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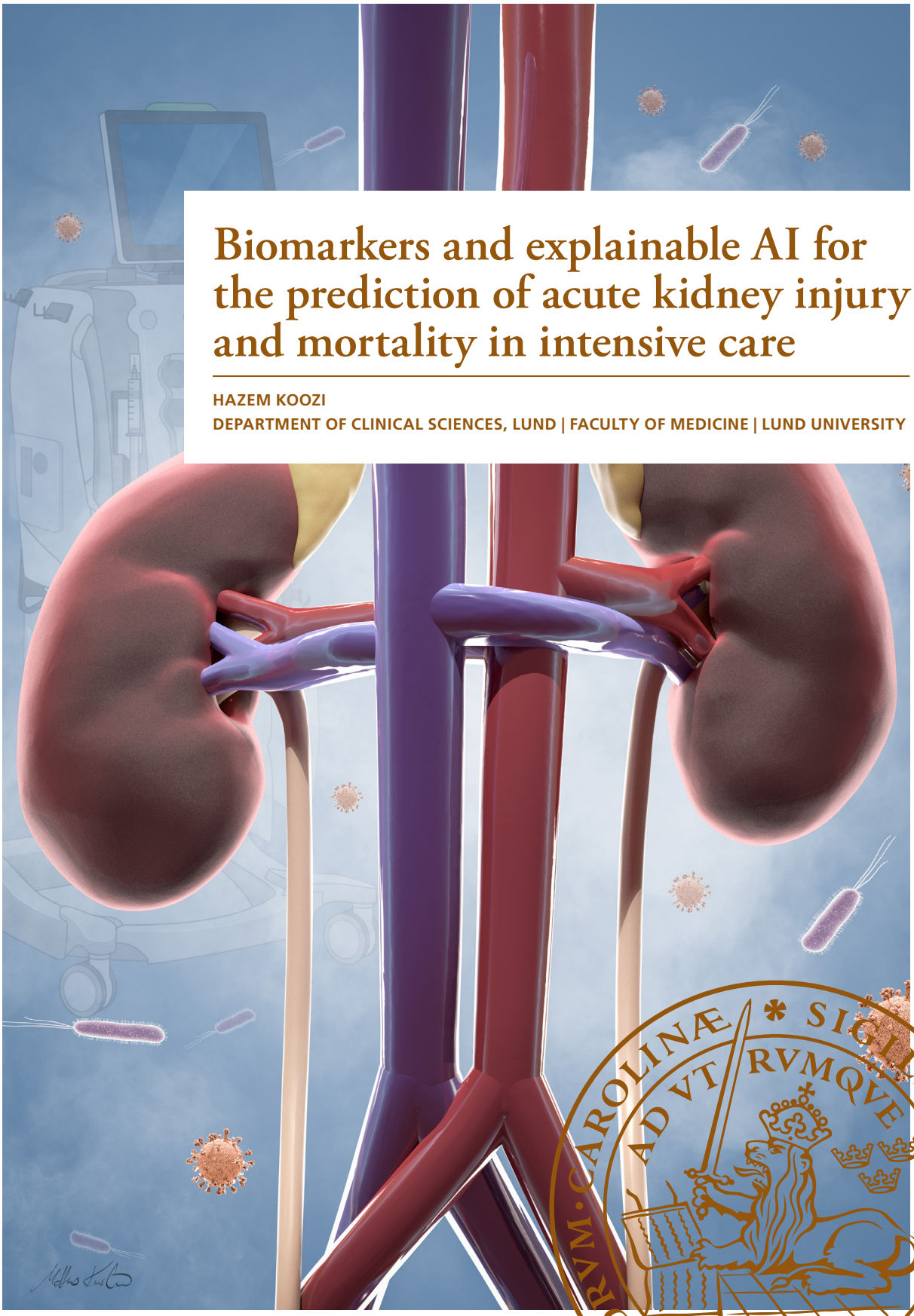

Biomarkers and explainable AI for the prediction of acute kidney injury and mortality in intensive care

HAZEM KOOZI
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

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Hazem Koozi

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Supervisor:
Associate Professor Attila Frigyesi

Co-supervisor:
Professor Hans Friberg

Faculty opponent:
Professor Martin Ingi Sigurðsson, University of Iceland

Examining committee:
Associate Professor Alain Dardashti
Senior Associate Professor Jan-Erik Karlsson
Professor Ulf Schött

Organisation
LUND UNIVERSITY
Faculty of Medicine
Department of Clinical Sciences, Lund
Anaesthesiology and Intensive Care
221 84 LUND, Sweden

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Hazem Koozi

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Abstract
Background: Mortality in the intensive care unit (ICU) remains high, and patient heterogeneity complicates risk stratification. Acute kidney injury (AKI) is a frequent complication, linked to substantial morbidity and mortality, but early detection remains challenging. Promising biomarkers exist, yet few are in clinical use.
Aims: 1) Evaluate plasma neutrophil gelatinase-associated lipocalin (NGAL) for predicting renal replacement therapy (RRT) and mortality in COVID-19. 2) Assess plasma endostatin at ICU admission for predicting new-onset AKI, RRT, and mortality in COVID-19 and a general ICU population. 3) Identify key predictors of new-onset AKI and RRT using biomarkers and explainable artificial intelligence (XAI) in a general ICU population.
Methods: ICU admissions from four hospitals were included prospectively (COVID-19) and retrospectively (general ICU). Logistic regression assessed whether NGAL and endostatin were associated with and predicted outcomes, adjusting for established renal markers and key confounders. eXtreme Gradient Boosting (XGBoost) and SHapley Additive exPlanations (SHAP) were used to improve prediction and identify top predictors.
Results: Approximately 500 COVID-19 and 4,700 general ICU admissions were included. In COVID-19, NGAL combined with creatinine and cystatin C predicted RRT (AUC 0.95), and combined with age and sex predicted 90-day mortality (AUC 0.83). Endostatin levels of 100–200 ng/mL were associated with AKI (OR 5.1), RRT (OR 3.5), and mortality (OR 4.2), and improved prediction of AKI and mortality. In the general ICU population, endostatin was associated with AKI (OR 1.7), stage 3 AKI (OR 1.4), and RRT (OR 1.2), but not mortality, and outperformed creatinine and cystatin C in predicting AKI (AUC 0.67 vs. 0.63, $p < 0.001$). XAI identified top predictors of AKI (urine output, endostatin, baseline creatinine, lactate, albumin) and RRT (creatinine, urine output, endostatin, NGAL) and enhanced prediction.
Conclusions: NGAL improved prediction of RRT and mortality in COVID-19. Endostatin was independently associated with AKI and RRT in both cohorts, and outperformed creatinine for AKI prediction in a general ICU population. XAI revealed key biomarkers for further clinical evaluation, including NGAL and endostatin.

Keywords
Intensive care, acute kidney injury, renal replacement therapy, mortality, biomarkers, NGAL, endostatin, explainable artificial intelligence.

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Generative AI tools (ChatGPT and Grammarly) were solely used for proofreading the text in this thesis. They were not used for producing text or images. Non-generative AI methods (XGBoost and SHAP) were applied for predictive modelling and explanation of results. Full responsibility for the content is taken by the author. A more detailed explanation of how and when the AI models were used can be found in the Methods and materials section.

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To my family

Table of contents

List of papers

This thesis is based on the following papers, referred to by their Roman numerals:

I. Engström J, **Koozi H**, Didriksson I, Larsson A, Friberg H, Frigyesi A, Spångfors M. Plasma neutrophil gelatinase-associated lipocalin independently predicts dialysis need and mortality in critical COVID-19. Sci Rep. 2024 Mar 20;14(1):6695.

II. **Koozi H**, Engström J, Zwawi A, Spångfors M, Didriksson I, Larsson A, Friberg H, Frigyesi A. Plasma endostatin at intensive care admission is independently associated with acute kidney injury, dialysis, and mortality in COVID-19. Intensive Care Med Exp. 2025 Apr 3;13(1):42.

III. **Koozi H**, Engström J, Larsson A, Spångfors M, Friberg H, Frigyesi A. Plasma endostatin and its association with new-onset acute kidney injury in critical care. J Intensive Care. 2025 Sep 2;13(1):48.

IV. **Koozi H**, Engström J, Friberg H, Frigyesi A. Explainable AI identifies key biomarkers for acute kidney injury prediction in the ICU. Manuscript submitted.

Related papers not included in this thesis:

1. **Koozi H**, Lengquist M, Frigyesi A. C-reactive protein as a prognostic factor in intensive care admissions for sepsis: A Swedish multicenter study. J Crit Care. 2020 Apr;56:73-79.

2. **Koozi H**, Lidestam A, Lengquist M, Johnsson P, Frigyesi A. A simple mortality prediction model for sepsis patients in intensive care. J Intensive Care Soc. 2023 Nov;24(4):372-378.

Populärvetenskaplig sammanfattning

Intensivvård ges till patienter med livshotande tillstånd som kräver avancerad övervakning och stöd av sviktande organ. Trots medicinska framsteg är dödligheten på intensivvårdsavdelningar (IVA) fortfarande hög, och många överlevande drabbas av långvariga komplikationer. Ett vanligt tillstånd på IVA är akut njurskada (acute kidney injury, AKI), som drabbar nästan hälften av patienterna och är förknippad med ökad risk för död. AKI är oftare en komplikation till kritisk sjukdom snarare än den primära orsaken till intensivvårdsbehov. Behandlingen av AKI är understödjande och inkluderar optimering av cirkulation och vätskebalans samt utsättning av läkemedel som är skadliga för njurarna. Specifik behandling saknas vanligen. En progredierande AKI leder så småningom till överskott av vätska, elektrolyter (såsom kalium) och slaggprodukter i kroppen. Svår AKI kräver ofta dialysbehandling på IVA för att ersätta njurarnas funktion, och dialyskrävande patienter representerar en grupp med särskilt hög risk för negativa utfall. Hög dödlighet och förekomst av AKI på IVA var framträdande under COVID-19-pandemin.

Idag används framför allt blodprovet kreatinin och mätning av urinmängden (diures) för att upptäcka AKI. Dessa kan benämnas som biomarkörer, det vill säga mätbara indikatorer på ett biologiskt tillstånd eller sjukdom. Kreatinin och diures identifierar dock AKI för sent, när skadan redan är omfattande. Den sena identifikationen av AKI anses vara en huvudorsak till att effektiva behandlingar saknas. För att möjliggöra tidigare diagnos och bättre riskbedömning har forskningen riktat in sig på nya biomarkörer. Neutrofilt gelatinasassocierat lipokalin (NGAL) och endostatin är två lovande laboratorieprover för att förutsäga AKI och död. NGAL är ett protein som både utsöndras från vita blodkroppar vid inflammation och njurceller vid AKI. Flera studier har visat att NGAL i blod och urin har potential att förutsäga AKI. Endostatin, ett fragment av ett viktigt kärlprotein som bland annat finns i njurarna, kan mätas i blodet och har i några

mindre studier kopplats till både AKI och ökad dödlighet.

Syftet med denna avhandling var att undersöka om NGAL och endostatin i blod kan fungera som tidiga och pålitliga biomarkörer för nydebuterad AKI, dialysbehov och död inom intensivvård. Vidare syftade vi till att undersöka om en kombination av flera nyare biomarkörer (inklusive NGAL och endostatin), tillsammans med en form av artificiell intelligens (eXtreme Gradient Boosting), kunde hitta de viktigaste faktorerna för tidig identifikation av nydebuterad AKI och dialysbehov. Studierna byggde på insamlade data och blodprover på cirka 500 COVID-19-inläggningar på IVA samt en generell IVA-population på cirka 4 700 inläggningar.

Resultaten visade att NGAL i kombination med rutindata kan förutsäga vilka COVID-19-patienter som kommer att behöva dialys eller avlida på ett träffsäkert sätt. Endostatin visade starka kopplingar till AKI och dialysbehov, både vid COVID-19 och i en generell IVA-population, men var mest värdefullt som tillägg till rutinblodprover för AKI. I den generella IVA-populationen var endostatin bättre än kreatinin på att förutsäga nydebuterad AKI på IVA inom 48 timmar. Dessutom var endostatin användbart för att förutsäga dödligt utfall vid COVID-19, vilket inte sågs i den generella IVA-populationen. En kombination av flera nyare biomarkörer och AI visade att de viktigaste riskfaktorerna för att utveckla nydebuterad AKI var diures, endostatin, kreatinin före IVA-inläggning, laktat och albumin. För dialysbehov var kreatinin, diures, endostatin och NGAL de viktigaste parametrarna. AI förbättrade dessutom förmågan att förutsäga nydebuterad AKI och dialysbehov, jämfört med den traditionellt mest använda statistiska metoden logistisk regression.

Sammanfattningsvis visar avhandlingen att NGAL, endostatin och AI-baserade modeller har stor potential att förbättra tidig riskbedömning av AKI och dialysbehov inom intensivvård. NGAL och endostatin förbättrar även identifikation av patienter med hög risk för dödligt utfall vid COVID-19. Ytterligare studier behövs dock för att bekräfta det prognostiska värdet av framför allt endostatin och AI. Dessa metoder kan i framtiden hjälpa vårdpersonal att tidigare identifiera högriskpatienter och vägleda forskning om nya behandlingar för AKI, vilket i sin tur kan bidra till att rädda liv och minska behovet av långvarig dialys.

Abbreviations

AI	Artificial Intelligence
AKI	Acute Kidney Injury
APACHE	Acute Physiology And Chronic Health Evaluation
AUC	Area Under the Curve
CI	Confidence Interval
CKD	Chronic Kidney Disease
CRP	C-Reactive Protein
GFR	Glomerular Filtration Rate
ICAM-1	Intercellular Adhesion Molecule 1
ICU	Intensive Care Unit
IDI	Integrated Discrimination Improvement
IL-18	Interleukin 18
IQR	Interquartile Range
KDIGO	Kidney Disease: Improving Global Outcomes
KIM-1	Kidney Injury Molecule 1
ML	Machine Learning
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NRI	Net Reclassification Improvement
OR	Odds Ratio
PasIva	Patient Administrative System for Intensive Care Units
RCT	Randomised Controlled Trial
ROC	Receiver Operating Characteristic
RRT	Renal Replacement Therapy

SAPS	Simplified Acute Physiology Score
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SHAP	SHapley Additive exPlanations
SOFA	Sequential Organ Failure Assessment
VCAM-1	Vascular Cell Adhesion Molecule 1
WBC	White Blood Cell
XAI	Explainable Artificial Intelligence
XGBoost	eXtreme Gradient Boosting

Background

Intensive care

What is intensive care?

Intensive care is a specialised form of medical care focused on continuous monitoring and support for patients with life-threatening conditions and severe organ failure. It is provided primarily, though not exclusively, in intensive care units (ICUs), where advanced equipment, medications, and techniques are used to maintain vital organ functions [1].

History

Intensive care medicine is widely regarded as having originated during the polio epidemic in Copenhagen in 1953. Dr Bjørn Ibsen, a Danish anaesthetist, established the world's first ICU, where patients with respiratory failure due to polio received mechanical ventilation. This pioneering unit featured a significantly higher nurse-to-patient ratio than other wards and marked a turning point in the care of critically ill patients. As a result of ICUs, polio mortality in Copenhagen declined from over 80% to approximately 40%.

In the decades following the polio epidemic, intensive care underwent a significant transformation. The increasing need to manage patients with prolonged respiratory failure led to technical innovations that allowed for more accurate support. The development of blood gas analysis, for example, contributed to better monitoring. Alongside technological advances, integrating physiotherapy into daily care routines helped improve outcomes, and the value of coordinated, multidisciplinary teamwork became increasingly apparent. The 1960s and 1970s saw a rapid expansion of ICUs across Europe and North America, supported

by the growing availability of trained anaesthetists and other specialists. This period was also marked by the introduction of new technology and medications, which made intensive care more advanced and adaptable to the needs of the patients. By the 1980s, intensive care had matured into a multidisciplinary speciality. Specialised roles in intensive care emerged for a wide range of healthcare professionals [2].

Complex decision-making, time-pressured interventions, and the presence of multiple failing organ systems characterise the nature of intensive care, carrying a high risk of iatrogenic harm. During the 1990s, there was a growing awareness and recognition of complications and unintended consequences in intensive care [3].

Modern intensive care

The past few decades of advancements in intensive care can be summarised by the "less is more" principle. Tidal volumes in mechanical ventilation, oxygenation targets, fluid therapy volumes, blood pressure goals, transfusion thresholds, sedation, and targeted temperature management have all been progressively reduced in response to increasing evidence of harm, or lack of benefit, associated with more aggressive treatment strategies [4–10].

Despite the evolution of intensive care since its origin, mortality rates among critically ill patients remain high [11]. Randomised controlled trials (RCTs) assessing promising interventions have often been negative. The pronounced heterogeneity among ICU patients and lack of better characterisation of common syndromes such as sepsis and acute respiratory distress syndrome (ARDS) are considered the main factors for the limited success of RCTs in intensive care [12]. Furthermore, re-analyses of RCTs have suggested that intervention effects may vary significantly across patient subgroups, emphasising the need for precision medicine approaches [13]. These findings underscore the need for enhanced patient characterisation, refined risk stratification, and the development of targeted therapies tailored to specific subgroups within intensive care. A global shortage of ICU beds, compounded by the resource-intensive nature and costs of ICU care, further highlights the importance of effective risk stratification [14].

COVID-19

The global shortage of ICU resources became particularly evident during the COVID-19 pandemic, where the surge in critically ill patients overwhelmed existing ICU capacities worldwide [14]. COVID-19 is a highly transmissible disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic, citing the alarming spread, severity, and urgent need for global action [15]. Critical COVID-19 carries a substantial risk of mortality [16]. As of March 2025, over 7 million confirmed COVID-19 deaths have been reported worldwide, with the actual toll likely higher due to underreporting and variations in surveillance systems [17]. Acute kidney injury (AKI) occurs in approximately 10% of hospitalised COVID-19 patients, with the incidence rising to 26% among those requiring ICU admission [18]. Renal replacement therapy (RRT) is commonly needed in critically ill COVID-19 patients with AKI [19]. Although the global incidence of COVID-19 has decreased thanks to widespread vaccination programmes, periodic outbreaks continue and are likely to persist, making it essential to identify tools to improve outcomes in critical COVID-19.

Acute kidney injury

AKI is a common and multifactorial syndrome affecting approximately 50% of adult ICU patients and 20% of hospitalised patients. The incidence of AKI is comparable worldwide, regardless of resource level, although the underlying causes differ between high- and low-income countries. AKI is more often a complication of critical illness rather than the primary reason for ICU admission, and contributes substantially to both short- and long-term morbidity and mortality. It represents a growing public health challenge with substantial healthcare costs. Acute consequences of AKI include fluid overload, electrolyte disturbances, metabolic acidosis, immune dysfunction, and increased risk of bleeding [20]. AKI recovery in ICU patients is highly variable, with about one-quarter achieving sustained early recovery, a similar proportion showing no recovery, and the rest experiencing late recovery or relapse [21]. Incomplete renal recovery may lead to chronic kidney disease (CKD), end-stage renal disease (ESRD) with chronic dialysis need, and increased long-term cardiovascular risk [20]. ICU mortality associated with AKI has been reported to be approximately 24%, although it varies depending on the severity and duration of AKI. Even mild AKI is associated with a significant increase in mortality [22, 23].

Definition and diagnosis

The most widely used markers of AKI are creatinine and urine output. Historically, the definition and classification of AKI have evolved to improve diagnostic consistency and prognostic accuracy. In 2004, the Risk, Injury, Failure, Loss, and End-stage kidney disease (RIFLE) criteria were introduced, defining AKI based on changes in serum creatinine levels and urine output. The RIFLE criteria provided a standardised framework for assessing AKI [24]. Subsequently, in 2007, the Acute Kidney Injury Network (AKIN) proposed modifications to the RIFLE criteria, emphasising the need for early detection. The AKIN classification defined AKI as an abrupt (within 48 hours) reduction in kidney function, indicated by an absolute increase in serum creatinine of $\geq$ 26.5 µmol/L (0.3 mg/dL), a $\geq$ 50% increase in creatinine, or urine output $<$ 0.5 mL/kg/h for $\geq$ 6 hours [25]. Building upon these frameworks, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines were established in 2012 to unify and refine AKI definitions. According to the KDIGO criteria, AKI is diagnosed if creatinine rises by $\geq$ 26.5 µmol/L (0.3 mg/dL) within 48 hours, increases to 1.5 times the baseline within 7 days, or urine output falls to $<$ 0.5 mL/kg/h for $\geq$ 6 hours. The KDIGO criteria have become the most widely accepted classification in both clinical and research settings. Furthermore, the KDIGO criteria stratify AKI into three stages based on changes in creatinine and urine output [26].

Table 1: Staging of acute kidney injury according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria.

Stage	**Creatinine**	**Urine output**
1	1.5–1.9 x baseline or Increase by $\geq$ 26.5 µmol/L	$<$ 0.5 mL/kg/h for 6–12 h
2	2.0–2.9 x baseline	$<$ 0.5 mL/kg/h for $\geq$ 12 h
3	$\geq$ 3.0 x baseline or Increase to $\geq$ 353.6 µmol/L or Initiation of RRT	$<$ 0.3 mL/kg/h for $\geq$ 24 h or Anuria for $\geq$ 12 h

RRT Renal Replacement Therapy.

AKI may progress to acute kidney disease (AKD), defined as kidney dysfunction

persisting for more than 7 days but less than 90 days following the initial injury. If renal impairment continues beyond 90 days, it is classified as CKD [27].

Pathophysiology and risk factors

Classically, the aetiologies of AKI have been divided into prerenal, intrarenal, and postrenal [28]. In reality, AKI in critically ill patients usually results from a multifaceted interplay of haemodynamic disturbances, inflammation, and cellular dysfunction. Haemodynamic instability leads to reduced renal perfusion and subsequent ischaemic injury. Venous congestion can also cause renal impairment by increasing interstitial pressure and reducing glomerular filtration. Systemic inflammation, particularly in sepsis, contributes to endothelial dysfunction and increased vascular permeability, aggravating renal injury. At the cellular level, tubular epithelial cells may undergo apoptosis or necrosis due to oxidative stress and mitochondrial dysfunction. Additionally, organ cross-talk, where dysfunction in one organ system adversely affects others, plays a significant role in the progression of AKI. Understanding these complex mechanisms, potentially through biomarkers, may improve early recognition of AKI and support the development of targeted therapeutic strategies [29].

In addition, a range of patient characteristics and clinical exposures influence susceptibility to AKI. Older age is a particularly important risk factor and often coexists with CKD, proteinuria, cardiovascular disease, diabetes, or hypoalbuminaemia. Nephrotoxic medications, such as angiotensin-converting enzyme (ACE) inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs), further increase the risk of AKI, particularly when combined. Acute triggers such as infection, hypotension, and hypovolaemia frequently precipitate renal injury [20].

Management

Early identification of AKI is essential in order to initiate nephroprotective strategies and reduce the risk of progression. However, delayed diagnosis and the heterogeneity of AKI have limited the success of specific therapeutic interventions. The prevention and management of AKI are therefore primarily supportive. Key strategies include maintaining optimal fluid balance and renal perfusion pressure, often achieved through cautious fluid resuscitation to correct hypovolaemia and the use of vasopressors. Timely identification and treatment of postrenal causes are also essential. Avoiding nephrotoxic agents is important to limit further renal injury. Dose adjustment of renally excreted medications

is often required. Supportive care further involves correcting complications such as electrolyte imbalances, acid–base disturbances, and fluid overload [28, 29].

Renal replacement therapy

Approximately 10-15% of critically ill patients with AKI require RRT [22, 30]. As mortality correlates with the severity of AKI, patients requiring RRT represent a high-risk subgroup with an estimated mortality rate of 50%. Indications for RRT include fluid overload, hyperkalaemia, and metabolic acidosis unresponsive to medical treatment, as well as severe uraemia and poisoning with a dialysable toxin. The two primary modalities of RRT are continuous renal replacement therapy (CRRT) and intermittent haemodialysis (IHD), each with distinct advantages and limitations. CRRT is generally preferred in the ICU, primarily due to better haemodynamic stability, although practice patterns vary across institutions and regions. Regardless of the RRT technique employed, the fundamental principle remains the removal of excess solutes and water via a semipermeable membrane, usually performed through a central venous dialysis catheter. The optimal timing and dosing of RRT remain subjects of ongoing debate [31, 32].

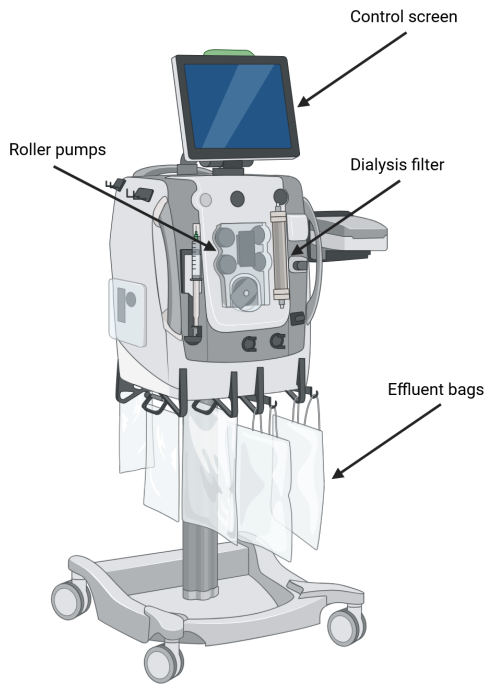

Figure 1: Continuous renal replacement therapy (CRRT) machine used in the management of critically ill patients with acute kidney injury. Created in BioRender.com.

Biomarkers

A biomarker is a measurable indicator of a biological state [33]. In intensive care, biomarkers are used to understand and quantify the processes that lead to organ failure, and many are proteins that can be measured in blood and urine [34]. Creatinine and urine output, both reflecting kidney function, have important limitations as biomarkers of AKI. Creatinine, a marker of glomerular filtration rate (GFR), is a breakdown product of muscle metabolism that is renally cleared. Significant decline in GFR usually precedes detectable increases in serum creatinine, with the timing and magnitude of these changes influenced by baseline kidney function [35]. Thus, creatinine may not accurately reflect acute changes in renal function. Additionally, creatinine levels are affected by factors such as muscle mass. Urine output can be influenced by fluid balance, diuretics, and be a transient physiological response to hypovolaemia without kidney injury [36]. These limitations underscore the need for other biomarkers to improve the early detection of AKI.

Emerging biomarkers hold significant promise for improving early AKI detection, refining risk stratification, and guiding the development and application of targeted therapies. They also reflect the pathophysiological events of AKI at different stages. However, despite extensive research into many promising biomarker candidates, few have been adopted in clinical practice or demonstrated benefit in clinical trials [36].

Cystatin C, a low-molecular-weight protein produced by nucleated cells, is freely filtered in the glomerulus and reabsorbed by the proximal tubules where it is degraded. It has a shorter half-life than creatinine, making it a potentially quicker indicator of GFR. Unlike creatinine, cystatin C levels are less influenced by muscle mass, age, or sex. Cystatin C has previously shown strong predictive performance for AKI in various settings [37, 38]. However, its role in AKI detection remains under investigation, and clinical application has been limited.

Neutrophil gelatinase-associated lipocalin (NGAL), initially identified in neutrophils, has been described as a "troponin-like" biomarker for AKI. Under normal conditions, NGAL is expressed at low levels in various human tissues, including the kidneys, gastrointestinal tract, liver, and lungs. However, its expression is markedly upregulated in response to tubular epithelial injury. Unlike creatinine and cystatin C, which are kidney function markers, NGAL is a direct indicator of kidney injury. NGAL has been a promising biomarker for AKI for over two decades, with multiple studies supporting the role of both plasma/serum and urine NGAL in predicting AKI and RRT in several clinical settings, including cardiac surgery and critical illness [39–43]. Still, the usefulness of plasma/serum

NGAL as a marker of AKI has been questioned, as its levels may reflect neutrophil activation rather than kidney-specific injury [44]. In COVID-19, urinary NGAL has been associated with AKI, and a few smaller studies have reported promising results for plasma/serum NGAL in predicting both AKI and RRT [45–48].

Endostatin is a fragment of collagen XVIII, a structural component of basement membranes found in various tissues, including blood vessels and renal structures such as the tubular epithelium and Bowman's capsule. It functions as a broad-spectrum angiogenesis inhibitor, is released during extracellular matrix remodelling, and can induce apoptosis [49]. Elevated plasma endostatin levels may reflect microvascular dysfunction and ongoing tissue remodelling, and have been linked to CKD, AKI, and renal recovery in AKI patients [50–53]. Nonetheless, findings across studies have varied [54]. Furthermore, most studies conducted in ICU populations have been small.

Vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) are endothelial proteins involved in leukocyte adhesion during inflammation. They both play important roles in the pathophysiology of AKI [55]. Elevated VCAM-1 levels have been linked to severe AKI in critically ill patients, mediating the relationship between fibroblast growth factor 23 and AKI [56]. Similarly, increased VCAM-1 and ICAM-1 levels have been associated with the development of AKI in both COVID-19 and non-COVID-19 ARDS, indicating their potential as biomarkers of AKI [57].

Other promising biomarkers for the early detection of AKI include kidney injury molecule 1 (KIM-1) and interleukin 18 (IL-18) in urine, as well as the NephroCheck test, which measures urinary levels of tissue inhibitor of metalloproteinases 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7). Elevated urinary levels of KIM-1 and IL-18 have been associated with AKI development in burn patients [58]. Combining IL-18 and KIM-1 has also been shown to improve predictive accuracy for severe AKI and mortality following cardiac surgery [59]. The Sapphire study demonstrated that NephroCheck could predict moderate to severe AKI within 12 hours in critically ill patients, outperforming other biomarkers such as cystatin C, NGAL, KIM-1, and IL-18 [60]. Similarly, the Topaz study validated NephroCheck's predictive value for moderate to severe AKI within 12 hours, confirming its utility in diverse ICU populations [61]. In a small feasibility study, elevated NephroCheck values triggered a care bundle associated with reduced AKI incidence in patients whose biomarker levels declined over time [62].

Mortality prediction

Scoring systems

As intensive care has grown more complex in the last decades, structured ways to evaluate patient outcomes have become necessary. This has led to the development of clinical scoring systems that compare patient groups by adjusting for illness severity, thereby improving both research quality and clinical benchmarking.

The Acute Physiology And Chronic Health Evaluation (APACHE) was introduced in 1981 to assess illness severity in ICU patients and estimate hospital mortality [63]. It was refined into APACHE II in 1985, which includes 12 physiological and laboratory variables such as mean arterial pressure, serum creatinine, white blood cell (WBC) count, and pH, along with age and chronic health status [64]. APACHE III followed in 1991, adding comorbidities, pre-ICU location, and additional biomarkers, including urine output, bilirubin, and albumin. It was validated in over 17,000 ICU patients from 40 hospitals in the United States, showing improved predictive performance, and is widely used [65].

The Simplified Acute Physiology Score (SAPS), developed in 1984, was designed as a simpler alternative to APACHE for estimating illness severity in the ICU [66]. SAPS II, published in 1993, incorporated 12 physiological and laboratory variables, age, admission type, and three chronic disease indicators to predict hospital mortality. Biomarkers included in SAPS II were serum creatinine, urea, sodium, potassium, bicarbonate, bilirubin, and WBC count [67]. SAPS 3, introduced in 2005, marked a significant revision. Unlike its predecessors, it was designed to predict mortality at ICU admission, using variables collected within the first hour of intensive care rather than 24 hours. The model was developed using data from over 16,000 patients across 35 countries, ensuring broad international applicability. SAPS 3 incorporates both physiological and contextual variables. Regarding biomarkers, SAPS 3 added platelet count, arterial pH, and the ratio of partial pressure of oxygen in arterial blood to the fraction of inspired oxygen. This score enables early risk stratification and supports accurate benchmarking across ICUs. SAPS 3 is designed to be calibrated to local ICU populations through region-specific logistic regression equations. This approach improves its accuracy in predicting hospital mortality across different healthcare settings [68]. Although SAPS 3 is designed to predict hospital mortality, the Swedish version uses 30-day mortality [69].

Table 2: Variables included in the Simplified Acute Physiology Score (SAPS) 3. Box I includes information before ICU admission, Box II covers reasons for ICU admission, and Box III contains physiological and laboratory values measured at ICU admission.

Box	Variables
I	Age, length of hospital stay before ICU admission, intra-hospital location before ICU admission, comorbidities (cancer therapy, metastatic cancer, haematologic cancer, chronic heart failure, cirrhosis, AIDS), and use of vasoactive drugs before ICU admission.
II	Planned or unplanned ICU admission, reason for ICU admission (e.g. septic shock, liver failure, trauma, stroke, coma, seizures), surgical status (no surgery, emergency surgery, scheduled surgery), type of surgery (e.g. cardiac, neurosurgery, transplant), and infection status (nosocomial, respiratory).
III	Systolic blood pressure, heart rate, Glasgow Coma Scale, body temperature, creatinine, bilirubin, white blood cell count, platelet count, arterial pH, and PaO_2/FiO_2 ratio combined with mechanical ventilation.

ICU Intensive Care Unit, *AIDS* Acquired Immunodeficiency Syndrome, PaO_2 Partial Pressure of Arterial Oxygen, FiO_2 Fraction of Inspired Oxygen.

Scoring systems like SAPS 3 and APACHE are primarily designed for risk adjustment and benchmarking in ICU cohorts. However, they are often used to estimate illness severity and are associated with mortality. Similarly, the Sequential Organ Failure Assessment (SOFA) score, although initially intended to assess organ dysfunction, also correlates with mortality [70].

Biomarkers

Prognostic biomarkers are integral to illness severity scores like APACHE, SAPS, and SOFA. They also offer objective measures of physiological stress and organ dysfunction, helping to risk-stratify critically ill patients.

Serum bilirubin is a routinely measured marker of hepatic function in the ICU. Beyond its prognostic role in liver dysfunction, elevated bilirubin levels are frequently observed in critically ill patients without obvious hepatic pathology and have been independently associated with increased mortality [71, 72]. WBC count, a marker of systemic inflammation, is also readily available in the ICU and has been shown to correlate with mortality [73]. Other prognostic biomarkers available in routine ICU care are albumin and lactate. Albumin levels have shown a strong association with poor outcomes in a large meta-analysis, and

are included in APACHE III but not SAPS 3 [74]. Elevated lactate levels have several aetiologies, including hypoperfusion, and have been strongly linked to poor outcomes. For instance, a lactate level above 2.5 mmol/L was identified as a threshold predicting mortality in severe sepsis and septic shock [75]. Furthermore, increasing lactate during critical illness is strongly correlated with mortality [76]. Lactate is neither included in APACHE III nor SAPS 3.

Several other biomarkers have shown prognostic value in the ICU. Cardiac biomarkers such as N-terminal pro–B-type natriuretic peptide (NT-proBNP) and troponin T are associated with mortality in ICU patients [77, 78]. Elevated soluble urokinase plasminogen activator receptor (suPAR) levels and neutrophil-to-lymphocyte ratio have been linked with poor outcomes in sepsis [79, 80].

Although NGAL has been shown to be a predictor of AKI and the need for RRT, its ability to predict mortality has been conflicting [40, 41]. Smaller studies have reported that endostatin could be a valuable predictor of mortality in both general ICU populations and patients with AKI [52, 81]. However, a prospective study found that endostatin had limited value for predicting 90-day mortality [54].

In COVID-19, multiple biomarkers have consistently been linked to disease severity and mortality. Elevated inflammatory markers such as C-reactive protein (CRP), interleukin-6, ferritin, and calprotectin are associated with adverse outcomes [82, 83]. Coagulation disturbances, particularly increased D-dimer levels, have been identified as independent predictors of mortality. Markers of organ injury, including creatinine, troponin, lactate dehydrogenase, and aspartate transaminase, indicate multiorgan dysfunction and have also been associated with poor prognosis [82]. Urinary NGAL has been shown to correlate with mortality in COVID-19, and one study found that NGAL in blood was associated with mortality in critical COVID-19 [46, 47, 84]. In addition, two studies have indicated that endostatin may be a marker of disease severity in critically ill COVID-19 patients [85, 86].

Explainable artificial intelligence

Artificial intelligence (AI) refers to computer systems that can perform tasks that typically require human intelligence. These tasks may include learning from data, recognising patterns, or making predictions. AI is increasingly used in medicine to support diagnosis, risk assessment, and outcome prediction based on large and complex datasets. A key subfield of AI is machine learning (ML), where

algorithms learn from data to improve their performance over time without being explicitly programmed with fixed rules. ML has become increasingly valuable for analysing complex clinical data and uncovering patterns that may not be apparent using traditional statistical methods [87].

Among ML techniques, eXtreme Gradient Boosting (XGBoost) is a particularly useful algorithm that builds a series of decision trees, where each tree attempts to correct the errors of the previous one. This powerful method, known as boosting, helps improve predictive accuracy and reduce overfitting [88]. XGBoost has shown effectiveness in analysing clinical data and improving outcome prediction, as shown in studies on spinal cord injury, myocardial infarction, and CKD [89–91].

However, many AI models, including XGBoost, are often considered "black boxes" because they produce predictions without clearly explaining how those predictions were made. This lack of transparency can be a barrier to clinical acceptance. Explainable artificial intelligence (XAI) refers to techniques that aim to make the inner workings of AI models understandable to humans. One such method is SHapley Additive exPlanations (SHAP), which quantifies how much each input variable contributes to a model's output. This makes it possible to understand the factors underlying individual predictions, improving the interpretability of complex AI models [92].

A few studies have demonstrated the effectiveness of XGBoost in predicting AKI using routine clinical data and commonly available biomarkers, including in trauma patients and general ICU populations [93–95].

Rationale

Despite advances in intensive care, high rates of AKI and mortality remain significant challenges. This was especially highlighted during the COVID-19 pandemic. The limitations of traditional biomarkers such as creatinine and urine output in the early detection of AKI have hindered timely interventions and the development of effective treatments. While NGAL is a well-studied biomarker of AKI, the role of plasma NGAL in predicting RRT and mortality in critically ill COVID-19 patients has not been fully evaluated. Endostatin has not been studied in this context, and results from general ICU populations have been inconsistent. Furthermore, the integration of emerging biomarkers with XAI to predict AKI and RRT has not been explored. Such methods hold promise not only for improving predictive accuracy but also for ranking the relative import-

ance of variables, identifying independently associated predictors, and uncovering key biomarkers that traditional statistical approaches may overlook. Studies in large and well-characterised multicentre ICU cohorts are needed to address these gaps.

Aims

Paper I

To investigate plasma NGAL at ICU admission and on ICU day 2 in critical COVID-19, primarily as an independent predictor of the need for RRT, secondarily as an independent predictor of 90-day mortality.

Paper II

To assess plasma endostatin at ICU admission as an independent predictor of new-onset AKI, the need for RRT, and 90-day mortality in critical COVID-19.

Paper III

To evaluate plasma endostatin at ICU admission as an independent predictor of new-onset AKI, the need for RRT, and 30-day mortality in critically ill patients.

Paper IV

To identify the most important predictors of new-onset AKI and the need for RRT in critically ill patients at ICU admission, using an XAI approach based on XGBoost. Additionally, to evaluate the predictive performance of XGBoost compared to logistic regression.

Methods and materials

Papers I & II

Study design and setting

A retrospective analysis of a prospectively collected multicentre cohort was conducted as part of the SWECRIT project [34, 96]. The study involved six ICUs in southern Sweden: two general, one cardiothoracic, and one infectious disease ICU at Skåne University Hospital (Lund and Malmö), as well as two general ICUs at Helsingborg Hospital and Kristianstad Hospital. ICU admissions were consecutively included between May 11, 2020, and May 10, 2021, with follow-up monitoring extending to at least 101 days post-ICU admission. Blood samples were collected at ICU admission, on ICU days 2 and 7, and at 3- and 12-month follow-up visits, and stored in the SWECRIT biobank for retrospective analyses. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed for Paper II [97].

Participants

Adult ICU admissions with SARS-CoV-2 infection were eligible for inclusion. General exclusion criteria were ICU admission primarily due to reasons other than COVID-19 or lack of consent. Study-specific exclusion criteria were missing all blood samples (Paper I, RRT analysis), missing SAPS 3 data (Paper I, mortality analysis), missing NGAL at ICU admission or on ICU day 2 (Paper I, mortality analysis), and missing endostatin at ICU admission (Paper II).

Data sources and variables

Data were obtained from the SWECRIT-COVID-IR database, as detailed by Didriksson et al. [98]. NGAL, endostatin, creatinine, and CRP were retrospectively batch-analysed from blood samples stored in the SWECRIT biobank. All other laboratory values were automatically extracted from electronic medical records. Confirmation of SARS-CoV-2 infection relied on real-time reverse transcriptase-polymerase chain reaction (RT-PCR) testing conducted on nasal or pharyngeal swabs or lower respiratory airway aspirates. SAPS 3 and SOFA parameters, survival data, age, and sex were automatically extracted from the Patient Administrative System for Intensive Care Units (PasIva). PasIva is a digital system where treating clinicians submit data to the Swedish Intensive Care Registry (SIR) during patients' ICU stay. PasIva is linked to the national Swedish population register. Data on comorbidities and treatments were collected manually from electronic medical records.

In Paper I, the difference in NGAL measured at ICU admission and on ICU day 2 was calculated by subtracting the admission value from the day 2 value. This difference was referred to as ΔNGAL.

In Paper II, endostatin was analysed as a categorical variable for logistic regression and net reclassification improvement (NRI) analyses. Values < 50 ng/mL represented a baseline state, as supported by studies reporting similar endostatin levels in healthy individuals and a mixed elderly population [99, 100]. A cut-off of 100 ng/mL was chosen based on evidence linking endostatin levels around 100 ng/mL to increased mortality in critically ill patients with AKI, as well as for pragmatic reasons [81].

Baseline creatinine was defined as the last creatinine value before ICU admission. Creatinine was analysed at ICU admission and on ICU days 2 and 7 for the study, while daily measurements were performed as part of routine intensive care. Urine output was recorded at ICU admission and daily until ICU discharge. If 24-hour monitoring was not possible, urine output was extrapolated from hourly measurements. AKI was defined as fulfilment of the KDIGO criteria [26]. If an admission met at least one KDIGO criterion on a given day, AKI was considered present, even if data for other criteria were missing. Missing daily AKI status was imputed when the same value (AKI or no AKI) was observed both before and after the missing entry, assuming no change in status. Missing AKI status on the day of ICU admission was imputed using the status from ICU day 2, provided AKI was not present on that day. For missing AKI status on the day of ICU discharge, the previous day's AKI status was carried forward. Patients with AKI at ICU admission were excluded from analyses of AKI on ICU day 1

to focus on new-onset AKI. RRT was regarded as AKI. For the outcome of RRT, we evaluated its initiation in the ICU after ICU admission.

ICU day 1 was defined as beginning at 6 a.m. on the morning following ICU admission; ICU day 2 and subsequent days were defined accordingly.

In Paper I, the primary outcome was RRT during the ICU stay, and the secondary outcome was 90-day mortality. The primary outcomes in Paper II were new-onset AKI on ICU day 1, RRT during the ICU stay, and 90-day mortality.

Study size

The sample size was determined by the number of ICU admissions due to COVID-19 during the study period, patient consent, and the completeness of NGAL (Paper I), SAPS 3 (Paper I), and endostatin (Paper II) data. For the analysis of new-onset AKI in Paper II, the sample size was further reduced by excluding admissions that had AKI at ICU admission.

Bias

Treating clinicians did not know NGAL or endostatin levels, but they had access to creatinine, CRP, and occasionally cystatin C levels as part of routine intensive care. Knowledge of these levels may have influenced clinical decisions. Trained data collectors performed the manual data recording. Guidelines for data collection were standardised and precisely outlined. The management of missing data was discussed and decided on collectively in the study group.

Papers III & IV

Study design and setting

A retrospective multicentre cohort study was conducted as part of the SWECRIT project [34, 96]. Consecutive ICU admissions between 2015 and 2018 were included from four general ICUs in southern Sweden: Skåne University Hospital in Lund and Malmö, Helsingborg Hospital, and Kristianstad Hospital. Blood samples were obtained at ICU admission and preserved in the SWECRIT biobank for retrospective analyses. The STROBE guidelines were followed [97].

Participants

All adult ICU admissions were screened for eligibility. Exclusion criteria included discharge alive from the ICU within 24 hours, the absence or incorrect handling of a biobank sample, withdrawal of consent, and transfer to another ICU without re-sampling.

Data sources and variables

Creatinine, cystatin C, NGAL, endostatin, ICAM-1, VCAM-1, CRP, calprotectin, and albumin were retrospectively batch-analysed from blood samples stored in the SWECRIT biobank. Clinical data, including SAPS 3, SOFA, survival, age, and sex, were automatically extracted from PasIva. Data on body weight, body mass index (BMI), diabetes mellitus, hypertension, and chronic dialysis were manually collected from electronic medical records. Baseline creatinine, lactate, and WBC were automatically extracted from electronic medical records.

For baseline creatinine, the value closest to ICU admission within 7 to 365 days before was recorded. If baseline creatinine was missing, it was estimated using the 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation, assuming a baseline estimated GFR (eGFR) of 75 mL/min/1.73 m^2 [101]. CKD was defined as eGFR $<$ 60 mL/min/1.73 m^2 [26]. Urine output was recorded at ICU admission and daily until ICU discharge. If 24-hour monitoring was not possible, urine output was extrapolated from hourly measurements. AKI was defined as fulfilment of the KDIGO criteria within 48 hours after ICU admission [26]. New-onset AKI was defined as the absence of AKI at ICU admission, followed by its subsequent development within 48 hours after ICU admission. Admissions with AKI or missing AKI status at ICU admission were excluded from analyses of new-onset AKI. For admissions to be classified as not having AKI at a certain time point, both creatinine and urine output data had to be available. For admissions to be classified as having AKI at a certain time point, missing creatinine or urine output data were accepted as long as they met KDIGO criteria. RRT was regarded as AKI unless the admission had a diagnosis of dialysable intoxication, in which case, they needed to fulfil another KDIGO criterion.

In Paper III, the primary outcomes were new-onset AKI and new-onset stage 3 AKI within 48 hours after ICU admission, RRT in the ICU within 7 days after ICU admission, and 30-day mortality. The primary outcomes in Paper IV were

new-onset AKI within 48 hours after ICU admission and RRT in the ICU within 7 days after ICU admission.

Study size

The sample size was determined by the number of ICU admissions during the study period, ICU length of stay, the validity of collected blood samples, and the number of patients who declined participation. For the analysis of new-onset AKI, the sample size was further reduced by excluding admissions that had AKI at ICU admission. Missing data on RRT status and 30-day mortality reduced the number of included admissions in the respective analyses.

Bias

Treating clinicians and data collectors did not know NGAL, endostatin, ICAM-1, VCAM-1, or calprotectin levels. However, they had access to creatinine, CRP, and occasionally cystatin C and albumin levels as part of routine intensive care. Knowledge of these levels may have influenced clinical decisions. Trained data collectors performed the manual data recording. Guidelines for data collection were standardised and precisely outlined. The management of missing data was discussed and decided on collectively in the study group.

Biomarker analyses

Blood samples were collected using ethylenediamine tetraacetic acid (EDTA) vacutainers and centrifuged to obtain EDTA plasma. The plasma samples were aliquoted and stored in the SWECRIT biobank at $-80\,^{\circ}\mathrm{C}$. ICU admission samples had to be collected within 6 hours of admission. In cases where the sampling time was missing, samples were included if the freezing time fell within the 6-hour time frame.

Creatinine, cystatin C, and albumin were analysed on a Mindray BS380 chemistry analyser (Mindray Medical International, Shenzhen, China) using traceable enzymatic creatinine reagents from Abbott Laboratories (Abbott Park, IL, USA), particle-enhanced turbidimetric cystatin C reagents from Gentian AS (Moss, Norway), and albumin reagents from Abbott Laboratories. NGAL and endostatin analyses were performed using commercial sandwich kits (DY1757/DY1098, R&D Systems, Minneapolis, MN, USA). ICAM-1 and

VCAM-1 were also analysed with commercial sandwich kits (DY720/DY809, R&D Systems). For the analysis of CRP and calprotectin, CRP reagents from Abbott Laboratories and calprotectin reagents from Gentian AS were used on a Mindray BS430/BS380 chemistry analyser (Mindray Medical International). All analyses were performed according to the manufacturers' recommendations.

Statistics

Statistical analyses were performed in R [102]. Significance was determined as a p-value of less than 0.05.

Median values were calculated for general characteristics, SAPS 3, physiological parameters, and laboratory values. Mean values were calculated for the Glasgow Coma Scale, SOFA score, and Charlson Comorbidity Index (CCI). The Wilcoxon rank-sum test or the Kruskal-Wallis test was used to compare medians, as appropriate. Pearson's chi-squared test or the chi-square test of independence was used to compare proportions, as appropriate. The unpaired t-test or the analysis of variance was used to compare means, as appropriate.

In Papers III and IV, when both baseline creatinine and body weight were missing, body weight was first imputed using linear regression based on age and sex. The imputed weight was then used to estimate baseline creatinine for AKI classification.

For logistic regression and XAI analyses in Paper IV, variables with more than 20% missing rates were excluded except baseline creatinine. Missing values in the remaining variables were imputed using a random forest–based method in two iterations using 100 trees.

Local polynomial regression with smoothing was employed to show trends in the association between NGAL, endostatin, creatinine, and outcomes [103].

Non-normally distributed continuous variables were log-transformed prior to logistic regression and XAI analyses. Subsequent z-normalisation was performed to facilitate the calculation and comparison of odds ratios (ORs) in Papers II and III.

Logistic regression was used to develop prediction models for new-onset AKI, RRT, and mortality and to assess associations between predictors and outcomes. In Paper I, the models included NGAL, cystatin C, creatinine, age, and sex (all at ICU admission), as well as ΔNGAL. In Paper II, the models included

endostatin, CRP, creatinine, age, and sex (all at ICU admission). In Paper III, the models included endostatin, cystatin C, creatinine, age, and SAPS 3 (all at ICU admission). In Paper IV, the models included all available variables at ICU admission.

For XAI analyses in Paper IV, the XGBoost package (version 1.7.9.1) was used [88]. Limited hyperparameter tuning by grid search was performed (boosting rounds of 100–300, maximum tree depth of 3–5, learning rate of 0.03–0.05, gamma of 0–1, and minimum child weight of 3–5). The final XGBoost model used 300 boosting rounds, a maximum tree depth of 3, a learning rate of 0.03, a gamma of 1, a subsample of 0.8, a column sample by tree of 0.8, and a minimum child weight of 5 for both new-onset AKI and RRT. Logistic regression was utilised alongside XGBoost to create prediction models of new-onset AKI and RRT. Model training was conducted using repeated 10-fold cross-validation with 20 repetitions.

Model discrimination was assessed using the area under the receiver operating characteristic (ROC) curve (AUC) with 95% confidence intervals (CI). In Papers III and IV, mean AUCs were estimated using 10-fold cross-validation with 20 repetitions, and 95% CIs were derived from the repeated validation runs. Differences in AUCs (or mean AUCs) were assessed using the method proposed by DeLong et al. [104]. NRI was calculated to assess the added predictive value of NGAL and endostatin in Papers I and II. Integrated discrimination improvement (IDI) was also used for model comparison in Paper I. Additionally, the population was stratified into tertiles based on the predicted mortality risk from the best-performing model in Paper I.

Ethics

Papers I & II

These studies were approved by the Swedish Ethical Review Authority (DNR 2020-011955, which was based on DNR 2014/47). Written informed consent was obtained at ICU admission. If the patient was too severely ill to consent at ICU admission, consent was obtained as soon as the patient's condition allowed. Presumed consent was applied if the patient died before consent could be obtained. In such cases, the patient's next of kin were informed and allowed to opt out on behalf of the deceased. Although the use of presumed consent for deceased patients can be ethically debated, it was considered ethically justified to enable the inclusion of the most critically ill patients. Excluding these patients would

reduce the generalisability of the findings and limit progress in improving care for those at the highest risk.

Papers III & IV

These studies were approved by the Swedish Ethical Review Authority (DNR 2015/267 and 2017/802) and followed a different consent procedure. Patients who survived at least three months after ICU discharge received an informational letter about the study and the option to withdraw their participation (opt-out). For deceased patients, presumed consent was applied, and next of kin were allowed to opt out on behalf of their relative. While the opt-out procedure may raise ethical considerations, it was deemed necessary to enable the development of a large and representative ICU biobank, given the practical and ethical challenges of obtaining informed consent from critically ill patients who are often unconscious or sedated at the time of admission. A procedure similar to that used in Papers I and II would not have been feasible due to the large number of patients included.

Results

Papers I & II

Participants

A total of 607 patients were admitted to the ICU with COVID-19 during the study period. Of these, 65 were primarily admitted for reasons other than COVID-19, 25 missed inclusion, and 19 patients did not provide consent, resulting in 498 eligible patients. For the RRT analysis in Paper I, 4 patients were excluded due to missing blood samples, leaving 494 patients. For the survival analysis in Paper I, 99 patients were excluded due to missing SAPS 3 or NGAL data, resulting in 399 patients available for analysis. See Figure 2. For Paper II, 14 patients with missing admission endostatin were excluded, resulting in a final study population of 484 patients. See Figure 3. The differences in sample size between the analyses reflect the slightly differing exclusion criteria applied in each study.

Descriptive data

Based on the study population from Paper II ($n = 484$), the median age was 66 years (interquartile range [IQR] 56–73), and 74% of the patients were male. The mean CCI was 2.9 (standard deviation [SD] 1.9). The median SAPS 3 score was 60 (IQR 50–69), and the mean SOFA score at ICU admission was 7.4 (SD 3.2). CKD was present in 4.0% of the cohort. The median NGAL at ICU admission was 110 ng/mL (IQR 83–170), and the median endostatin was 61 ng/mL (IQR 50–83). The median CRP at ICU admission was 150 mg/L (IQR 83–230), and the median creatinine was 77 µmol/L (IQR 65–100). The rate of invasive mechanical ventilation was 72%.

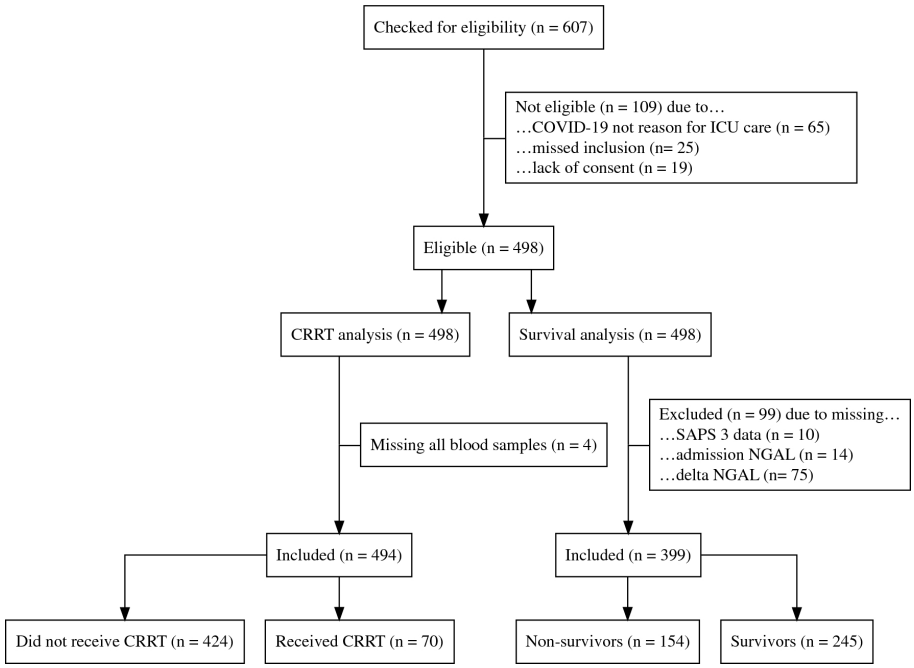

Figure 2: Flow chart of included and excluded patients in Paper I. *ICU* Intensive Care Unit, *CRRT* Continuous Renal Replacement Therapy, *SAPS* Simplified Acute Physiology Score, *NGAL* Neutrophil Gelatinase-Associated Lipocalin.

Outcomes

Based on the study population of Paper II ($n = 484$), AKI during the ICU stay occurred in 61%, with 36% of cases present at ICU admission and 25% developing on ICU day 1. AKI status during the ICU stay was missing for 15% of the population. A total of 13% of the study population received RRT. Ninety-day mortality was 39%. The relationships between endostatin, AKI, RRT, and mortality are visualised in Figure 4.

New-onset AKI

Logistic regression analysis ($n = 307$), adjusted for age, sex, CRP, and creatinine, showed that endostatin levels of 100–200 ng/mL were associated with new-onset AKI on ICU day 1 (OR 5.1, 95% CI 1.5–18, $p = 0.0097$). See Table 3. Adding endostatin to creatinine resulted in an NRI of 14% (95% CI 2.6–24). Adding endostatin to a model containing age, sex, CRP, and creatinine resulted in an NRI of 13% (95% CI 1.0–23).

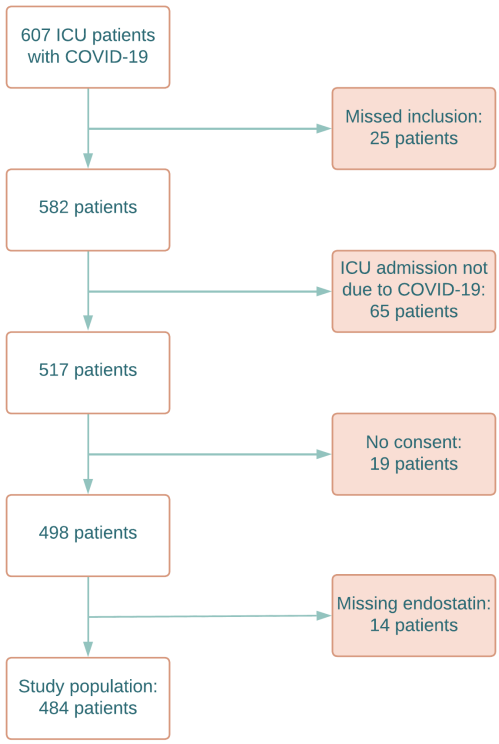

Figure 3: Flow chart of included and excluded patients in Paper II. *ICU* Intensive Care Unit.

Table 3: Association between plasma endostatin at intensive care unit admission and new-onset AKI on ICU day 1, RRT, and 90-day mortality.

Variable	**AKI OR (95% CI)**	**RRT OR (95% CI)**	**90-day mortality OR (95% CI)**
Endostatin (ng/mL)			
< 50	1.0 (reference)	1.0 (reference)	1.0 (reference)
50–100	1.5 (0.65–3.6)	2.4 (1.0–6.5)	1.6 (0.93–2.7)
100–200	5.1 (1.5–18)	3.5 (1.1–12)	4.2 (1.6–11)
> 200	2.5 (0.63–9.5)	2.1 (0.29–10)	2.2 (0.69–6.8)
Age	1.0 (0.99–1.0)	1.0 (0.98–1.0)	1.1 (1.1–1.1)
Male sex	0.67 (0.34–1.4)	1.3 (0.64–2.6)	0.81 (0.49–1.3)
C-reactive protein	1.2 (0.84–1.7)	0.84 (0.65–1.1)	0.86 (0.68–1.1)
Creatinine	2.8 (1.8–4.6)	1.8 (1.4–2.5)	1.1 (0.84–1.4)

P-values are reported in Paper II. AKI Acute Kidney Injury, *RRT* Renal Replacement Therapy, *OR* Odds Ratio, *CI* Confidence Interval.

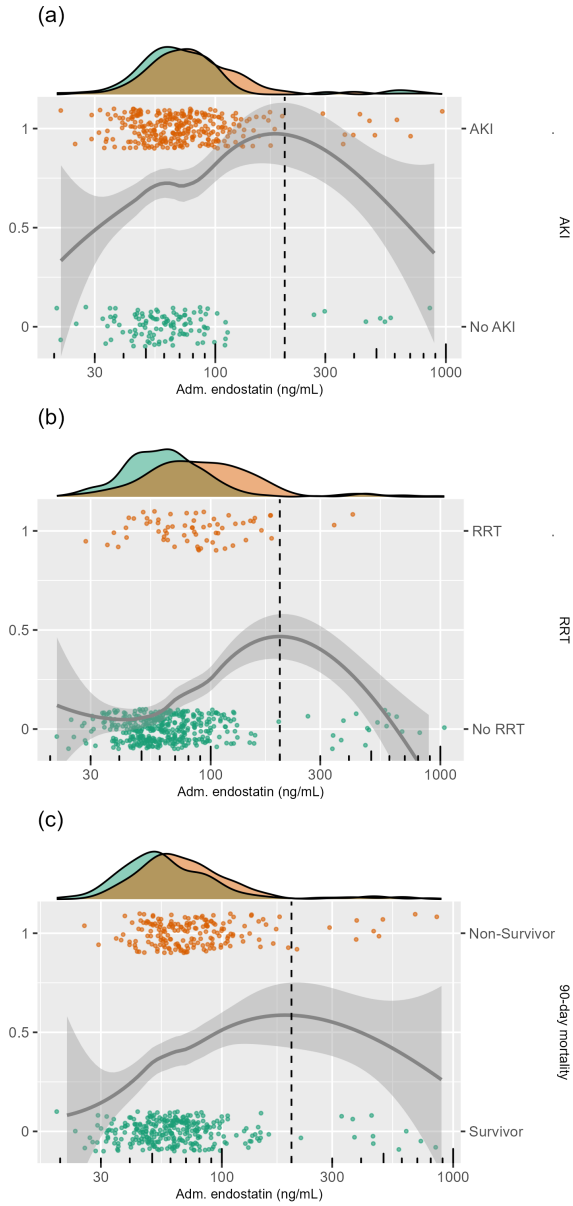

Figure 4: The relationships between plasma endostatin at intensive care unit admission and AKI, RRT, and 90-day mortality visualised in scatter and density plots. The x-axes have logarithmic scales. The points show the outcome (AKI, RRT, or 90-day mortality) versus endostatin level in individual patients. Continuous lines represent smoothed conditional means, while the accompanying shading indicates the corresponding 95% confidence intervals. The dashed vertical line indicates 200 ng/mL. *Adm* Admission, *AKI* Acute Kidney Injury, *RRT* Renal Replacement Therapy.

RRT

The RRT prediction models (n = 494) achieved AUCs ranging from 0.87 to 0.95. The highest AUC (0.95) was observed in the model incorporating NGAL, creatinine, and cystatin C as independent variables. Compared to the best-performing model without NGAL (which included creatinine and cystatin C), the inclusion of NGAL resulted in an IDI of 7.4%. See Figure 5.

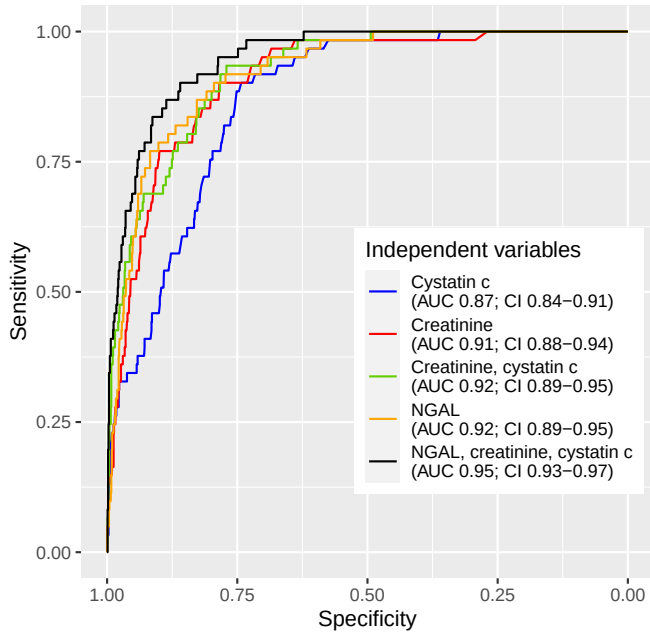

Figure 5: Receiver operating characteristic (ROC) curves for predicting renal replacement therapy. *P*-values: cystatin C vs. creatinine < 0.001, creatinine and cystatin C < 0.001, NGAL 0.002, NGAL, creatinine, and cystatin C < 0.001; creatinine vs. creatinine and cystatin C 0.021, NGAL 0.271, NGAL, creatinine, and cystatin C 0.001; creatinine and cystatin C vs. NGAL 0.50, NGAL, creatinine, and cystatin C 0.004; NGAL vs. NGAL, creatinine, and cystatin C 0.007. *AUC* Area Under the Curve, *CI* Confidence Interval, *NGAL* Neutrophil Gelatinase-Associated Lipocalin.

Logistic regression analysis (n = 483), adjusted for age, sex, CRP, and creatinine, showed that endostatin levels of 100–200 ng/mL were associated with RRT (OR 3.5, 95% CI 1.1–12, p = 0.039). See Table 3. Adding endostatin to creatinine resulted in an NRI of -1.7% (95% CI -18–15). Adding endostatin to a model containing age, sex, CRP, and creatinine resulted in an NRI of 8.7% (95% CI -6.3–24).

Mortality

The 90-day mortality prediction models ($n = 399$) yielded AUCs ranging from 0.63 to 0.83. The highest AUC (0.83) was achieved by the model including age, sex, NGAL at admission, and ΔNGAL. Compared to the best-performing model without NGAL (which included only age and sex), this model showed an IDI of 2.0%. When predicted risk was divided into tertiles, the NRI between the same two models was 8.1%. The second-best model included age, sex, and NGAL at admission as independent variables (AUC 0.80, $p = 0.005$). See Figure 6.

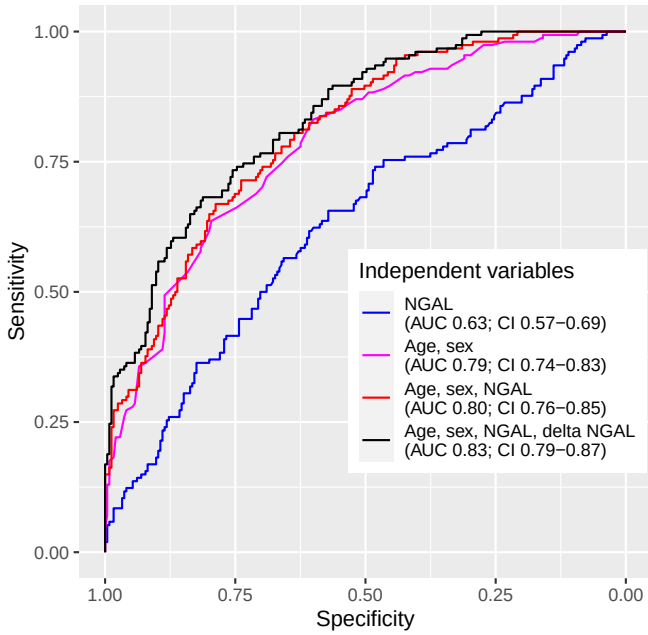

Figure 6: Receiver operating characteristic curves (ROC) for predicting 90-day mortality. *P*-values: NGAL vs. age and sex < 0.001, age, sex, and NGAL < 0.001, age, sex, NGAL, and ΔNGAL < 0.001; age and sex vs. age, sex, and NGAL 0.030, age, sex, NGAL, and ΔNGAL 0.001; age, sex, and NGAL vs. age, sex, NGAL, and ΔNGAL 0.005. *AUC* Area Under the Curve, *CI* Confidence Interval, *NGAL* Neutrophil Gelatinase-Associated Lipocalin.

Logistic regression analysis ($n = 483$), adjusted for age, sex, CRP, and creatinine, showed that endostatin levels of 100–200 ng/mL were associated with 90-day mortality (OR 4.2, 95% CI 1.6–11, $p = 0.0037$). See Table 3. Adding endostatin to a model containing age, sex, CRP, and creatinine resulted in an NRI of 7.6% (95% CI 1.6–13).

Papers III & IV

Participants

Of the 8,360 ICU admissions identified during the study period, 4,732 were included in the study. See Figure 7. A total of 321 admissions (6.8%) were ICU readmissions. In Paper III, new-onset AKI was analysed in 2,601 admissions, new-onset stage 3 AKI in 3,942 admissions, RRT in 4,714 admissions, and mortality in 4,729 admissions. In Paper IV, new-onset AKI was analysed in 2,603 admissions and RRT in 4,716 admissions.

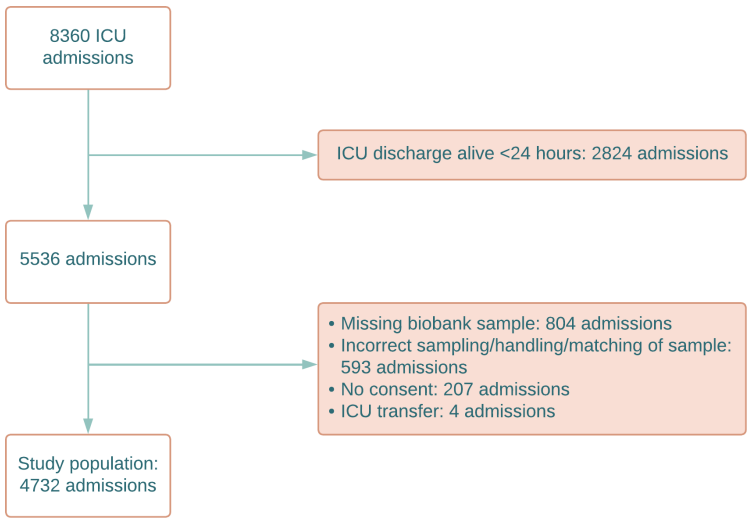

Figure 7: Flow chart of included and excluded patients in Papers III and IV. *ICU* Intensive Care Unit.

Descriptive data

The median age was 68 years (IQR 56–75), with males comprising 60%. CKD was present in 17% of patients. The rate of Sepsis-3 was 42%, the rate of cardiac arrest was 11%, and the rate of trauma was 5.4%. The three most common primary ICU diagnoses were cardiac arrest (10%), septic shock (9.9%), and respiratory failure (5.3%). The median SAPS 3 score was 64 (IQR 52–75), and the mean SOFA score at ICU admission was 6.9 (SD 4.0). Median

baseline creatinine was 79 µmol/L (IQR 62–110), while median creatinine at ICU admission was 100 µmol/L (IQR 69–160).

Outcomes

The overall rate of AKI was 62%, with 40% of admissions having AKI at ICU admission. New-onset AKI occurred in 22% of admissions. Among admissions with AKI, 35% had stage 1, 23% had stage 2, and 42% had stage 3. The rate of missing AKI status was 4.8%. RRT was initiated in 14% of patients during the ICU stay, and in 12% within 7 days after ICU admission. The median ICU length of stay was 2.5 days (IQR 1.5–4.8). Mortality within 30 days was 30%.

New-onset AKI

Adjusted ORs were 1.7 (95% CI 1.5–1.9) for endostatin, 1.4 (95% CI 1.2–1.6) for creatinine, 1.0 (95% CI 0.88–1.2) for cystatin C, and 1.5 (95% CI 1.4–1.7) for SAPS 3. Endostatin had superior discrimination compared to creatinine (mean AUC 0.67 vs. 0.63, $p < 0.001$) and cystatin C (mean AUC 0.67 vs. 0.63, $p < 0.001$). Adding endostatin to creatinine improved mean AUC compared to creatinine alone (0.68 vs. 0.63, $p < 0.001$). See Figure 8.

See Figure 9 for a SHAP summary plot of the top 10 predictors of new-onset AKI. An XGBoost model including all eligible predictor variables demonstrated superior discrimination compared to a logistic regression model with the same variables (mean AUC 0.76 vs. 0.74, $p < 0.001$). An XGBoost model including only the top 5 SHAP-ranked variables (urine output, endostatin, baseline creatinine, lactate, and albumin) also outperformed logistic regression using the same variables (mean AUC 0.74 vs. 0.73, $p < 0.001$). See Figure 10.

See Figure 11 for SHAP dependency plots for key predictors of new-onset AKI.

New-onset stage 3 AKI

Adjusted ORs were 1.4 (95% CI 1.2–1.6) for endostatin, 2.0 (95% CI 1.6–2.4) for creatinine, 1.5 (95% CI 1.3–1.8) for cystatin C, and 1.7 (95% CI 1.5–1.8) for SAPS 3. Endostatin had inferior discrimination to creatinine (mean AUC 0.73 vs. 0.77, $p < 0.001$) and cystatin C (mean AUC 0.73 vs. 0.77, $p < 0.001$). Adding endostatin to creatinine improved mean AUC (0.79 vs. 0.77, $p < 0.001$) compared to creatinine alone. See Figure 8.

RRT

Adjusted ORs were 1.2 (95% CI 1.05–1.4) for endostatin, 2.7 (95% CI 2.3–3.2) for creatinine, 1.4 (95% CI 1.1–1.7) for cystatin C, and 1.5 (95% CI 1.3–1.6) for SAPS 3. Endostatin's discrimination was inferior to creatinine (mean AUC 0.78 vs. 0.86, $p < 0.001$) and cystatin C (mean AUC 0.78 vs. 0.84, $p < 0.001$). Adding endostatin to creatinine did not enhance prediction (mean AUC 0.86 vs. 0.86, $p = 0.10$). See Figure 8.

See Figure 9 for a SHAP summary plot of the top 10 predictors of RRT. An XGBoost model including all eligible predictor variables demonstrated superior discrimination compared to a logistic regression model with the same variables (mean AUC 0.92 vs. 0.90, $p < 0.001$). Similarly, an XGBoost model using only the top 5 SHAP-ranked variables (creatinine, urine output, endostatin, NGAL, and SAPS 3) outperformed logistic regression using the same variables (mean AUC 0.90 vs. 0.89, $p < 0.001$). See Figure 10.

See Figure 11 for SHAP dependency plots for key predictors of RRT.

Mortality

For 30-day mortality, the adjusted OR was 1.1 (95% CI 0.98–1.1) for endostatin. Endostatin was inferior to SAPS 3 (mean AUC 0.61 vs. 0.79, $p < 0.001$). Adding endostatin to SAPS 3 did not improve mean AUC (0.79 vs. 0.79, $p = 1.0$). Combining endostatin with age was also inferior to SAPS 3 (mean AUC 0.66 vs. 0.78, $p < 0.001$). See Figure 8.

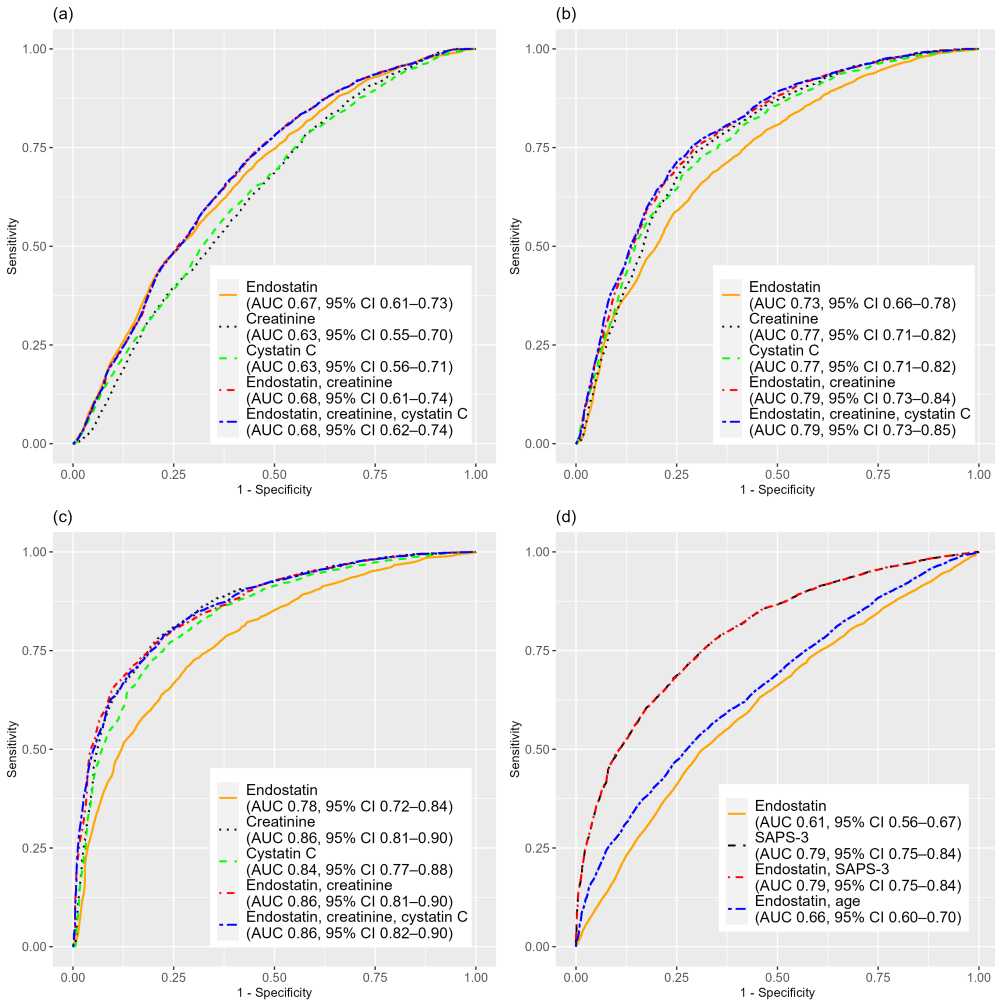

Figure 8: Receiver operating characteristic (ROC) curves and mean AUCs for endostatin and other variables at intensive care unit admission for the prediction of new-onset AKI (a), new-onset stage 3 AKI (b), renal replacement therapy (c), and 30-day mortality (d). *AUC* Area Under the Curve, *CI* Confidence Interval, *AKI* Acute Kidney Injury, *SAPS-3* Simplified Acute Physiology Score 3.

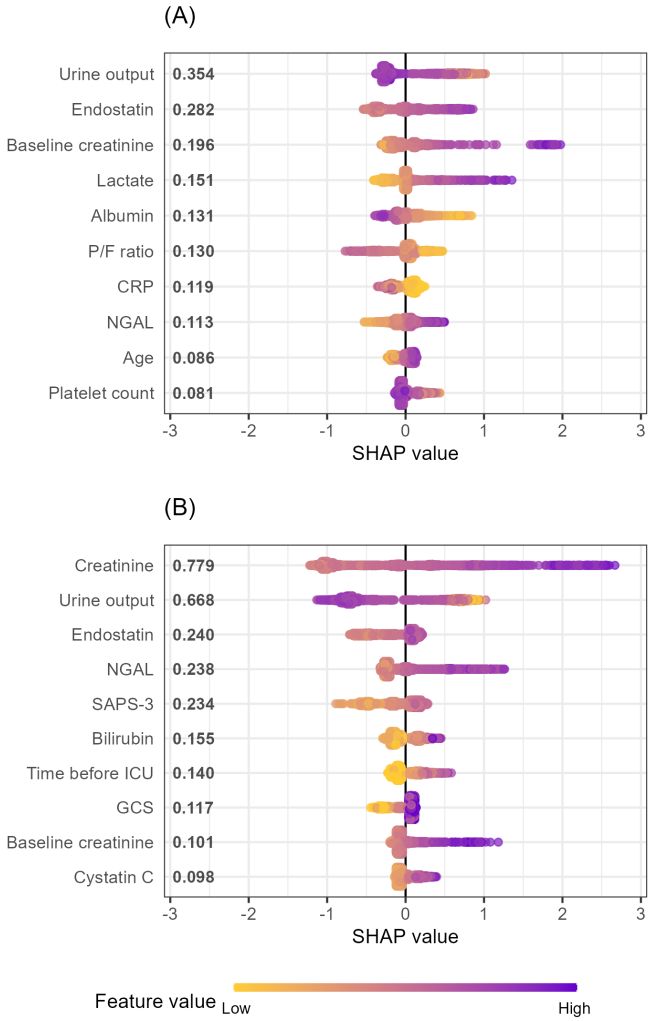

Figure 9: SHapley Additive exPlanations (SHAP) summary plots for eXtreme Gradient Boosting (XGBoost) models predicting new-onset acute kidney injury (A) and renal replacement therapy (B). Each shows the top 10 features ranked by mean absolute SHAP value, with feature values colour-coded from low (yellow) to high (purple). Positive SHAP values indicate an increased risk, while negative values indicate a decreased risk. *P/F ratio* PaO_2/FiO_2 ratio, *CRP* C-reactive protein, *NGAL* Neutrophil Gelatinase-Associated Lipocalin, *SAPS-3* Simplified Acute Physiology Score 3, *ICU* Intensive Care Unit, *GCS* Glasgow Coma Scale.

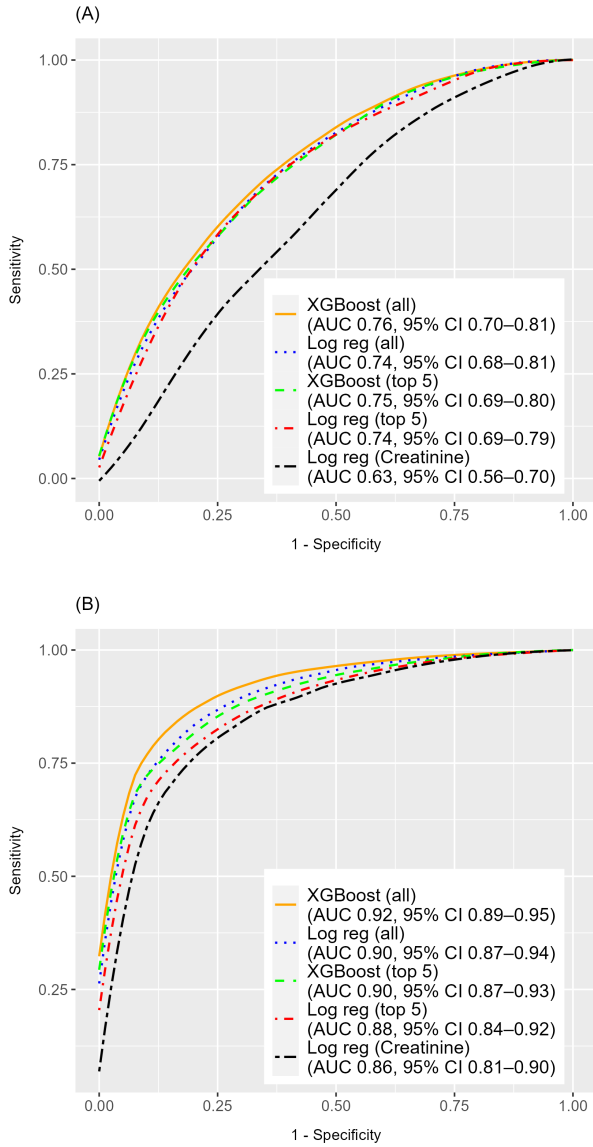

Figure 10: Receiver operating characteristic (ROC) curves for the prediction of new-onset acute kidney injury (A) and renal replacement therapy (B). Five models were compared: eXtreme Gradient Boosting (XGBoost) and logistic regression with all variables, XGBoost and logistic regression with the top 5 SHAP-ranked variables, and logistic regression with only creatinine. AUC values represent means, and CIs were derived from repeated cross-validation. Curves represent smoothed averages across validation folds. *Log reg* Logistic regression, *AUC* Area Under the Curve, *CI*, Confidence Interval, *SHAP* SHapley Additive exPlanations.

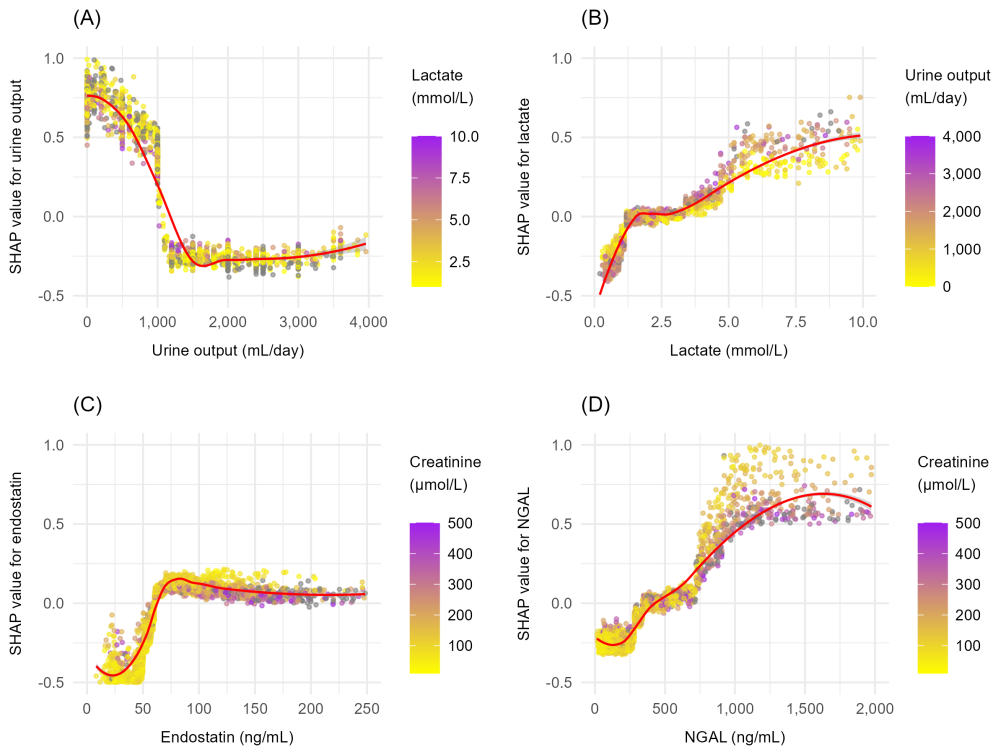

Figure 11: SHapley Additive exPlanations (SHAP) dependency plots for key predictors in eXtreme Gradient Boosting (XGBoost) models for acute kidney injury (A, B) and renal replacement therapy (C, D). Each point represents a patient, with SHAP value on the y-axis indicating the feature's impact on the prediction. Feature values are colour-coded from low (yellow) to high (purple). Smoothing curves (red) illustrate the associations between feature values and SHAP contributions. *NGAL* Neutrophil Gelatinase-Associated Lipocalin.

Discussion

This thesis investigated the early predictive value of biomarkers, with a special focus on NGAL and endostatin, for new-onset AKI, RRT, and mortality in ICU patients. In patients with critical COVID-19, NGAL in plasma at ICU admission and an early increase in NGAL improved the prediction of RRT and 90-day mortality when added to conventional markers. In the same COVID-19 population, plasma endostatin levels of 100–200 ng/mL at ICU admission were independently associated with new-onset AKI on ICU day 1, RRT, and 90-day mortality. The addition of endostatin improved the prediction of new-onset AKI on ICU day 1 and 90-day mortality, but not RRT. In a large general ICU population, plasma endostatin was independently associated with new-onset AKI, new-onset stage 3 AKI, and RRT. Endostatin showed better predictive performance for new-onset AKI than creatinine and cystatin C. Adding endostatin to creatinine improved the prediction of new-onset AKI and stage 3 AKI, but not RRT. The value of endostatin for prediction of 30-day mortality in a general ICU population was limited. In the same general ICU population, XGBoost and SHAP identified that the strongest predictors of new-onset AKI were urine output, endostatin, baseline creatinine, lactate, and albumin. The most important predictors of RRT were creatinine, urine output, endostatin, NGAL, and SAPS 3. XGBoost outperformed logistic regression in predicting new-onset AKI and RRT, and SHAP revealed non-linear associations, especially notable for endostatin and urine output.

NGAL, initially identified in neutrophils, is upregulated in renal tubular cells after AKI and detectable in urine and plasma soon after injury. Unlike creatinine, which reflects GFR, NGAL captures active injury processes, which may explain its early predictive value as a marker of structural kidney damage [42]. The performance of NGAL in predicting RRT and mortality in COVID-19, as well as RRT in a general ICU population, aligns with previous research. Both blood and urine NGAL have shown strong predictive value for AKI and RRT across various settings, as demonstrated in several meta-analyses [40, 43]. In COVID-

19, two studies conducted outside the ICU have reported associations between urinary NGAL and both AKI and RRT [45, 46]. Additionally, one ICU-based study found that urinary NGAL was independently associated with mortality in COVID-19 patients [84]. However, only two smaller studies have evaluated NGAL in blood in the context of COVID-19. In one, serum NGAL measured in emergency department patients showed adequate predictive performance for AKI and RRT [48]. Another study involving 103 ICU patients reported that NGAL in blood was associated with severe AKI, RRT, and mortality [47].

To our knowledge, Paper I represents the most extensive study investigating NGAL in COVID-19. In contrast to several earlier studies, NGAL was evaluated alongside established renal biomarkers and key clinical confounders, underscoring its added predictive value. Serial measurements enabled analysis of NGAL dynamics, further strengthening its prognostic utility. Practically, plasma NGAL may be preferable to urinary NGAL in critically ill patients. Blood samples are routinely collected in the ICU and readily integrated into clinical workflows. Importantly, plasma NGAL remains available even in the setting of anuria, a common complication in severe AKI. In addition, NGAL can be analysed from the same blood samples used for other routine tests, potentially facilitating implementation and enhancing its clinical applicability. Although the use of plasma/serum NGAL has occasionally been questioned, two reviews have concluded that its predictive performance is comparable to that of urinary NGAL [40, 41, 44]. In the general ICU population analysed in Paper IV, plasma NGAL ranked the eighth most important predictor of new-onset AKI and the fourth most important for RRT. Together with the broader body of evidence establishing NGAL as a robust predictor of AKI and RRT across diverse settings, these findings support its potential clinical implementation. Nonetheless, Paper IV also shows that several other biomarkers had a more substantial predictive influence for new-onset AKI and RRT in a general ICU population.

Endostatin is a considerably less studied biomarker in critical illness compared to NGAL. Previous findings on its utility as a marker of AKI and mortality in the ICU have been inconsistent. In a general ICU setting, a 2016 study found that adding endostatin to a predictive model improved AKI prediction [51]. Similarly, a more recent study reported that endostatin, combined with age and conventional biomarkers, effectively predicted AKI, RRT, and 30-day mortality at ICU admission [52]. One study has also reported an association between dynamic changes in endostatin levels and mortality in AKI [81]. In the context of critical COVID-19, elevated endostatin levels have been linked to hypoxia and mortality [85]. In contrast, a larger prospective study reported limited predictive value of endostatin for AKI, RRT, and mortality in a general ICU population

[54]. Several factors may explain the differences between this conflicting study and the results of this thesis. The cohorts in this thesis had higher rates of AKI and RRT, along with higher median endostatin levels, potentially reflecting more severely ill populations. The AKI observation period also differed. In addition, Paper III compared endostatin with creatinine and cystatin C and assessed its added predictive value when combined with these markers, an approach not used in the conflicting study.

The addition of endostatin improved the prediction of 90-day mortality in the COVID-19 population. In contrast, no added predictive value was observed in the general ICU population, suggesting limited utility for mortality prediction in a more heterogeneous ICU population. In COVID-19, endostatin showed a non-linear, U-shaped relationship with key outcomes, with the highest risk observed around 200 ng/mL. This pattern was not observed in the general ICU population. Thus, endostatin may reflect pathophysiological processes specific to COVID-19, such as endothelial dysfunction or systemic inflammation, which could explain the differing results between cohorts. However, its independent association with new-onset AKI and RRT suggests that elevated endostatin levels represent disease mechanisms beyond general illness severity. While endostatin did not improve RRT prediction in Paper III, it emerged as the third most important predictor of RRT in Paper IV. This discrepancy likely reflects differences in analytic approach. Paper III used traditional regression models, where endostatin was independently associated with RRT but did not improve overall predictive performance. In contrast, Paper IV identified endostatin as a top predictor of RRT, suggesting that non-linear relationships or interactions captured by XAI may reveal predictive value not apparent in conventional models.

Endostatin is a fragment of collagen XVIII, a protein integral to the kidney's glomerular and tubular basement membranes [49]. In experimental models of AKI, renal expression of collagen XVIII and endostatin increases early following kidney injury [105]. Moreover, elevated endostatin levels in AKI may reflect increased collagen XVIII turnover. However, because collagen XVIII is widely expressed in basement membranes throughout the body, circulating endostatin likely reflects broader tissue remodelling, limiting its specificity for kidney injury. Given its low molecular weight (20 kDa), endostatin is expected to be relatively freely filtered by the glomerulus, suggesting that plasma levels inversely reflect GFR [51, 106]. In addition, endostatin is a potent angiogenesis inhibitor and may serve as a marker of endothelial injury and microvascular dysfunction [85]. Its release during basement membrane remodelling further supports a potential role in capillary leakage and vascular stress associated with inflammation [107].

Among the top predictors of new-onset AKI and RRT identified in Paper IV,

several markers stood out for their expected and unexpected contributions. Urine output emerged as one of the strongest predictors of new-onset AKI and RRT, consistent with its well-established role in AKI diagnosis and monitoring [108, 109]. However, in contrast to both clinical expectations and the findings of Paper IV, previous studies have not consistently identified urine output as a major predictor of the need for RRT [110, 111]. Although creatinine was the strongest predictor of RRT, it did not rank among the top 10 predictors of new-onset AKI, underscoring its limited sensitivity for early kidney injury [35, 36]. Similarly, despite being a well-established biomarker and a potentially quicker indicator of GFR, cystatin C was not among the top predictors of AKI and ranked only tenth for RRT. This contrasts with earlier studies reporting stronger predictive performance for cystatin C [37, 38]. Both creatinine and cystatin C primarily reflect changes in GFR, whereas NGAL and endostatin may capture structural kidney injury before GFR declines. The findings of this thesis particularly highlight endostatin in this role, as it ranked fourth among predictors of new-onset AKI, while NGAL ranked eighth.

Lactate and albumin are established markers of illness severity in the ICU [74, 112]. However, none of them are routinely used for AKI risk stratification. In Paper IV, lactate emerged as the fourth most important predictor of new-onset AKI. Dynamic changes in lactate levels have previously been linked to AKI, and the lactate-to-albumin ratio has been shown to predict the need for RRT in septic shock [113, 114]. Albumin ranked fifth among predictors of new-onset AKI, aligning with findings from a large meta-analysis demonstrating the independent association between hypoalbuminemia and AKI [115].

Paper IV is the third study to apply XGBoost for AKI prediction in a general ICU population and the first to extend its use to RRT prediction. Previous studies also found that XGBoost outperformed other ML algorithms and logistic regression for the prediction of AKI. A 2022 study identified ICU length of stay, creatinine, albumin, electrolytes, blood urea nitrogen, and glucose as top predictors, while a more recent study highlighted diuretic use, mechanical ventilation, vasopressor use, age, and antibiotics [94, 95]. These differences likely reflect key methodological variations. Both earlier studies relied solely on creatinine to define AKI and did not include urine output, which may have impaired detection. They also included variables collected after ICU admission, limiting their relevance for early risk assessment. Most importantly, differences in available predictors likely contributed to the divergent results. While the previous studies were larger, the present study focused on ICU admission and uniquely included emerging biomarkers, providing broader clinical relevance and novel insights into the early prediction of AKI and RRT.

Strengths and limitations

This thesis included the largest studies to date of NGAL and endostatin in COVID-19 and, by far, the most extensive study to evaluate endostatin in critically ill patients overall. Patients were included from multiple ICUs of varying sizes and case mixes, supporting generalisability within comparable healthcare systems. The comprehensive data incorporated prospectively collected blood samples, electronic medical records, and national registry data. In the COVID-19 population, the prospective design allowed for systematic follow-up and particularly detailed collection of comorbidities, laboratory parameters, and daily AKI status throughout the ICU stay. Multivariable analyses were consistently adjusted for established renal biomarkers and key clinical confounders, and model performance in Papers III and IV was internally validated using repeated cross-validation. With the exception of ΔNGAL in Paper I, all predictors were based on data available at ICU admission, enabling early risk assessment at a clinically important time point. A key methodological strength is the focus on new-onset AKI, achieved by incorporating pre-ICU baseline creatinine and excluding patients who already had AKI at admission from AKI analyses. This approach likely reduced misclassification and enhanced the relevance of the findings for identifying patients at risk of deterioration and guiding early intervention.

Integrating XAI, emerging biomarkers, and routine clinical data in Paper IV offered a novel and clinically relevant approach to early risk stratification. It provided insight into the relative importance of individual predictors and uncovered non-linear and independent associations. XGBoost, a robust and widely used ML algorithm, was chosen for its ability to model complex interactions, accommodate missing data, and outperform conventional statistical methods in structured data analysis [88]. The methodology was carefully described to ensure reproducibility, with transparent reporting of data preprocessing, variable selection, model parameters, and cross-validation strategy. Hyperparameters were tuned conservatively to favour generalisability over maximum performance, although further tuning or ensemble approaches might have yielded small performance gains. SHAP was used to enhance model interpretability, a critical step for clinical translation.

Using new-onset AKI as a primary outcome in Papers II to IV also had limitations. While it enabled a clearer assessment of early predictors, it excluded a substantial proportion of ICU patients from AKI analyses. It may also have underestimated the predictive value of creatinine, given that AKI criteria such as KDIGO rely heavily on creatinine. Nonetheless, this exclusion was necessary to enable comparisons between creatinine and other biomarkers. In Paper I, AKI

was not used as an outcome, which may be considered as a limitation. Instead, only RRT was chosen as it was considered a more patient-centred and clinically tangible outcome, representing severe AKI. RRT was also considered less susceptible to misclassification than the KDIGO criteria. In subsequent studies, the need to identify patients at risk before they progressed to severe AKI became more apparent. In Paper II, new-onset AKI on ICU day 1 was used as the primary outcome, as we hypothesised that endostatin would show its strongest association with new-onset AKI during this time window. In contrast, Papers III and IV defined new-onset AKI within 48 hours after ICU admission, reflecting the availability of reliable creatinine and urine output data. This time frame is consistent with the KDIGO criteria, which define AKI partly based on changes in creatinine within 48 hours, and is widely used in ICU-based AKI research [26, 116–118]. To improve data quality and meet the KDIGO time requirement for oliguria ($\geq$ 6 h), the urine output criterion was not used for defining AKI at ICU admission [26]. Consistent with previous studies, urine output data at the time of ICU admission were often missing or unreliable, which may have contributed to underrecognition of AKI present at ICU admission [119, 120]. Finally, patients on chronic dialysis may have been inadvertently included in the outcome of RRT. Data on chronic dialysis status were frequently missing in Papers III and IV, and this exclusion could therefore not be applied reliably.

All studies were conducted in ICUs within a single geographic region, which may limit generalisability to other healthcare settings. The absence of external validation in an independent cohort further restricts the applicability of the findings beyond the studied populations. However, endostatin was evaluated in two clinically distinct ICU populations: patients with COVID-19 in Paper II and a general ICU population in Paper III. NGAL was also assessed across both settings through its inclusion in the models presented in Paper IV. While these comparisons do not represent formal external validations, they offer some support for the consistency of findings across different patient groups. Repeated cross-validation was applied in Papers III and IV, but not in Papers I and II, which may limit the robustness of those results.

In Papers III and IV, ICU admissions discharged alive within 24 hours were excluded to avoid inclusion of low-risk patients primarily admitted for observation. Although this limits generalisability to less severely ill ICU populations, it aligns with the study's objective of evaluating early AKI risk in higher-risk patients, where timely detection may offer the most significant clinical benefit. Rather than individual patients, ICU admissions were included in Papers III and IV. While this design choice may introduce clustering due to repeat admissions, the overall readmission rate was low, and each ICU stay was considered a distinct

clinical episode to reflect the dynamic nature of AKI risk.

Although robust methodologies were employed to mitigate bias, the retrospective design of Papers III and IV was subject to inherent limitations, including incomplete data. Variables such as comorbidities not included in SAPS 3, body weight, and baseline creatinine were frequently missing. In particular, missing baseline creatinine may have resulted in misclassification of CKD as AKI, potentially leading to an overestimation of AKI at ICU admission. However, the outcome of new-onset AKI is unlikely to have been significantly affected by this. In Papers III and IV, admissions missing both baseline creatinine and body weight required imputation of body weight to estimate baseline creatinine using the CKD-EPI formula, which represents a notable limitation [101]. In Paper IV, imputation was also applied, primarily to variables with less than 20% missingness. While XGBoost can natively handle missing data, logistic regression lacks this functionality, making imputation necessary for a fair comparison between the two modelling approaches.

Endostatin was analysed as a categorical variable in Paper II. This approach was appropriate given the observed non-linear, U-shaped relationship between endostatin levels and outcomes, where risk increased to approximately 200 ng/mL and then declined. The cut-offs were based on prior studies from varying settings and pragmatic considerations, allowing for clinical interpretability despite the non-linear pattern. However, categorising a continuous biomarker may lead to loss of information and reduced statistical power, and the choice of thresholds could have influenced the results. Furthermore, the group with endostatin levels above 200 ng/mL in Paper II was relatively small, which limits the strength of conclusions that can be drawn about the observed U-shaped relationship. In contrast, endostatin was analysed as a continuous variable in Paper III. This decision reflected the lack of a clearly defined baseline or normal endostatin levels in critically ill patients and the absence of a U-shaped association with outcomes in the general ICU population.

The decision not to use AUC for model performance evaluation in Paper II was also based on the non-linear relationship between endostatin and key outcomes in COVID-19. AUC assumes a monotonic association between a predictor and outcome across all values. As very high values may not consistently contribute to model discrimination, this could lead to an underestimated AUC. To better assess the incremental predictive value of endostatin in Paper II, we chose to analyse NRI. Unlike AUC, NRI does not assume a linear relationship between predictor and outcome [121]. In Paper III, NRI was not applied because endostatin was analysed as a continuous variable and showed a more linear association with outcomes in the general ICU population, making AUC an appropriate measure

of model performance. Cross-validated mean AUCs in Paper III also allowed for robust internal model comparisons. However, without NRI, improvements in risk classification may not have been fully captured. Calibration was not assessed in any of the papers. While it is an important aspect of model evaluation, this thesis focused on assessing whether biomarkers and models showed adequate discriminatory ability or added predictive value, which is typically the first step in evaluating predictive performance. Moreover, widely used calibration tests such as the Hosmer–Lemeshow test can be problematic in large datasets, as even small and clinically irrelevant differences between predicted and observed outcomes may yield statistically significant results [122]. For these reasons, calibration analyses were not performed.

Although the conclusions of this thesis were based on statistically significant differences between models and measures of incremental predictive value, some of the observed AUCs, absolute AUC differences, and NRIs were relatively modest. AUC is a relative measure, and its interpretation depends heavily on the comparator. For example, while the AUC for endostatin in predicting new-onset AKI may be considered low in absolute terms, it exceeded that of creatinine and cystatin C. Similarly, the absolute increases in AUC for new-onset AKI and RRT prediction using XGBoost were small, yet highly statistically significant, and might have been greater if additional emerging AKI biomarkers had been available. These findings should therefore be interpreted within the appropriate context. While NGAL may already have sufficient evidence to support consideration for clinical use, endostatin and the XAI methodology applied in this thesis have not been presented as immediately practice-changing or ready for clinical adoption. Instead, they should be regarded as potentially useful tools for improving the prediction of important outcomes and as candidates for further clinical evaluation. Despite extensive research into promising candidates, the absence of new biomarkers and predictive tools in intensive care may partly reflect an unrealistic expectation of a "silver bullet" capable of transforming care single-handedly. A more pragmatic approach may be to gradually integrate multiple complementary biomarkers and prediction tools to enhance risk stratification.

Future directions

The adoption of new biomarkers for AKI into routine clinical practice has been limited. One major challenge is the variability in performance across studies, with biomarker accuracy influenced by differences in patient populations, measurement timing, assay methods, and confounding factors. In many cases, clinically validated cut-off values have not been established. Standardised assays

and point-of-care testing devices are also lacking for some biomarkers, and many tests remain confined to research settings [36]. Financial considerations are frequently cited as a barrier, although a recent cost-utility analysis suggests that biomarker-guided prediction of AKI may be cost-effective [123]. These limitations highlight the need for targeted research to address technical, logistical, and economic challenges before emerging biomarkers can be integrated into intensive care.

The findings of this thesis suggest several important directions for future research. Most importantly, external validation is needed to confirm the predictive performance and generalisability of endostatin and XAI-based models in independent ICU populations. While this thesis demonstrated that XAI methods such as XGBoost and SHAP can enhance risk prediction and identify key predictors of AKI, these tools should be tested in prospective studies to assess clinical reliability and real-world applicability. Paper IV included multiple emerging biomarkers, but several other promising candidates for AKI, such as KIM-1, IL-18, TIMP-2, and IGFBP7, were not assessed. Including these in future studies may further enhance predictive performance, especially when combined with XAI.

Once robust validation is achieved, further work should focus on defining optimal cut-off values and clinically actionable thresholds for NGAL and endostatin. Future studies should also evaluate their impact on real-world decision-making, for example, by assessing whether biomarker-guided risk stratification influences treatment decisions or improves patient outcomes. Ideally, these strategies should be tested in prospective clinical trials comparing care with and without access to emerging biomarkers and XAI tools [29]. Incorporating biomarkers with early prognostic value into clinical workflows may support more personalised care by guiding decisions about ICU admission and enabling earlier recognition of deterioration. Biomarker-guided risk stratification may also enhance the design of interventional trials by identifying patient subgroups most likely to benefit from targeted therapies, particularly for interventions that have failed to show benefit in heterogeneous ICU populations. Furthermore, improving illness severity scores such as SAPS 3 with emerging biomarkers of prognostic value may strengthen ICU benchmarking, support meaningful comparisons between units, and improve the accuracy of morbidity assessments in intensive care research. Although this work focused on ICU populations, the early predictive value of NGAL, endostatin, and XAI-based risk models may also improve risk stratification in other acute care settings, including emergency departments and hospital wards.

Finally, emerging biomarkers and XAI may provide new insights into the patho-

physiology of AKI, potentially uncovering novel disease pathways and therapeutic targets [36]. Integrating proteomic profiling with XAI represents a particularly promising strategy, with the potential to identify novel biomarkers, improve prediction, and refine disease phenotyping [124, 125]. However, as AI use expands in medicine, its implementation must be guided by validation, transparency, and interpretability. Ensuring that XAI models are accurate and clinically understandable will be essential to gaining trust among clinicians and ensuring safe integration into patient care.

Conclusions

This thesis highlights the potential of NGAL and endostatin as early predictors of new-onset AKI, the need for RRT, and mortality in intensive care. In patients with critical COVID-19, NGAL improved the prediction of both RRT and mortality beyond conventional markers. Endostatin was consistently and independently associated with new-onset AKI and RRT in both COVID-19 and a general ICU population. Furthermore, endostatin improved the prediction of new-onset AKI, outperforming creatinine and cystatin C in a general ICU population. XAI with XGBoost and SHAP further improved the prediction of new-onset AKI and RRT, identified key predictors such as NGAL and endostatin, and revealed clinically relevant non-linear relationships. Given the extensive existing evidence, NGAL should be considered for clinical implementation in both COVID-19 and general intensive care. Endostatin and XAI hold promise for improving the early identification of AKI, which remains a significant challenge in intensive care. External validation and prospective studies are warranted to determine the clinical utility of endostatin and XAI-based models. Future research should also explore how these promising tools can be integrated into clinical workflows. Their implementation may support more effective risk stratification, enable targeted clinical trials, and promote a more personalised approach to intensive care, ultimately contributing to improved outcomes in the ICU.

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About the author

HAZEM KOOZI is a medical doctor undertaking specialty training in anaesthesiology and intensive care at Kristianstad Hospital, Sweden. He received his medical degree from Lund University, where he later pursued doctoral studies. His PhD thesis focuses on biomarkers and explainable artificial intelligence to improve the early prediction of acute kidney injury and mortality in critically ill patients.

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