, versus 0.02 U/mg Cr in those who did not.

The same authors studied urine NAG and Kidney Injury Molecule-1 (KIM-1) in 201 consecutive adult patients with AKI on the nephrology consult service [29]. The AUC for NAG for the composite outcome of RRT requirement or hospital death was 0.71 (95% CI, 0.63-0.78), better than that of sCr (0.60, 95% CI, 0.52-0.68) or urine output (0.65, 95% CI, 0.57-0.73). There were significantly higher odds (Odds Ratio [OR] 9.1, 95% CI,3.7-22.7) for RRT or death in the NAG fourth quartile group compared to the first

Table 3: Selected other urine biomarkers for prediction of RRT.

Reference	Biomarker	Population		Endpoint	Results	Adjustments in multivariate analysis
Vaidya 2008 [21]	NAG	AKI patients on nephrology consult service (at least RIFLE-Risk)	Adults	RRT or death (47/102=46%)	Significant predictor for RRT, death and composite	Age
Liangos 2007 [29]	NAG	AKI patients on nephrology consult service	Adults	RRT or death (96/201=48%)	AUC 0.71 (95% CI, 0.63-0.78); unadjusted OR of 4th quartile vs 1st quartile 9.1 (95% CI, 3.7-22.7); adjusted ORs range from 4.8 to 7.2	APACHE II, MOF, cirrhosis, sepsis, oliguria, mechanical ventilation
Herget-Rosenthal 2004 [27]	NAG	Non-oliguric ATN patients who reported to nephrology department	Adults	RRT (26/73=36%)	AUC 0.81 (95% CI, 0.73-0.88); at cut-off 4.5 U/mmol Cr, sensitivity 85%, specificity 62%	None
Nickolas 2008 [30]	NAG	Emergency room patients	Adults	Nephrology consultation, ICU admission, RRT or death*	Not an independent predictor (OR = 1.07, 95% CI, 0.52-2.18)	sCr, BUN, serum leukocyte count, NGAL, α_1-Microglobulin, α_1-Acid glycoprotein
Vaidya 2008 [21]	KIM-1	AKI patients on nephrology consult service (at least RIFLE-Risk)	Adults	RRT or death (47/102=46%)	Significant predictor for death, but not a significant predictor for RRT or composite	Age
Liangos 2007 [29]	KIM-1	AKI patients on nephrology consult service	Adults	RRT or death (96/201=48%)	AUC 0.61 (95% CI, 0.53-0.69); unadjusted OR of 4th quartile vs 1st quartile 3.2 (95% CI, 1.4-7.4); not significant on adjusted analyses	APACHE II, MOF, cirrhosis, sepsis, oliguria, mechanical ventilation
Herget-Rosenthal 2004 [27]	α_1-microglobulin	Non-oliguric ATN patients who reported to nephrology department	Adults	RRT (26/73=36%)	AUC 0.86 (95% CI, 0.78-0.92); at cut-off 20 g/mmol Cr, sensitivity 88%, specificity 81%	None
Nickolas 2008 [30]	α_1-microglobulin	Emergency room patients	Adults	Nephrology consultation, ICU admission, RRT or death*	Not an independent predictor (OR = 1.85, 95% CI, 0.80-4.31)	sCr, BUN, serum leukocyte count, NGAL, NAG, α_1-Acid glycoprotein

AKI – acute kidney injury; APACHE - Acute Physiology And Chronic Health Evaluation; AUC – area under the receiver operator characteristic curve; BUN – blood urea nitrogen; CI – confidence interval; Cr – creatinine; ICU – intensive care unit; MOF – multisystem organ failure; NAG - N-acetyl-β-D-glucosaminidase; NGAL - Neutrophil gelatinase-associated lipocalin; OR – odds ratio; RIFLE – Risk Injury Failure Loss Endstage renal disease; RRT – renal replacement therapy

quartile. This persisted, though attenuated, after adjustment APACHE II scores, MOF scores, and other variables including oliguria and sepsis (OR 4.8, 95% CI, 1.7-13.1).
In the above-mentioned study by Herget-Rosenthal et al, the urinary excretion of NAG was significantly higher in patients requiring RRT [27]. On ROC curve analysis, the AUC for NAG was 0.81 (95% CI,0.73-0.88), slightly inferior to that of uCyC and α_1-microglobulin. At a cut-off of 4.5 U/mmol Cr, NAG was a sensitive (85%) but non-specific (62%) predictor for RRT. The authors concluded that tubular proteinuria was superior to tubular enzymuria in differentiating the need for RRT in AKI patients. A possible explanation might be that the early peak of the enzymuria was missed in these patients who already had established AKI at enrollment. In a study of 635 unselected patients presenting to the emergency room, urine NAG was not predictive of a composite outcome of nephrology consultation, ICU admission, RRT initiation and mortality on multivariable analysis which included sCr and blood urea nitrogen (BUN) [30] (Table 3). Urine NAG also appears to hold promise as added clinical information to aid in early decision-making for RRT initiation; however, similar to prior studies, further confirmatory data would be needed that focuses specifically on the additive/synergistic value of urine NAG for predicting RRT alone rather than as part of a composite outcome.

Kidney Injury Molecule-1 (KIM-1)

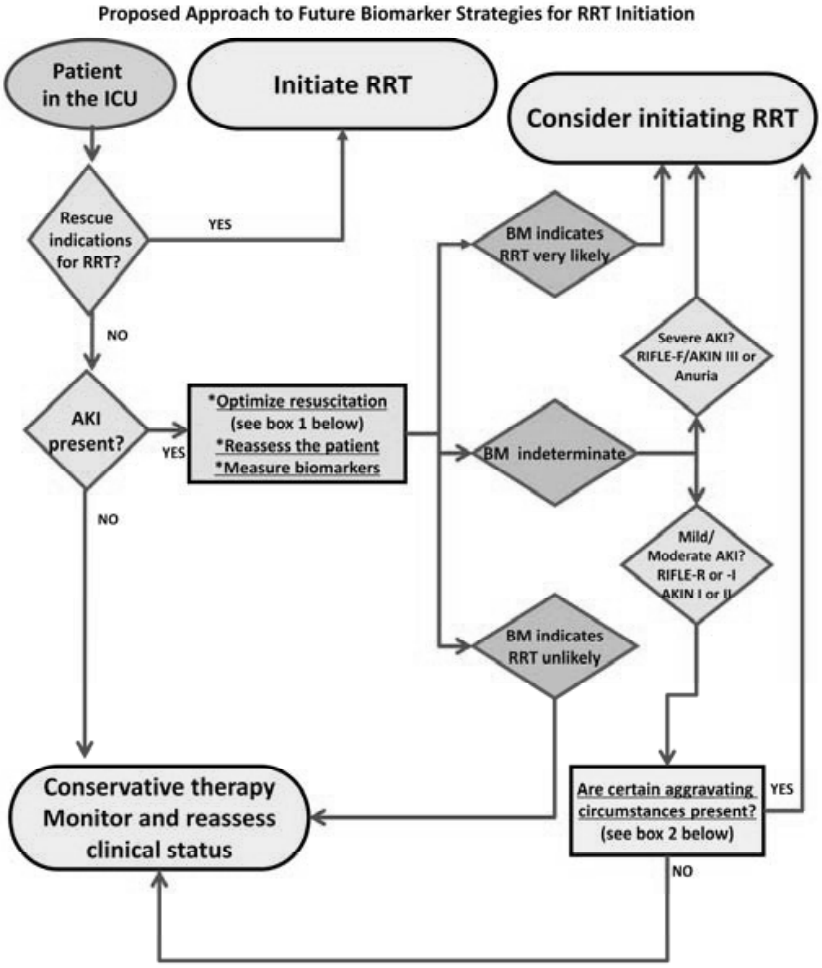
KIM-1 is a type I transmembrane glycoprotein with a cleavable ectodomain which is localized in the apical membrane of dilated tubules in acute and chronic injury [31]. There are two homologues forms of KIM-1 with differences in their C terminal portion; one is expressed in the liver, the other in the kidney. Further expression of those homologous forms is reported in the cochlea, in clear cell-type renal carcinoma which is associated with proximal tubular cell dedifferentiation and by subpopulations of T-cells (TIM-1) [32]. KIM-1 and its soluble ectodomain in urine (90 kDa) are believed to play a role in the regeneration processes after epithelial injury.
In the study by Liangos et al, the AUC for prediction of RRT or death for KIM-1 was 0.61 (95% CI, 0.53-0.61), comparable to that of sCr and urine output [29]. On adjusted analysis, patients in the highest KIM-1 quartile had a 3.2 fold higher odds (95% CI, 1.4-7.4) for the composite outcome compared to patients with the lowest quartile. On multivariate analysis, this was no longer significant. In another study by the same authors, KIM-1 was not a significant predictor for RRT, but was a significant predictor for mortality [21] (Table 3).

Creating biomarker strategies for RRT initiation

Although results are encouraging for some biomarkers, there remains important challenges to creating and testing biomarker-based strategies for RRT initiation. First, while a "hard" outcome such as RRT may be perceived as relatively uniform across various studies, this still has the limitations of a surrogate outcome. Due to the wide practice variation in timing of initiation of RRT and the lack of broad consensus, the initiation of RRT remains somewhat subjective as a hard endpoint. The decision to start RRT is strongly influenced not only by individual physician practice, but also by sCr and urine output, the very same conventional markers whose shortcomings we lament. Furthermore, such a decision may also be affected by factors unrelated to either patient or physician, such as organizational or logistical issues [33]. Second, a limitation of these studies is the variable timing of specimen collection. These include at ICU admission and at the time of nephrology consultation, the timing of which can vary from institution to institution. Third, most analyses have been based on a single specimen. Because different biomarkers may have varying kinetics following AKI, timing of specimen collection with respect to the relevant kidney insult may significantly affect their predictive values. Fourth, in many of these studies the number of days from biomarker measurement to RRT initiation was not stated. Therefore we cannot comment as to whether it could help the clinician make an "early" decision to initiation RRT. Fifth, some studies have a conceptual approach to the analysis for prediction of RRT. While analysis by quartiles [29] or by "per 1-SD increase"[24] can potentially confirm that a higher biomarker level is predictive of RRT, it does not provide clinically meaningful cut-off values which can be used at the bedside. Lastly, only two of these studies adjusted the analysis for "traditional" measures used to decide when to initiate RRT, such as sCr, BUN [30] or oliguria [29].

Timing of RRT initiation has been the focus of many scientific publications. In general, the currently available data, many from observational studies, suggest a trend towards reduced mortality and better renal recovery with earlier initiation of RRT [34-36]. However, no sufficiently powered RCT to date has shown a survival or kidney recovery benefit to earlier RRT initiation compared with standard of care or for more conventional indications. Currently, there exists no broad consensus to guide clinicians on this important issue. Accordingly, we have recently proposed an opinion-based clinical algorithm to aid in the decision on when to consider initiation of RRT in critically ill adults, giving a more quantitative characterization of "timing" using RIFLE/ AKIN, as well as incorporating several patient-specific factors [33]. One interesting question to ponder is whether biomarkers would be useful to better inform this decision.

Studies have demonstrated better outcome in patients who were started on RRT while they were still in RIFLE-Risk or Injury, as compared to those who started RRT when



Cruz et al, Seminars in Dialysis 2011, 24:124

Box 2:

Are certain aggravating circumstances present?

Rapidly worsening AKI
Rapidly worsening illness severity
Hypercatabolic state
Refractory fluid overload and/or accumulation
Severe sepsis
Permissive hypercapnea
Reduced renal reserve
Low probability for rapid renal recovery
Dialyzable toxins

Box 1:

Optimize resuscitation:

Intravascular volume
Cardiac output
Mean arterial pressure
Intra-abdominal pressure

Re-assess the patient :

AKI severity & trend
Illness severity & trajectory
Initial response to initial resuscitation therapy

Figure 1: Proposed approach to future biomarker strategies for RRT. AKI – acute kidney injury; BM – biomarker; ICU – intensive care unit; RIFLE – Risk Injury Failure Loss Endstage renal disease; RRT – renal replacement therapy. Box 1: Optimize resuscitation: intravascular volume, cardiac output, mean arterial pressure, intra abdominal pressure. Re-assess patient: AKI severity and trend, illness severity and trajectory amd initial response to resuscitation therapy. Box 2: Are mitigating circumstances present?: Rapidly worsening AKI, or illness severity, hypercatabolic state, refractory fluid overload, severe sepsis, permissive hypercapnea, reduced renal reserve, low probablityt for rapid renal recovery and dialyzable toxins.

they were in RIFLE-Failure, although interpretation of these studies is confounded by the failure to include patients who recover renal function or die without ever receiving RRT [37, 38]. But clearly we cannot provide renal support for all critically ill patients with Risk/ Stage 1 or Injury/ Stage 2. A considerable proportion of these patients are likely to spontaneously recover renal function before developing significant sequelae such as fluid overload or uremia, and therefore the potential benefits of RRT may not exceed the potential risks and/or additional health resource expenditure. On the other hand, if we could easily identify patients with sustained and severe renal injury, and therefore a high probability of needing RRT, it may be prudent to consider starting RRT even at milder stages of AKI to provide renal support and in order to prevent, rather than correct, AKI-related complications [33].

Such biomarkers may be integrated into clinical decision algorithms that have previously relied only on conventional clinical parameters [33], enhancing further our ability to predict need for RRT. A potential approach to future biomarker strategies for RRT is shown (Figure 1). For this, two cut-offs are needed, one that identifies a high likelihood of needing RRT, and another indicative that RRT is very unlikely. This is analogous to the approach currently taken with other biomarkers already in clinical use, such as BNP [39]. These cut-offs may be either absolute values, or an absolute or relative change from a baseline value. The first cut-off would aid in the early identification of patients who have persistent and severe renal injury in whom rapid spontaneous recovery of renal function is improbable. The second distinguishes those with milder tubular injury who are expected to experience prompt renal recovery. These may also vary depending on the clinical setting, e.g. ICU vs cardiac surgery, etc. For patients with values in the indeterminate range, the presence of extenuating factors assist further in the decision for RRT. Patients treated with conservative therapy should be monitored and reassessed, which may include repeated biomarker measurements. Further studies are needed to first, establish appropriate thresholds, and second, to test this approach in prospective rigorous study.

Summary

A number of novel kidney injury specific biomarkers have recently been characterized that can be detected in the blood and urine. Several of these biomarkers have shown value for the early detection and diagnosis of AKI, when benchmarked against conventional measures of loss of kidney function, such as sCr, urea or urine output. However, fewer data have evaluated the potential value of these biomarkers, either alone or integrated with additional clinical factors or traditional surrogate measures of kidney injury for early prediction of whether patients will develop severe AKI requiring

RRT. Selected studies, examining NGAL, cystatin-C, NAG, KIM-1, and α_1-microglobulin, among others, have suggested these novel biomarkers have the potential to distinguish patients in whom RRT will be needed. This would imply that these biomarkers may be integrated into clinical decision algorithms, and could additively or even synergistically improve our current ability to predict need for RRT. However, published studies have recognized limitations , which preclude our ability to adopt their findings into clinical practice today. These include, but are not limited to, differences in patient populations (adult vs pediatric),clinical setting (cardiac surgery, nephrology consult, etc), type of specimens (blood vs urine), type of assays (standardized vs research platform-based assays), small sample sizes, and lack of adjustment for relevant clinical factors in the analyses. Clearly more data are needed. While currently available data are not sufficient to conclude that biomarkers should be used routinely for clinical decision making for RRT initiation today, additional data may significantly modify the clinical variability for initiation of RRT, and potentially translate into improved outcomes and cost-effectiveness.

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Chapter 7

A nomogram for risk assessment of RRT in adult critically ill patients

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Abstract

Background and objectives

The decision to initiate renal replacement therapy (RRT) depends on several factors and is still ambiguous among clinicians. Patient related outcome however is negatively influenced by a cumulating fluid balance. In patients with oliguric AKI fluid balance control is an issue, especially when the decision to initiate RRT is postponed or even doubted by other factors. It might therefore be helpful for clinicians to consult a prognostic model at ICU admission for risk assessment of need for RRT initiation within seven days of admission.

Design, setting, participants and measurements

All consecutive admitted patients to an academic adult ICU between September 2007 and April 2008 were included. The univariable predictive value of several predictors for the need of RRT was explored with logistic regression models. The additional predictive value of NGAL over these clinical predictors was calculated with multivariable logistic regression and expressed in increase in AUC (area under receiver operating characteristics curve). A prediction model was developed and its performance was quantified with respect to discrimination (AUC). The model was tested in an independent dataset, and a nomogram was developed.

Results

Patients initiated on RRT within the first 24 hours of admission were excluded, leaving 654 patients for the development of a prognostic model. Sepsis and MDRD eGFR at admission were the most important predictors for the need of RRT (Odds ratio's respectively 7.3 en 0.96). Plasma NGAL did not have any additional predictive value on top of these (AUC=0.92 to 0.92).

Conclusions

At the time of ICU admission the need for RRT within 7 days can be predicted accurately using the admission eGFR and a clinical diagnosis of sepsis by applying the presented prognostic model and nomogram.

Introduction

Acute kidney injury (AKI) is common among adult critically ill patients (30%) and about 5% of the entire population is temporarily depending on renal replacement therapy (RRT) during their stay. The mortality rate of patients requiring RRT in the Intensive Care Unit (ICU) is high (50%) with an accumulating fluid balance (FB) being associated with even worse outcome figures. In oliguric AKI the risk of daily fluid overload is high, especially when the decision to initiate RRT is postponed or doubted if clinicians decide to first challenge the kidneys' urine production with high doses of diuretics.

Several studies tried to address the issue of timing of RRT in relation to its effect on outcome such as mortality rates. However it seems difficult to interpret these data due to the wide variation in applied definitions of a timely initiation. Clearly observed evidence however is the association of a cumulating positive fluid balance with worsening outcome. Therefore it would perhaps be a challenge to investigate to effect of early fluid balance control on endpoints such as ventilator free days, ICU stay, and hospital stay and survival rates. In that context, an accurate prognostic scoring system for risk assessment for need for RRT within seven days of admission would be needed and helpful.

Several studies describe the possible role for biomarkers for AKI, such as plasma NGAL, to participate in a predictive model for risk assessment for the need for RRT. With this study we tried to create an externally validate an easy to use prognostic clinical model and nomogram for risk assessment of initiation of RRT within seven days of ICU admission using two large cohorts of adult critically ill patients and to assess the additive role in this prediction for plasma NGAL.

Methods

Participants

The institutional review board of the Erasmus MC University Medical Center in Rotterdam, the Netherlands approved the study. All consecutive admitted patients to the general adult ICU (which treats general surgical, trauma, medical, neurological and neurosurgical patients, but not cardiac surgery patients) between September 2007 and April 2008 were eligible for enrollment. Exclusion criteria included age under 18 years, refusal of consent, nephrectomy, chronic kidney disease (CKD stages 3, 4 and 5 based on eGFR criteria calculated with the baseline SCr values) and renal transplantation. Deferred consent was used, and written informed consent was obtained from all participants or their heath care proxy according to our deferred consent procedure [40].

Data collection

Immediately following admission (T=0), blood samples were collected in EDTA tubes, processed and stored at -80°C. Serum creatinine (SCr) concentrations were measured at admission and thereafter daily at 6:00 am using the Roche enzymatic kit which is traceable to a reference method based on isotope dilution-mass spectrometry. Plasma NGAL was quantified with the Triage® point-of-care immunoassay [41]. The corresponding estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study Equation (MDRD) [1]. Baseline SCr was defined as the patient's steady state level within the four weeks prior to ICU admission. If this was not available, the admission value was used as the baseline SCr value. Other recorded variables included age, gender, body mass index (BMI), clinical diagnosis of sepsis, bicarbonate levels (HCO_3^-), Urea, white blood cell count (WBC), Acute Physiology and Chronic Health Evaluation score (APACHE II), sequential organ failure assessment score (SOFA) and cumulative urine output (UP). The primary outcome variable was the need for RRT within seven days after ICU admission. AKI was classified as using AKIN criteria (Mehta) for increases in SCr only [42]. The diagnosis of sepsis was scored applying the international consensus criteria [43].

NGAL quantification

Plasma NGAL concentrations were measured using the Triage® NGAL Test point-of-care fluorescence immunoassay on a Triage MeterPlus (Biosite Inc, San Diego USA) using a commercially available NGAL cartridge. The devices used reported plasma NGAL concentrations between 46 and 1300 ng/ml. Urine NGAL levels were measured using a non-commercial Triage® immunoassay cartridge with a sandwich format immunoassay.

Statistical analysis

Patients with plasma NGAL concentrations missing at admission were excluded; other missing data were statistically imputed using their expected values conditional on the observed NGAL levels, clinical parameters and the occurrence of AKI. The univariable predictive value of NGAL for the occurrence of RRT was explored. The Area Under the receiver operating characteristic Curve (AUC) and its 95% confidence was used to assess the ability of the models to discriminate between RRT and no-RRT, and to quantify the difference between models with and without NGAL. The 95% CI of AUC was calculated by a bootstrap resampling method. A prediction model was developed based on multivariable logistic regression. Internal validation was assessed using bootstrap resampling. The model was fitted to an independent data from a single center prospective observational study of consecutive admitted adult ICU patients from Vicenza, Italy. Patients were enrolled between January and July 2007. This trial was originally designed as a prospective observational study evaluating consensus definitions for AKI

[44]. Patients with end stage renal disease on chronic RRT were excluded in the original study. Patients with CKD stages 3 or more were further excluded for the purpose of this study. Only plasma NGAL was available in this study. To assess the applicability of the model to the independent dataset, we compared the regression coefficients between both datasets and tested for interactions between predictors and dataset. Finally, a scoring system was developed on the basis of regression coefficients in the multivariable model fitted on the combined datasets, including possible interactions, to obtain best estimates for both datasets and optimal precision. Statistical analyses were performed in R statistical software (R Foundation, Vienna, Austria).

Results

Descriptives

In total 663 patients were included in the study of which 558 had no-AKI at admission and 654 did not receive RRT within the first 24 hours after admission. Thus for developing the nomogram 654 participated in the analysis, their mean age was 56 years and 58% of this cohort was of male gender. Two hundred and two (30%) patients developed AKI and 66 (10%) were diagnosed with sepsis. The mean APACHE II and SOFA scores for the first 24 hours of admission were 19 (sd=7.5) and 6.2 (sd=3.8) respectively. At admission mean SCr concentrations were 90 (sd=68) μmol/L with a corresponding MDRD eGFR of 100 (sd=84) mL/min/1.73 m^2. One hundred forty five patients (22%) died during the study (Table 1).

Neutrophil gelatinase associated Lipocalin

Plasma NGAL concentrations at admission were elevated in patients who were initiated on RRT compared to patients who were not. The relationship between NGAL and the probability of developing AKI was approximately linear.

Prediction model

Based on the literature and previous work we identified sepsis, MDRD eGFR as established clinical predictors of severe AKI and RRT. Both parameters were strong predictors of AKI or RRT in our data, and the additional predictive value of NGAL over these clinical predictors was limited. The AUC did not increase in the validation cohort when adding plasma NGAL to the model, and only 0.2 points in the external validation cohort (AUC 0.87 to 0.89). Since the additional predictive value of NGAL was limited our final prediction model was based on the clinical predictors; sepsis (Odds ratio (OR) =5.0, $p<0.01$), MDRD eGFR (OR=0.96, $p<0.01$) measured at ICU admission (Table 2.) The AUC of the model at T=0 was 0.92 and adding plasma NGAL did not add any

Table 1: Descriptive statistics of clinical characteristics and outcome

	Derivation cohort		Validation cohort	
	All patients (n=663)	AKI=0 at T0 (n=558)	All patients (n=279)	AKI=0 at T0 (n=200)
Mean age (sd)	56 (17)	56 (17)	58 (20)	54 (20)
Males (%)	387 (58%)	329 (59%)	195 (67%)	142 (71%)
Mean BMI (sd)	26 (17)	26 (18)	-	-
Mean SCR (µmol/l) (sd)	90 (68)	71 (24)	105 (67)	
Mean HCO3 (sd)	22 (5.3)	22 (4.8)	-	-
Mean Urea (mmol/l) (sd)	8.3 (7.0)	6.6 (3.9)	8.6 (6.7)	6.2 (3.0)
Mean WBC (sd)	12 (8.0)	12 (5.7)	-	-
Mean Apache II (sd)	19 (7.5)	18 (7.2)	18 (7.3)	16 (7.3)
Mean SOFA (sd)	6.2 (3.8)	5.6 (3.4)	5.7 (2.7)	5.2 (2.3)
Mean Urine production first 24 hours (ml) (sd)	2113 (1296)	2214 (1284)	2541 (1178)	2545 (1144)
Mean MDFDeGFR (sd)	100 (84)	111 (87)	83 (62)	95 (65)
Sepsis (%)	66 (10%)	31 (6%)	16 (6%)	6 (3%)
Mean NGAL plasma (sd)	264 (270)	205 (177)	187 (246)	116 (122)
Mean Days in ICU (sd)	8.3 (11)	7.5 (9.8)	11 (11)	10.3
AKIN 0 (%) AKIN 1 (%) AKIN 2(%) AKIN 3 (%)	461 (70%) 96 (14%) 45 (7%) 61 (9%)	455 (82%) 68 (12%) 22 (4%) 13 (2%)	81 (65%) 55 (20%) 20 (7%) 22 (8%)	167 (84%) 21 (15%) 0 (0%) 1 (0.5%)
CVVH (%) Mean time to CVVH (hours) (sd)	29 (4%) 70 (64)	8 (1%) 85 (46)	9 (3%)	1 (0.5%)
Mortality (%)	145 (22%)	101 (18%)	44 (16%)	23 (12%)

BMI: Body Mass Index; SCr: serum creatinine; $HCO3^-$: plasma bicarbonate concentration; Urea: Plasma urea concentration; WBC: white blood cell count; APACHE II: Acute Physiology and Chronic Health Evaluation score; SOFA: sequential organ failure assessment score; UP-24: urine production over first 24 hours; MDRD eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation measured at ICU admission; RIFLE: Risk-Injury-Failure classification based upon a percentage increase in serum creatinine, NGAL: neutrophil gelatinase associated lipocalin. Values are expressed in mean (standard deviation) or proportions in percentage.

Table 2: Multivariable odds ratio's from logistic regression model for prediction of AKIN 0-3 and CVVH.

	Odds ratio	P value	Odds ratio	P value
Outcome AKIN	Derivation cohort AKI=0 at T0 (n=558)		Validation cohort AKI=0 at T0 (n=200)	
Sepsis	5.5	<0.01	11.9	0.05
MDRD eGFR	0.973	<0.01	0.941	<0.01
+ NGAL				
Sepsis	3.1	0.01	4.9	0.23
MDRD eGFR	0.975	<0.01	0.942	<0.01
pNGAL	1.003	<0.01	1.005	0.01
Outcome CVVH	No CVVH within 24 hours (n=654)		No CVVH within 24 hours (n=277)	
Sepsis	5.0	<0.01	4.5	0.01
MDRD eGFR	0.955	<0.01	0.957	0.08
+ NGAL				
Sepsis	3.9	0.01	4.2	0.126
MDRD eGFR	0.960	<0.01	0.97	0.106
pNGAL	1.001	0.20	1.002	0.001

MDRD eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation measured at ICU admission; NGAL: neutrophil gelatinase associated lipocalin; AKI: acute kidney injury

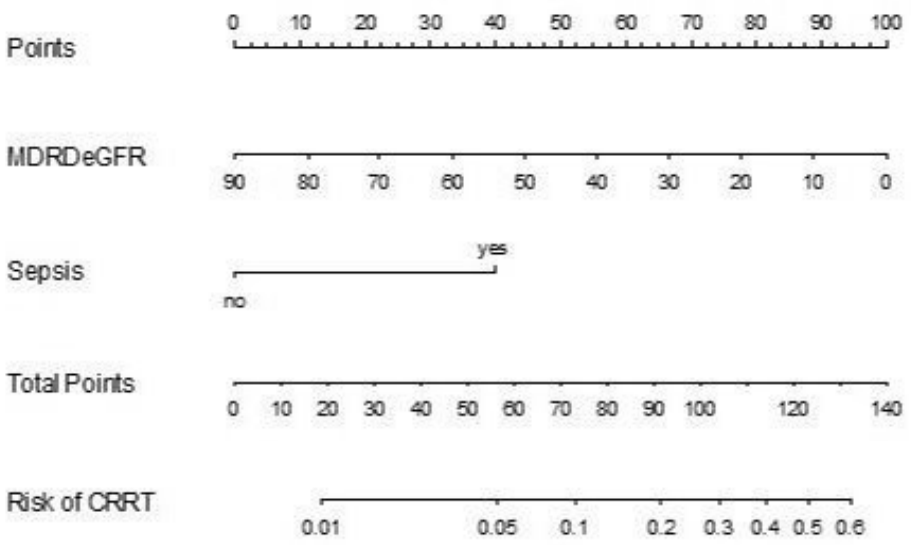

Figure 1: Nomogram for risk assessment of initiation of RRT within 7 days of ICU admission in patients without AKI at admission. MDRD eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation measured at ICU admission; NGAL: neutrophil gelatinase associated lipocalin; AKI: acute kidney injury; CRRT: continuous renal replacement therapy.

predictive value. The validation data set comprised 279 patients, of which 9 (3%) were initiated for RRT within 7 days after admission, which was very comparable to our own data. The baseline characteristics were highly comparable between the two datasets. The incidence of sepsis was comparable between both cohorts, the mean MDRD eGFR was lower in the validation data (83 versus 100 ml/min/1.73 m^2) and mean urine production was higher (2541 versus 2113). In the validation set, only plasma NGAL at admission was available. Mean plasma NGAL was 187 (sd=246) in the total population, 545 (sd=411) in patients who developed severe AKI and 151 (sd=187) in patients who did not, which was compatible with our data. With regard to the predictive value of NGAL, we confirmed our finding that although high NGAL is associated with a higher probability of developing severe AKI, the predictive value over clinical variables is limited. The prediction model performed well in validation set. Calibration was reasonable and discrimination was good (AUC admission model=0.87, AUC 24h model =0.75).

Nomogram for RRT risk assessment

The final nomogram was developed on the combined development and validation data. The scores for individual patients can be calculated as shown in figure 1 and the corresponding predicted probabilities of RRT within 7 days after admission can be obtained (Figure 1).

Table 3a and b: Predictive performance of the models.

A:

	Cutoff	Sepsis + eGFR	Sepsis + eGFR + NGAL
Internal validation	AKIN 0 vs. ≥1	0.75	0.78
	AKIN ≤1 vs. ≥2	0.76	0.83
	AKIN ≤2 vs. ≥3	0.84	0.89
External validation	AKIN 0 vs. ≥1 AKIN ≤1 vs. ≥2	0.82	0.86
	AKIN ≤2 vs. ≥3	0.81	0.93
		0.81	0.93

B:

	Sepsis + eGFR	Sepsis + eGFR + NGAL
Internal validation	0.92	0.92
External validation	0.87	0.89

MDRD eGFR: estimated glomerular filtration rate by the Modification of Diet in Renal Disease Study Equation measured at ICU admission; NGAL: neutrophil gelatinase associated lipocalin; AKI: acute kidney injury.

Discussion

The nomogram for risk assessment of RRT within 7 days of ICU admission at the time of ICU admission is an internally and externally validated predictive model that uses acute phase and easy-to-obtain clinical characteristics, which might be applied supporting the decision to start early RRT. On the basis of the MDRD eGFR value at admission and the clinical diagnosis of sepsis, the model accurately predicts the progression to need of RRT. This model provides a practical tool for physicians to identify individual patients with a high risk of severe AKI right at ICU admission.

In four previous studies NGAL either measured in plasma or urine was consistently identified as a strong predictor for (severe) AKI in general adult critically ill patients [12, 41, 45, 46]. However, uncertainty still existed on the true additive value of NGAL for the prediction at the time of ICU admission, and accordingly the clinical applicability, on top of the conventional clinical characteristics routinely measured. The current study provides clarity on this matter and offers a model to predict outcome on the basis of an empirically weighted combination of predictors based on a large cohort with detailed and accurate information. The finding that NGAL levels do not add predictive value to clinical characteristics in the ICU setting was very robust, as shown by replication of the results in the different sensitivity analyses and in the independent data from Italy.

Further improvement of predictive models would require the inclusion of additional variables, although it is unlikely that these variables would be clinical characteristics or currently available biomarkers. Our analysis shows that the additional early contribution of NGAL measured in plasma is limited in the setting of admission to the adult Intensive Care. Although several clinical studies in other patient cohorts such as pediatric and adult cardiopulmonary bypass patients have shown differently [5]. It is fair to say that, at least in our cohort, patients likely to develop severe AKI are admitted to the ICU already in an advanced stage of their illness.

One conclusion from this study is that for an accurate prediction baseline eGFR values are not necessary. In the clinical practice of emergency medicine steady state baseline SCr and the corresponding estimated GFR are often not available which is required to assess AKIN scores. Furthermore, these scoring systems are developed as primarily retrospective tools for the purpose severity of disease scoring purposes.

The nomogram developed here was based upon a distinct group of ICU patients, which might restrict clinical applicability. First, chronic kidney disease (CKD stage 3 or more) patients were excluded from the analysis. However, it is likely that those patients are easily identified at ICU admission due to the knowledge of patient chart history which will create a natural awareness of susceptibility for renal injury and progression of AKI on top of CKD. Second, the model was based on adult patients only and it is therefore uncertain whether it is applicable for the identification of pediatric patients. Third, the

nomogram might only be directly applicable to patients treated in hospitals with high-level of supportive and therapeutic care. And fourth, perhaps a further refinement of this proposed model is needed for example taking into account the gender differences in absolute SCr values. Unfortunately even this presented patient cohort was not large enough to perform a meaningful subset analysis.

The nomogram was well applicable to the independent data set from Italy, although there were some differences between the development and validation data. Mean eGFR was lower in the validation data, probably due to the inclusion of some chronic renal patients in the validation cohort. Nevertheless the predictive effect of eGFR was similar in both data sets. Urine production was higher in the validation data and also the predictive effect of low urine production was smaller. We expect this to be due to the more prevalent administration in Italy, compared to the Netherlands. Diuretics artificially increase urine production and mask the high probability of AKI in these patients. The number of patients with sepsis was substantially higher in the validation data, and the predictive effect much smaller. We suspect that this is due to the differences in clinical setting (academic vs tertiary hospital). A limitation of the validation set is the limited number of patients (9) who were initiated on RRT after 24 hours of admission. Future trials could now focus on new forms of treatment or early intervention such as the initiation of RRT for these very poor prognostic subgroups, especially if such treatments or interventions are potentially hazardous and patients with high chance of recovery should be excluded.

Conclusions

The nomogram for RRT risk assessment is an accurate and easy to use scoring system to be used at ICU admission. It is based upon clinical parameters readily available at ICU admission without the requirement of steady state baseline SCr values. Furthermore, although plasma NGAL is an accurate predictor for (severe) AKI at ICU admission its additional value over clinical predictors is limited.

Contributors

HdG participated in creating the study protocol, patient inclusion, data collection, data management, data analysis and model development. HL and EWS participated in data analysis and model development. DC facilitated the external validation of the analysis and participated in data interpretation. MB and JB participated in data analysis and interpretation. All authors contributed to the writing of the manuscript and read and approved the final manuscript.

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Chapter 8

Neutrophil gelatinase-associated lipocalin clearance during veno-venous continuous renal replacement therapy in critically ill patients

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Predicting recovery of renal function in patients with acute kidney injury (AKI) supported with renal replacement therapy (RRT) is one of the top ten questions in the field of current AKI research. However, defining renal recovery and accordingly the best time for discontinuation of RRT is difficult as reliable biomarkers are lacking.

The Best Kidney study identified urine production (>500 ml/24 hrs) as the best predictor for successful discontinuation from RRT (AUC 0.81) in contrast to SCr (AUC 0.64), however it's predictive value is severely confounded by the use of diuretics [47]. Neutrophil Gelatinase associated Lipocalin (NGAL), a 25 kDa protein involved in iron transportation, is a potential tool for the determination of initiation of RRT [46, 48]. However, once a patient is initiated on RRT it remains to be elucidated how plasma NGAL concentrations will change. In order to determine plasma NGAL clearance and sieving coefficient (SQ) during CRRT, we measured pre-filter and effluent NGAL concentrations in three subjects after informed consent was obtained. Patients were hemofiltered with zero fluid balance, 2 liter post dilution mode in continuous veno-venous hemofiltration. Blood flow was set at 200 ml/min and regional citrate anticoagulation was used. The filters were ETO sterilized Aquamax HF 19 with an in vitro cut-off point of 55 kDa. NGAL levels were measured using a research based Enzyme-Linked Immunosorbent Assay with a detection limit of 10 to 4000 ng/ml. NGAL clearance per minute was estimated using the assumed stable plasma concentrations during CRRT over the first 12hrs and the calculated amount of NGAL present in the total effluent. SQ was calculated dividing the NGAL effluent concentration by the NGAL pre-filter plasma concentration.

Three male anuric patients were studied (age 40-64 years). Their admission diagnoses were hemorrhagic shock, cardiopulmonary resuscitation and multitrauma. Baseline SCr values were 0.57, 0.66 and 0.68 mg/dL with SCr at time of CRRT initiation of 3.7, 9.7 and 12.3 mg/dL. The estimated median plasma NGAL clearance (SD) was 4.8 (1.89) ml/min with a median SQ (SD) of 0.147 (0.04). The pre-filter and effluent NGAL concentrations were plotted against time (Fig.1).

Normally, plasma NGAL is cleared through glomerular filtration and entirely processed by the proximal tubular cells [49]. However in case of AKI, plasma concentrations quickly rise even when eGFR is still normal and tend to quickly decrease when the injurious event subsides [5].Therefore, NGAL might have the potential to be an early indicator of renal recovery in critically ill patients supported by RRT. Our results suggest that plasma NGAL is hardly cleared during continuous veno-venous hemofiltration. A possible explanation lies in the fact that larger serum proteins effectively lower the passage of small proteins by forming a gel layer over the membrane pores.

In conclusion, we have shown that CRRT does not substantially influence a patients' plasma NGAL concentration and therefore doesn't need to be taken into account when kinetics of plasma NGAL levels are used as an indicator for persistent renal injury or renal recovery in hemofiltered critically ill patients.

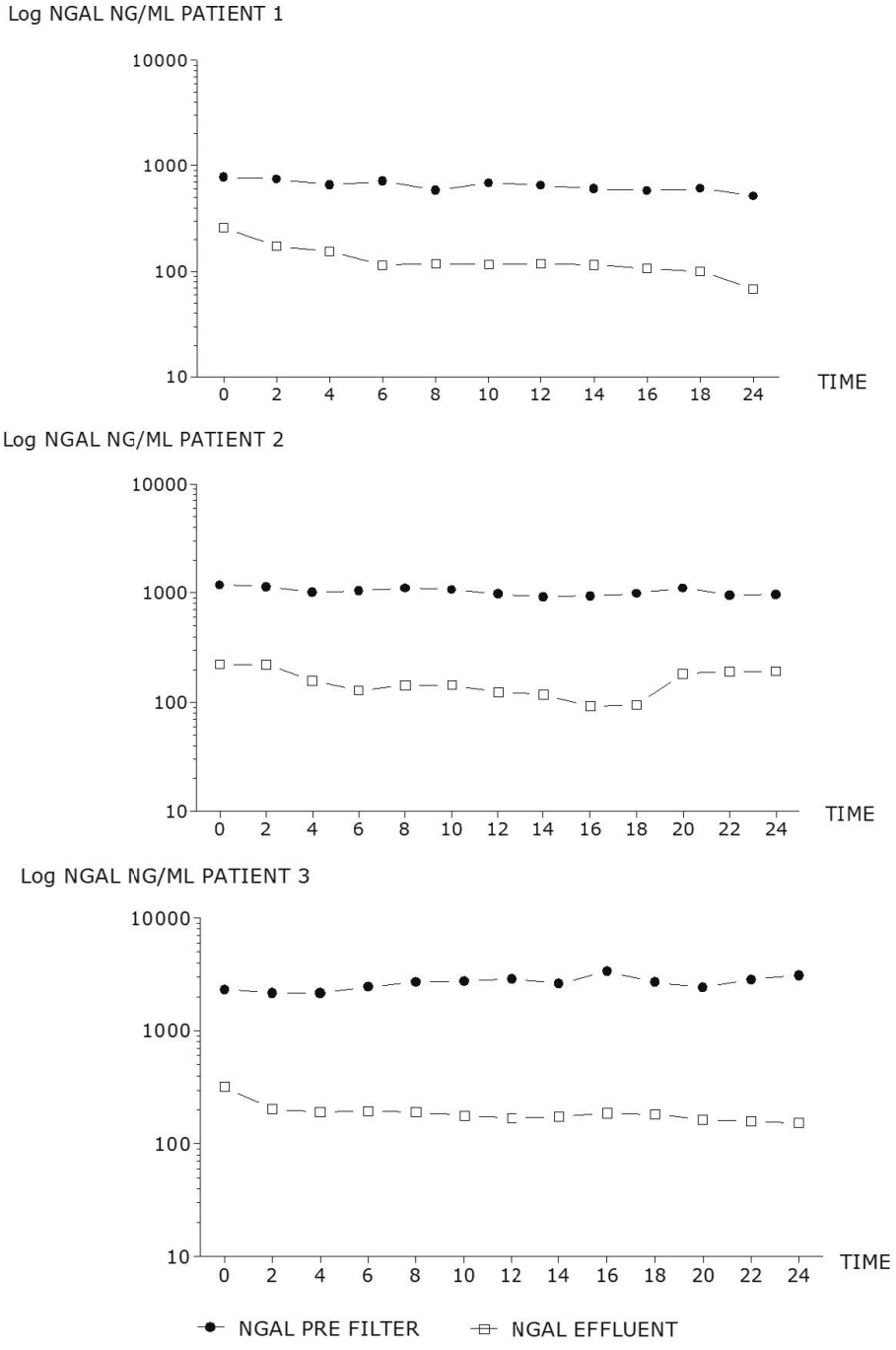

Figure 1: Temporal patterns of the pre-filter plasma and effluent NGAL concentrations (ng/ml) in hours after initiation of continuous veno-venous renal replacement therapy in 3 individuals with anuric AKI.

Summary

Introduction

In this thesis we describe the role of biomarkers in the prediction and diagnosis of acute kidney injury in adult critically ill patients. The main goals were to provide an overview of the available literature, to study the predictive properties of biomarkers in a large cohort of adult critically ill patients and to rationalize the limitations for their applicability in clinical practice. The second part of this thesis discusses the applicability of biomarkers in clinical decision modelling for (early) initiation of renal replacement therapy.

Overview of the literature

The first chapter describes the, at that time available, literature concerning the predictive value of several biomarkers for AKI in adult critically ill patients. The difference between early prediction and (late) diagnosis is emphasized. This is an evident issue since the inclusion of patients with an already risen SCr influences "predictive" test properties (John Kellum: There is no use in predicting rain when it is already raining). This is one of the explanations for the widely varying predictive test results of individual biomarkers.

Biomarkers and AKI

Chapter two describes the most important study of this thesis based upon a prospectively enrolled cohort of 700 adult critically ill patients recruited from 1 September 2007 until 1 April 2008. In all consecutively admitted patients blood and urine was sampled for biomarker measurements at 8 time points after ICU admission. Both plasma and urine Neutrophil gelatinase associated lipocalin concentrations were measured and tested for their predictive and diagnostic value for AKI. An important difficulty was the uncertain sample-time-to-injury relationship compared to a fixed model of cardiopulmonary bypass patients. Therefore the time-relationship between measurement and the predicted endpoint of AKI is important in the interpretation of the generated data. In this study the predictive value of biomarker concentrations measured at the time of ICU admission was evaluated. The results show that NGAL is just as good in predicting AKI as the functional marker eGFR, emphasizing however that patients with a slightly elevated SCr were not excluded for this analysis. NGAL concentrations in plasma and urine significantly increase when AKI is more severe classified by the RIFLE score. In patients with a seemingly normal eGFR at the time of ICU admission, NGAL's predictive performance is better compared to eGFR; even in a multivariable model with several clinical predictors for AKI. Approximately 60% of the patients who developed AKI during the first 7 days of their ICU stay already had a reduced eGFR at the time of ICU admission. Sepsis increases the levels of urine NGAL irrespective of the presence of AKI according to the RIFLE classification.

The definition for AKI has been a matter of debate for a long time now. The currently accepted international consensus definition is based upon a dichotomous outcome measure: The presence or absence of AKI based upon a rise in SCr compared to a "steady state" baseline value with an artificially chosen cut-off point of 50% rise in SCr. This assumption ignores the pathofysiological background of AKI which shows that the process of AKI is a gradual phenomenon wherein there is cumulative and progressive damage to the cells of the proximal and distal tubules. Although it is commonly accepted that SCr is not a sensitive measure of acute less serious tubular damage, there is at present no alternative. It is therefore conceivable that under certain circumstances there might be a degree of tubular cellular damage resulting in excretion of hypersensitive biomarkers which is not yet reflected in an increase in SCr.
In Chapter three is discussed whether plasma NGAL, urine NGAL and plasma and urine CyC are distinctive for transient and sustained kidney damage. Plasma NGAL appears to be increased for a short period of time in patients with transient AKI and is therefore in the early phases of AKI not very distinctive between both conditions in contrast to urine NGAL. The behaviour of plasma CyC is similar to that of SCr, which seems logical since both are functional markers instead of acute damage markers. Urine CyC is unfit for early distinction and displays a late expression in sustained AKI as well.

Plasma and urine NGAL are, although there is some overlap in patients with pre-existing albuminuria, of different origin. Plasma NGAL originates from several different kinds of epithelial tissue (lung, intestine and kidney) and from specific granulae of neutrophils. Therefore there are concerns about the effect that a condition such as a systemic inflammatory response syndrome has on the diagnostic properties of plasma NGAL for AKI. Chapter four describes the effect of severe sepsis and septic shock on the diagnostic test properties of plasma NGAL for AKI. Evidently the production of plasma NGAL is increased indeed in patients with severe sepsis and septic shock compared to patients with other conditions, even irrespective of the presence of SCr based AKI. Despite the higher levels of plasma NGAL in these patients, it turns out that the diagnostic test properties for AKI remain to be similar. However, optimal cut-off values are higher in patients with sepsis and septic shock. Therefore, it is of outmost importance to realize that for the applicability of absolute biomarker concentrations in clinical decision models stratification for underlying disease state is justified (sepsis vs non-sepsis).

In Chapter five is tried to find an answer to the question why the reported predictive test property of biomarkers for AKI such as urine NGAL, KIM-1, pi- and alpha GST varies so widely and why they are so mutually different. In this study patients with an increased SCr at ICU admission and a diagnosis of sepsis where excluded. Furthermore, in order to study the biomarker expression pattern before the time of first rise in SCr time points were

recoded as such. NGAL turned out to be the most consistent marker which is expressed earlier compared to the other three biomarkers. The predictive performance of NGAL increased closer to the endpoint of (first) AKI. KIM-1 is a late marker discriminating for AKI only at the time of AKI. The expression of the constitutional enzymes pi and alpha GST is exhibited in a very short time frame. Even at peak levels the predictive value for AKI is modest for both enzymes. Time and site of tubular injury should be taken into account for the interpretation of reported predictive test properties for AKI. This study demonstrates different expression patterns of biomarkers which might imply different fields of clinical utility.

Biomarkers and RRT

Chapter six provides an overview of the available literature which describes how biomarkers predict the need for renal replacement therapy (RRT). Worldwide, there is a wide variation in clinical practice when it comes to the time of RRT initiation. Several research groups have tried to study the effect of "early" initiation of RRT on hard outcomes such as mortality and ICU length of stay. However, without a single answer. Problematic in the interpretation of these data is that the applied definitions of early versus late are so diverse that the results of these studies are incomparable. This chapter explores the probable contribution of biomarker based decision models for prediction of initiation of RRT. The literature review shows that different biomarkers have the potential to perhaps contribute to the decision process. But at the same time, it must be recognized, supported in part by the results described in chapter four, that there are limitations in the in advance incorporation of cut-off values in these kind of clinical models. Actually, there is still too little literature available that can support that biomarkers should be routinely applied in clinical decision making for initiation or termination of invasive supportive therapy such as renal replacement therapy.

With chapter six in mind, an effort was made to study in the available dataset which predictors are associated with the initiation of RRT. Chapter seven describes the results of this analysis and concludes that especially in septic patients the combination of this diagnosis and the available eGFR at the time of admission alone is the strongest predictor for the need for RRT. The clinical algorithm generated from the "Rotterdam cohort" has been validated in a similar cohort of adult critically ill patients in the San Bartolo Hospital in Vicenza, Italy resulting in similar results. By combining both datasets a nomogram could be developed, which might be a practical tool for clinical application. The additive contribution of plasma NGAL to this model is negligible. Therefore, it seems, at least in predicting the need for RRT in sepsis related AKI, there is no additive role for plasma NGAL.

If the kidney is completely failing in critically ill patients this may, albeit imperfect compared to the healthy kidney, temporarily be replaced with a machine for renal replacement therapy. The principle of dialysis or hemofiltration depends on taking blood through a filter where waste, in the form of small molecules dissolved in the plasma, is washed out. These filters possess a certain pore size with the aim above all to remove small molecules but not larger substances such as proteins and red or white blood cells. Because creatinine travels easily through these filters it is often unclear, while being initiated on a RRT device, if recovery of renal function has occurred. Furthermore, urine production is often suppressed in the diseased kidney while being supported by RRT, due to the fact that when a patient is euvolemic there is simply not an evident excessive need to do so. Plasma NGAL is a small protein that accumulates in patients with acute renal failure. When a decreasing concentration of plasma NGAL might perhaps imply endogenous renal recovery this might be a potential predictor for successfully weaning off the extracorporeal support. However, at first it should be investigated if plasma is cleared (molecular seize 35 KDa) in patients still supported by RRT. Three case studies were performed and described in Chapter 8 in patients initiated on CRRT (CVVH) using a high cut-off filters (EFO Aquamax HF-19, in vitro cut-off point of 55 kDa). NGAL concentrations were measured before the hemofilter and in the effluent volume at 12 time points after initiation of CVVH. The calculated clearance of plasma NGAL is 4.8 ml-min. Thus there is some permeability, but the effective clearance is low so that potentially substantial decrease in plasma NGAL concentrations over a period of time might be tested as a predictor for renal recovery. And associated with that a potential tool for the decision to terminate CRRT successfully.

Samenvatting

Inleiding

In dit proefschrift wordt de rol van biomarkers bij het voorspellen en diagnostiseren van acute nier schade bij volwassen kritisch zieke patiënten beschreven. De doelstellingen waren om een overzicht te geven van de beschikbare literatuur, de predictieve waarde te bestuderen in een grote populatie volwassen intensive care patiënten en om te rationaliseren wat de beperkingen zijn voor het gebruik in de klinische praktijk. Het tweede gedeelte van dit proefschrift behandelt de toepassing van biomarkers in een klinisch besliskundige model voor (vroege) initiatie van nierfunctievervangende therapie.

Literatuur overzicht

Het eerste Hoofdstuk beschrijft de op dat moment beschikbaar literatuur over de predictieve waarde van verschillende biomarkers voor acute nierschade bij volwassen intensive care patiënten. Daarin wordt benadrukt dat er een verschil bestaat tussen vroege predictie en (late) diagnose stelling. Dit is van belang omdat de inclusie van patiënten waarbij het SCr al is verhoogd de test eigenschappen beïnvloedt (John Kellum: There is no use in predicting rain when it is already raining). Dit is mede een verklaring voor de uiteenlopende test resultaten die gepubliceerd zijn.

Biomarkers en AKI

In Hoofdstuk twee wordt de belangrijkste studie van dit proefschrift gepresenteerd die gebaseerd is op een cohort van 700 volwassen IC patiënten gerekruteerd van 1 september 2007 tot 1 april 2008. Bij alle opeenvolgende opnames werd op een acht tal tijdspunten bloed en urine afgenomen voor biomarker bepalingen. Zowel plasma als urine neutrophil gelatinase associated lipocalin werden gemeten en beoordeeld naar hun voorspellende en diagnostische waarde voor acute nierschade. Een belangrijke moeilijkheid in dit cohort is dat er geen vastgesteld moment is waarop de nierschade begint zoals dat het geval is in cardiochirurgische patiënten. Daarom is de tijdsrelatie tussen de meetpunten en het te voorspellen eindpunt van belang voor de interpretatie van de gegevens. In deze studie is vooral gekeken naar de predictieve waarde van de concentratie direct bij binnenkomst op de IC. Hieruit blijkt dat NGAL even goed voorspellend is voor AKI als de klaringsmaat eGFR, aantekenend dat de mensen met reeds aanwijzingen voor nierschade niet geexcludeerd zijn. De concentraties van NGAL in plasma en urine nemen significant toe met de ernst van AKI gescoord volgens RIFLE. Bij patiënten met een normale eGFR bij IC opname voorspelt NGAL wel beter; zelfs in een multivariabel model met andere klinische parameters. Ongeveer 60% van de patiënten die AKI ontwikkelen op de IC hebben al een verminderde klaring bij IC opname. Sepsis induceert de excretie van NGAL in de urine onafhankelijk van de aanwezigheid van AKI volgens het RIFLE score systeem.

De definitie van AKI staat al langere tijd ter discussie. De huidige internationaal geaccepteerde consensus is die van een dichotome classificatie: Er is wel of geen AKI gebaseerd op een toename van serum creatinine ten op zichten van een "steady state" baseline waarde. Deze aanname gaat voorbij aan de pathosfysiologische achtergrond van AKI die leert dat het proces een geleidelijk fenomeen is waarbij er een cumulatieve en progressieve schade optreedt aan tubulus cellen. Hoewel het duidelijk is dat SCr geen gevoelige maat is voor acute minder ernstige schade is er op dit moment geen alternatief. Het is dan ook voorstelbaar dat er mate van schade is die zich (nog) niet uit in een toename van SCr maar wel in de productie en of excretie van hypersensitieve biomarkers. In Hoofdstuk drie is daarom gekeken of plasma en urine NGAL en Cystatine-C onderscheidend zijn voor lichte nierschade van voorbijgaande aard (Transient AKI) en aanhoudende nierschade (Sustained AKI) met een groter beschadigend effect. Plasma NGAL blijkt kortdurend verhoogd te zijn in patiënten met transient AKI en is daarom in de beginfase niet erg onderscheidend tussen beide. Dit in tegen stelling tot urine NGAL. Het gedrag van plasma Cystatine-C is vergelijkbaar met dat van SCr, wat logisch lijkt aangezien beide functionele biomarkers zijn in plaats van acute schade markers. Urine Cystatine-C is ongeschikt en is zelfs voor sustained AKI een biomarker die laat tot expressie komt.

Voor plasma en urine NGAL geldt dat zij, hoewel er overlap is beschreven in patiënten met pre-existente albuminurie, van verschillende origine zijn. Omdat NGAL zich ook bevindt in andere epithelia zoals longweefsel en darmweefsel en in granulae van specifieke witte bloedcellen bestaat er zorg over het effect dat een systemic inflammatory response syndrome (SIRS) heeft op de diagnostische waarde van plasma NGAL voor AKI. Hoofdstuk vier beschrijft het effect van severe sepsis en septic shock op de test eigenschappen. Duidelijk naar voren komt dat er inderdaad meer plasma NGAL gegenereerd wordt bij severe sepsis en septic shock in vergelijking met patiënten met andere aandoeningen, zelfs bij de patiënten die volgens de SCr definitie geen AKI ontwikkelen. Ondanks deze verschillen blijkt de diagnostische waarde voor AKI gelijk te zijn in de groep patiënten met sepsis versus de groep patiënten zonder. Echter, de optimale cut-off waardes voor de diagnostische toets zijn hoger bij sepsis in vergelijking tot non-sepsis. Belangrijk om te realiseren is dan ook dat voor het gebruik van absolute concentraties van plasma NGAL voor klinische beslissingen er op zijn minst gestratificeerd zou moeten worden voor onderliggende ziekte (sepsis vs non-sepsis).
Met Hoofdstuk vijf wordt er voor verschillende urine biomarkers (NGAL, KIM-1, alpha- en pi-GST) gezocht naar een antwoord op de vraag waarom de predictieve waarde van deze markers in de literatuur zo uiteenloopt en onderling zo verschillend is. Daartoe zijn alle patiënten met een gestegen SCr bij opname geexcludeerd, evenals patiënten met sepsis. Er is een recodering toegepast om de expressie van de afzonderlijke

biomarkers inzichtelijk te maken voor het tijdspunt dat er voor het eerst een stijging van SCr detecteerbaar is. Van de ge-upreguleerde proteïnen is NGAL de vroegste en meest consistente biomarker. Waarbij de predictieve waarde beter wordt naar mate het meetpunt dichter bij het eindpunt komt. KIM-1 is een late marker die juist op het moment van eerste stijging van SCr het eerste discriminerende moment kent. De constitutionele enzymen vertonen een expressie in een zeer kort tijdsbestek. Zelfs op het punt van de hoogst gemeten concentraties is de predictieve waarde bescheiden. De tijd en plaats van tubulaire schade in relatie tot het meten van een biomarker in urine is dus van invloed op test eigenschappen. Het is voorstelbaar dat de met deze studie aangetoonde verschillende eigenschappen van de biomarkers een verschillend terrein van toepassing geïdentificeerd kan worden.

Biomarkers en RRT

Hoofdstuk zes geeft een overzicht van de beschikbare literatuur die beschrijft hoe biomarkers voorspellen voor de noodzaak tot start van nierfunctievervangende therapie (RRT). Wereldwijd bestaat er een grote variatie in de klinische praktijk als het gaat om het moment van starten van RRT. Verschillende onderzoeksgroepen hebben getracht om het effect van vroeger of later starten van RRT te bestuderen op harde uitkomsten zoals mortaliteit of ICU ligduur, echter zonder een eenduidig antwoord. Want, problematisch bij de interpretatie van die data is dat de definities van vroeg of laat starten zo ver uiteenlopen dat de onderzoeksgegevens nauwelijks onderling te vergelijken zijn. Daarom wordt in dit hoofdstuk geëxploreerd wat de bijdrage van biomarkers voor AKI en of RRT zou kunnen zijn in een algoritme dat zou kunnen ondersteunen in de klinische besluitvorming voor RRT. Het literatuur overzicht laat zien dat verschillende biomarkers de potentie hebben om wellicht bij te dragen aan deze besluitvorming. Maar tegelijkertijd moet erkend worden, mede ondersteund door de resultaten beschreven in Hoofdstuk vier, dat er limitaties zijn bij het op voorhand incorporeren van cut-off waarden van biomarkers in dit soort modellen. Eigenlijk is er nog te weinig literatuur voorhanden die kan ondersteunen dat biomarkers routine matig moeten worden toegepast in de klinische besluitvorming voor het starten of stoppen van een invasieve ondersteunende therapie zoals nierfunctievervanging.

In het licht van de achtergronden uit Hoofdstuk zes is er gekeken in het NGAL cohort welke van de beschikbare predictoren geassocieerd zijn met het starten van RRT. Hoofdstuk zeven beschrijft de uitkomsten van deze analyse en concludeert dat vooral het hebben van sepsis in combinatie met de waarde van de estimated glomerular filtration rate (eGFR) bij ICU opname het meest predictief zijn voor de initiatie van RRT. Het klinische beslismodel dat gegenereerd is uit het NGAL cohort uit Rotterdam is in deze studie gevalideerd in een vergelijkbaar cohort van volwassen ICU patiënten in het

San Bortolo Hospital te Vicenza, Italië met als resultaat zeer vergelijkbare uitkomsten. Door beide cohorten te combineren is er een nomogram ontwikkeld, wat een praktische tool zou kunnen zijn voor klinische toepassing om op het moment van ICU opname een kans berekening te maken. De additieve bijdrage van plasma NGAL aan het model is verwaarloosbaar. Het lijkt er dus op dat in ieder geval bij het voorspellen van de noodzaak tot RRT in patiënten met sepsis gerelateerde AKI er geen rol is weggelegd voor plasma NGAL.

Als er een volledige nierinsufficiëntie is opgetreden bij kritisch zieke patiënten kan dit, zij het kwalitatief veel minder goed dan een gezonde nier, tijdelijk vervangen worden met een machine voor nierfunctie vervangende therapie. Hiermee wordt bloed ontrokken uit de bloedbaan en door een filter geleid waar afvalstoffen, in de vorm van kleine in het bloed opgeloste moleculen, over worden uitgespoeld. Deze filters hebben een bepaalde porie grootte met als doel vooral kleine moleculen te verwijderen en stoffen zoals eiwitten niet uit te wassen. Tijdens een dergelijke behandeling is het vaak niet duidelijk of er herstel van nierfunctie is opgetreden, dit omdat de klassieke functionele parameter voor nierfunctie (Serum kreatinine) heel effectief door de machine wordt uitgewassen. Daarbij is ook de urine productie een moeizame maat voor herstel van nierfunctie omdat als de behandeling goed wordt uitgevoerd er voor de nier geen noodzaak is om urine te produceren. Plasma NGAL is een klein eiwit dat in verhoogde concentraties aanwezig is bij patiënten met een volledige nierinsufficiëntie. Om in de toekomst te kunnen bepalen of veranderingen in de plasma concentratie van de biomarker plasma NGAL een predictor zou kunnen zijn voor endogeen herstel van nierfunctie is er in Hoofdstuk acht bestudeerd of dit kleine eiwit (~35 kDa) geklaard wordt door de high-cut off filters (EFO Aquamax HF-19 met in vitro cut-off Point van 55 kDa) die worden toegepast in de kliniek. Concentraties zijn gemeten voor het filter en in het effluente volume, waaruit blijkt dat de klaring uitkomt op 4.8 ml/min. Er is dus enige doorlaatbaarheid, maar de effectieve klaring is zo laag bij deze porie grootte dat in potentie wezenlijke veranderingen in plasma NGAL concentraties een maat zouden kunnen zijn voor herstel van nierfunctie.

Appendices

List of publications

Publications related to this thesis

1. **de Geus HR**, Betjes MG, Bakker J. Neutrophil gelatinase-associated lipocalin clearance during veno-venous continuous renal replacement therapy in critically ill patients. ICM 2010, 36: 2156-7.
2. **de Geus HR**, Bakker J, Lesaffre EM, le Noble JL. Neutrophil Gelatinase-associated Lipocalin at ICU Admission Predicts for Acute Kidney Injury in Adult Patients. Am J Respir Crit Care Med 2011, 183: 907-14.
3. **De Geus HR**, Woo JG, Wang Y, Devarajan P, Betjes MG, le Noble JL, Bakker J. Urinary neutrophil gelatinase associated lipocalin on admission to the ICU accurately discriminates between sustained en transient AKI in adult critically ill patients. Nephron extra 2011, 1: 9-23.
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5. **De Geus HR**, Betjes MG, Bakker J. Biomarkers for the prediction of acute kidney injury: a narrative review on current status en future challenges. Clin Kidney J, 2012, 5: 102-108.
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2. **De Geus HR**, Giard RW, Jacons FA, Lonnee ER, Dees A. Abnormalities in tattooed skin: sometimes sarcoidosis. Ned Tijdschr Geneesk 2005, 14:1113-7.
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7. Yuruk K, Bezemer R, Euser M, Milstein DM, **De Geus HR**, Scholten EW, de Mol BA, Ince C. The effects of conventional extracorporeal circulation versus miniaturized extracorporeal circulation on microcirculation during cardiopulmonary bypass-assisted coronary artery bypass graft surgery. Interact Cardiovasc Thorac Surg 2012, 15: 364-70.
8. Fortrie G, Stads S, **de Geus HR**, Groeneveld ABJ, Zietse R, Betjes MG. Determinants of renal function at hospital discharge of patients treated with renal replacement therapy in the intensive care unit. J Crit Care 2013, 28: 126-32.
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Abstracts

1. Ngal in urine and plasma as a predictive biomarker of acute kidney injury in adult ICU patients. **HRH de Geus**, J. le Noble, F. J. Zijlstra, J. Bakker. Oral presentation, ESICM Lissabon 2008.
2. The early diagnostic value of Neutrophil Gelatinase Associated Lipocalin (NGAL) in adult ICU patients with Acute Kidney Injury (AKI). **H de Geus**, J le Noble, F Zijlstra, C Ince, J Bakker. ISICEM Brussels 2009. Critical Care Supplement 1. 13. S105.
3. Use of deferred proxy consent in two emergency critical care trials. E Kompanje, T Jansen, J le Noble, **H de Geus**, J Bakker. ISICEM Brussels 2009. Critical Care Supplement 1. 13. S196.
4. Measurement of admission Neutrophil Gelatinase-Associated Lipocalin value for risk assessment of severe Acute Kidney Injury in an adult heterogeneous population admitted to the Intensive Care Unit. **HR de Geus**, J le Noble, K Kupfer, B Nolan, K Little, C Ince, J Bakker. World Congress Nephrology Milan 2009.
5. Are admission plasma and urine values of Neutrophil Gelatinase Associated Lipocalin (NGAL) associated with mortality in adult ICU patients requiring Continuous Renal Replacement Therapy? **H de Geus**, J le Noble, F Zijlstra, C Ince, J Bakker. CRRT Congress San Diego 2009. Blood Purification 2009; 27: 271-305.
6. NGAL, no role as biomarker for early detection of complications in elective esophagectomy patients admitted to the ICU. M van Genderen, **H de Geus**, A Lima, D Gommers, J van Bommel. ESICM Barcelona 2010. Intensive Care Medicine S143.
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9. Neutrophil Gelatinase-Associated Lipocalin as an early indicator for mortality in adult critically ill patients with Acute Kidney Injury. WP Ridder, **HRH de Geus**, J Bakker. ISICEM Brussels 2010. Critical Care Supplement; S.
10. High anion gap metabolic acidosis secondary to pyroglutamic aciduria (5-oxoprolinuria) in an adult receiving antibiotic therapy. Neth J Crit Care. December 2010. NVIC dagen February 2011.
11. Increased plasma neutrophil gelatinase-associated lipocalin levels in poor grade aneurysmal subarachnoid hemorrhage on admission to ICU. M Terwiel, **HRH de Geus**, J Bakker, M van der Jagt. ISICEM Brussels 2011.
12. NGAL predicts the need for RRT in critically ill patients. D Cruz, **HR de Geus**, C Ronco, ABJ Groeneveld. Oral presentation, ERA EDTA Paris 2012.

13. Urinary π-Glutathione-s-transferase predicts developing (septic) acute kidney injury at least as well as urinary neutrophil gelatinase associated lipocalin. **HRH de Geus**, G Fortrie, MGH Betjes, ABJ Groeneveld. ASN San Diego 2012.
14. The predictive value of urinary tubular proteins compared to neutrophil gelatinase-associated lipocalin for septic and non-septic acute kidney injury in the critically ill. G Fortrie, **HRH de Geus**, MGH Betjes, RHN van Schaik, ABJ Groeneveld. Oral presentation, ERA EDTA Istanbul Turkey 2013.
15. The expression pattern of urinary biomarkers preceding AKI influences predictive test properties. **HRH de Geus**, G Fortrie, MGH Betjes, ABJ Groeneveld. ESICM Paris France October 2013.

Invited oral presentations

1. NGAL in urine and plasma as a predictive biomarker of acute kidney injury in adult ICU patients. ESICM Lissabon Portugal September 2008.
2. Emerging data on the use of NGAL as a biomarker for AKI in the ICU. World Congress Nephrology Milan Italy 25 May 2009.
3. NGAL, the biomarker for AKI in ICU. COEUR Erasmus University Rotterdam Netherlands September 2009.
4. NGAL, the biomarker for AKI in ICU. German congress for Nephrology Gottingen Germany September 2009.
5. NGAL, an emerging biomarker for acute kidney injury in the ICU. Cardiorenal symposium Amsterdam Netherlands 26 November 2009.
6. Biomarkers for AKI. NVIC dagen Ede Netherlands 11 February 2010.
7. NGAL, an emerging biomarker for acute kidney injury in the ICU. CRRT conference, San Diego, USA February 2010.
8. NGAL, an emerging biomarker for acute kidney injury in the ICU. Vierde Rotterdamse Internisten symposium Rotterdam Netherlands March 2010.
9. NGAL, emerging data on AKI prediction in the critically ill. Asia pacific congress of nephrology Seoul South Korea June 2010.
10. Expanding knowledge of NGAL in the ICU: the Erasmus experience. Critical Care Nephrology course Vicenza Italy June 2010.
11. AKI in the septic adult ICU patient. First Erasmus Critical Care Days Rotterdam Netherlands June 2011.
12. Sepsis, does it affect the use of NGAL? CRRT conference San Diego, USA, February 2012.
13. Biomarkers in AKI. NVIC nier, lever en darm dagen Ede June 2012.
14. AKI related outcome. NVIC nier, lever en darm dagen Ede June 2012.
15. Acute nierschade bij volwassenen. Second Erasmus Critical Care Days Rotterdam Netherlands June 2013.

Awards

1. Winner best case report price: Sporadic porphyria cutanea tarda due to haemochromatosis. NIV dagen Maastricht Netherlands, 2005.
2. Molenwater de Monchy penning awarded by the Erasmus University medical center board of directors for successfully evacuating ICU patients during a hospital fire on the 19th November 2006.
3. Young researchers price for best presentation and scientific study. Cardiorenal symposium Amsterdam Netherlands November, 2009.
4. Travel grant: The predictive value of urinary tubular proteins compared to neutrophil gelatinase-associated lipocalin for septic and non-septic acute kidney injury in the critically ill. ERA EDTA Istanbul Turkey 2013.

Book contributions

1. Geus de HR, Bakker J. Hoofdstuk 20; 287-314. Manipulação da oferta tissular de oxigênio em pacientes críticos. Choque Circulatorio. ISBN 978-85-372-0193-0. 2008

Curriculum Vitae

Curriculum Vitae

Hilde Rosetta Hendrica de Geus was born on 21 November 1973 in Rotterdam. After graduating from secondary school (VWO, C.S.G. Comenius Capelle a/d IJssel) she studied Medicine between 1992 and 1998 at the Erasmus University Rotterdam. After a two year internship she obtained her qualification as a Medical Doctor. She started her professional carrier as a resident in Internal Medicine at Reinier de Graaf Gasthuis Delft, followed by her specialty training in Internal Medicine working at St Clara Hospital Rotterdam, Leids Universitair Medisch Centrum Leiden and Ikazia Hospital Rotterdam. The last year of her training she dedicated to Intensive Care Medicine at the Erasmus University Medical Center Rotterdam under supervision of Prof. Jan Bakker where she onwards started her extended specialty. She became a member of staff in 2008 and obtained her European EDIC (I and II) certified diploma as an Intensivist the same year. During her fellowship intensive care she started her PhD trajectory as described in this thesis at the department of Intensive Care under supervision of Prof. Jan Bakker and Prof. Johan Groeneveld. Currently she serves as an Intensivist register and permanent member of the adult ICU staff at the Erasmus University Medical Center Rotterdam with special focus on Critical Care Nephrology.

Dankwoord

Dankwoord

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Commercial partners

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Tot slot

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'Give light, and the darkness will disappear of itself.'

- Desiderius Erasmus 1466 – 1536 -