Application of new specific biomarkers for organ damage after open and endovascular thoracoabdominal aortic aneurysm surgery as model for more accurate perioperative patients' surveillance

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Dissertation

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AKI :	Acute kidney injury
APACHE II:	Acute Physiology and Chronic Health Evaluation
AUC:	Area under Curve
BIO-ADM	Bio-Adrenomedullin
cDPP3:	Circulating dipeptidyl peptidase 3
CSFD :	Cerebrospinal fluid drainage
CVVH:	Continous venovenous hemofiltration
DGG:	Deutsche Gesellschaft für Gefäßchirurgie
DPP3-LIA:	DPP3 luminescence immunoassay
ECC:	Extracorporeal circulation
fEVAR:	Fenestrated endovascular aortic aneurysm repair
bEVAR:	Branched endovascular aortic aneurysm repair
IGFBP7:	Insulin-like growth factor-binding protein 7
ICP :	Intracranial pressure
ICU:	Intensive care unit
IIT:	Investigator-Initiated Trial
IQR:	Interquartile ranges
MAP:	Mean arterial pressure
MEP:	Motor evoked potential
MIF:	Macrophage-migration inhibiting factor
NGAL.	Neutrophil gelatinase associated lipocalin
PI:	Principle Investigator
RNase 1:	Ribonuclease 1
RNH1:	RNase inhibitor 1
ROC:	Receiver operating characteristic
SD:	Standard deviations
SIRS:	Systemic inflammatory response syndrome
SLPI:	Secretory leucocyte peptidase inhibitor
sNGAL:	Serum neutrophil gelatinase-associated lipocalin

SOFA:	Sequential organ failure assessment
TAAA:	Thoracoabdominal aortic aneurysm
TIMP-2:	Tissue inhibitor of metalloproteinase 2
uNGAL:	Urine neutrophil gelatinase-associated lipocalin

Content

Clinical outcome and the role of biomarkers in the management of patients with complex aortic aneurysms

Chapter 1

General Introduction

In the 21st century, endovascular techniques have been displacing open repair of thoracoabdominal aortic aneurysm (TAAA) (1). Long-term results after successful endovascular TAAA repair are still pending, and large trials thus far have failed to suggest adequate long-term patency after complex fenestrated and branched aortic aneurysm repair (2) (3). The current trend is to a decreasing number of open aortic procedures performed annually, resulting in a significant lack of surgical education opportunities (4). Even if endovascular techniques could cover all elective and emergency aortic procedures within the next decade, some pathologies will remain inappropriately treated, including connective tissue disease–related TAAA and TAAA following failed endovascular repair or infected endovascular prostheses. These pathologies still require open repair to achieve definite and curative therapy, but the risk for perioperative morbidity and mortality will be increased (5). Large trials have shown mortality rates up to 16% depending on the extent of disease, a need for mass transfusion in more than 40% of surgeries, and acute kidney failure requiring renal replacement therapy in more than 20% (6).

Therefore, a better understanding is needed of the physiological and pathophysiological processes arising during open TAAA repair under elective and emergency conditions. An enhanced understanding of how surgical repair affects the aorta, viscero-renal organs, and spinal cord could lead to improved surgical techniques and better perioperative and postoperative surveillance. Some studies have highlighted the possibility of detecting complications early after major surgery based on different biomarkers (7). The impact on the aortic endothelium and systemically of open TAAA repair and the required surgical steps,

such as aortic cross-clamping and heparinisation, are still unknown, and opportunities to track the peri-operative inflammation cascade are limited (8).

In this context, the application of different biomarkers could be useful for assessing the peri-operative organ-specific and general condition of patients during and after open TAAA repair (9-11). Thus far, organ-specific biomarkers that can be used in complex surgery, especially open complex aortic surgery, are scarce, and clear recommendations for using specific biomarkers are not available. For critically ill patients, some good evidence exists for using biomarkers to enable early detection of different organ dysfunctions, such as acute kidney injury (AKI) (12, 13). One obstacle to implementing standardised biomarker assessment in the early postoperative phase after aortic surgery is the lack of transferability of findings from one surgical specialty to another, or from critical-care patients to postoperative aortic patients, which delays the establishment of scientifically reliable recommendations. Clinical application has stalled despite demonstration of clinical need in multiple settings and findings showing that timely detection of organ failure using organ-specific biomarkers can lead to improved patient outcomes after major surgery, such as open TAAA repair. In my clinical career, I have cared for TAAA patients pre-operatively and postoperatively on the normal ward, in the operating room, and in the intensive care unit. During the first days after major aortic surgery, the peri-operative inflammation cascade can lead to an unpredictable postoperative course, with severe organ failure and patient death. In the first 72 hours after surgery, even if the surgical repair went well without severe intra-operative complications such as mass transfusion, prolonged organ ischaemia, or major surgical complications, an important and incompletely understood inflammatory reaction follows ischaemia-reperfusion during aortic cross-clamping. Known triggers of this cascade include disturbed organ perfusion distal to the aortic cross-clamping despite selective perfusion, cellular damage from the extracorporeal heart-lung machine, and the effect of heparin and surgical exposure (14, 15). Further organ damage can arise from several cellular effects, such as dysregulated

inflammation mediator release following tissue damage of varying origin or apoptosis from ischaemia. Such damage, including acute kidney, bowel, and lung injury, can lead to acute kidney failure, paralytic ileus, or pneumonia (16, 17). These complications may upend an initially uneventful postoperative course and impair recovery. Timely and early detection of specific organ failure could improve outcomes and enable targeted therapy before clinically recognisable signs emerge (12). The examination and establishment of biomarkers of acute organ failure and outcomes after open TAAA repair have motivated me and my research team to pursue investigations that we hope will improve understanding and outcomes for patients undergoing major aortic surgery throughout the world.

Outline of the thesis

In this thesis, the potential relevance of biomarkers for detecting early organ dysfunction after open TAAA repair is assessed. The first chapters focus on using novel biomarkers of peri-operative outcome. In seven studies, we evaluated the potential benefits of peri-operative and even pre-operative use of assessable biomarkers for earlier detection or risk stratification during and after complex aortic surgery. The main focus of the thesis is acute kidney insufficiency, which can arise after open or endovascular TAAA repair. In the past, retrospective study designs and inappropriate classification tools may have led to underestimation of AKI incidence in these situations. This incidence has been evaluated more appropriately in recent years, and an internationally recommended classification system was applied in the corresponding studies (6). Despite regular application of nephro-protective measures, the reported frequency of AKI has remained stable or increased. The relevance of AKI for patient outcomes after TAAA repair is evident, and each effort to reduce incidence is important (18). Clinically established biomarkers represent one possible application for incidence reduction. Our findings in retrospective and prospective studies indicate that a multitude of biomarkers could be used in TAAA surgery for this purpose. Based on the two

clinical research manuscripts included in this thesis, the impact of open TAAA surgery with regard to postoperative complications should be underlined, especially the high rate of organ failure (e.g., AKI and acute respiratory distress syndrome). We also include our data from a prospective multicentre study conducted between 2019 and 2021. In three aortic centres in Germany, a bedside kit was used successfully for early detection of AKI within a few hours after open TAAA repair, illustrating the clinical applicability and reliability of biomarkers for early detection of organ dysfunction. The momentum for this thesis was improvements in postoperative surveillance after major aortic surgery. Endovascular techniques have evolved in the last two decades and significantly changed the treatment of patients suffering from aortic aneurysm. Nevertheless, open aortic aneurysm repair remains of undisputed relevance for some patients, and optimizing the pre-, intra-, and postoperative setting seems mandatory for them to achieve the best possible outcome. The research in this thesis may contribute to continuous improvement of patient outcomes after open aortic surgery.

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Chapter 2:

Perioperative and long-term outcome after ascending aortic and arch repair with elephant trunk and open thoracoabdominal aortic aneurysm repair

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Article Highlights

Type of research:

Multicenter, retrospective, non-randomized observational study

Key Findings:

Open thoracoabdominal aortic aneurysm (TAAA) repair in 32 cases after frozen elephant trunk (FET) repair because of acute or chronic pathologies was related to a perioperative mortality rate of 18.75%. During median follow up of 1.29 years, no procedure-related re-interventions and one aortic-related death could be assessed. The estimated 1-year survival rate of all patients was 78.1% (95%-CI = [63.9%, 95.6%]).

Take home Message:

Open TAAA repair following aortic arch repair including elephant trunk or FET because of acute or chronic aortic pathologies is associated with a relevant peri-operative morbidity and mortality rate. During follow up a low aortic-related mortality rate and procedure-related re-intervention rate were observed, indicating a favorable long-term outcome.

Table of Content Summary:

In this multicenter, retrospective study assessing 32 patients treated because of acute or chronic artic pathologies by frozen elephant or elephant trunk and open thoracoabdominal aortic repair a relevant perioperative mortality rate of 18.75 % could be observed. The outcome during long-term follow up seems to be favorable.

Abstract

Objective: Describe outcome of open thoracoabdominal aortic aneurysm (TAAA) repair following previous aortic arch repair including elephant trunk (ET) or frozen elephant trunk (FET) for acute and chronic pathologies.

Methods: Retrospective observational multicenter study including 32 patients treated between 2006 and 2019 in two aortic centers using identical surgical protocols. Assessment focused on peri-operative and long-term outcome, namely in-hospital morbidity and mortality as well as procedure-related re-intervention rate and aortic-related mortality rate. Kaplan-Meier curves with 95%-confidence intervals were used to analyse the overall survival after surgery within the cohort.

Results: 32 patients (mean age was 45.0 ± 13.6, 20 males [62.5%]) were treated because of acute (34.38 % [n = 11]) or chronic (65.62 % [n = 21]) aortic pathologies including residual dissection following acute, symptomatic type A dissection (n = 7), symptomatic mega aortic syndrome (MAS) (n = 4) as well as post-dissection TAAA (n = 18) and asymptomatic MAS (n = 3). 28 patients (87.5 %) received type II repair and 4 patients (12.5%) received type III repair after previous ascending aorta and arch repair including ET/FET. Concomitant infrarenal and iliac vessel repair was performed in 38.7 % (n = 12), respectively 29.4 % (n = 10).

In-hospital mortality rate was 18.75 % (n = 6). Spinal cord ischemia occurred in two cases, both after one-stage emergency procedure with one case of permanent paraplegia. Temporary acute kidney injury occurred in 41.94 % (n = 13).

The estimated 1-year survival rate was 78.1% (95%-CI = [63.9%, 95.6%]) with a median follow-up time of 1.29 years (IQR: 0.26 – 3.88).

No procedure-related re-interventions and one case of aortic-related mortality, namely sepsis because of graft infection, was observed.

Conclusion: Open TAAA repair following aortic arch repair including ET or FET because of acute or chronic aortic pathologies is associated with a relevant peri-operative morbidity and mortality rate. During follow up a low aortic-related mortality rate and procedure-related re-intervention rate were observed.

Introduction:

TAAA repair following ascending aortic and aortic arch repair including ET and FET is an extensive procedure, associated with major complications and in-hospital mortality, even in an elective setting (1). The European Society of Vascular Surgery (ESVS) notes in their current consensus paper focusing on aortic arch repair, that ET or FET can be used as landing zone for endovascular TAAA repair (2). According to small series of patients treated in a strictly elective surgical setting, endovascular repair as second stage procedure may be favorable if compared with open TAAA repair (3). Connective tissue disease (CTD), anatomical conditions or complex dissection may thwart an endovascular approach and only open repair remains as treatment option. Little is known regarding the impact of open TAAA repair in elective and emergency setting after previous aortic arch repair including ET and FET, which was the motivation for this study. The impact of extensive aortic aneurysm repair on long-term mortality and procedure-related re-intervention rate was the second purpose.

Methods:

The risk for bias in this retrospective study has been addressed by reporting the findings according to the STROBE criteria, additionally all information regarding patients' outcome

peri-operatively and during follow up were assessed by at least two physicians (4). Furthermore, the limitations section underlines the remaining confounders.

Between May 2006 and December 2019 32 patients were included in this retrospective, observational multicenter study. The study was approved by the local internal review board (EK004/14). Due to the retrospective character of this study informed consent was only obtained from the surviving patients during the post-interventional follow up. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Patients were included if an elective or emergency open TAAA repair was performed after previous aortic arch repair including ET or FET. All elective and emergency cases were discussed in an interdisciplinary panel involving vascular and cardiac surgeons, radiologists and anesthesiologists, except three emergency cases treated within 24h without any delay because of life-threatening emergency. Due to unfavorable anatomical conditions such as lack of proximal or distal landing zone, severe kinking or tortuosity and complex aortic dissection, an endovascular procedure was no option for each included patient. TAAA was defined according to the Crawford classification (5). The exclusion criteria for study inclusion were age below 18 years, pregnancy, chronic kidney disease requiring permanent dialysis treatment and ongoing immunosuppressive medication therapy. Findings regarding perioperative outcome such as complications are presented combined for both steps of the surgical repair (aortic arch repair including ET or FET and TAAA repair). Hereby the authors want to enable a risk assessment of the whole surgical procedure rather than a non-expedient selective assessment. Separate demonstration of the major complications after aortic arch repair including ET and FET can be found in the first section of the results.

Data collection

The standard physiological variables, demographics (age, sex, body mass index, and medical history including history of cardiovascular disease, diabetes and chronic kidney insufficiency) and type of surgery were determined based on medical records and electronic bedside flow charts (table 1). Elective and emergency procedures were defined based on the time between the first and the second step of treatment, while a minimum of 6 months between the surgical procedures was considered as desirable regarding patients' recovery. No patient died or dropped out between both surgical steps. One patient was treated after 33 days for the second stage of the aortic aneurysm repair, although he was asymptomatic. This patient was referred to our department from a distant European country. As he recovered fast from the first surgical repair, the second step could be performed early in his case. Another patient was treated under emergency conditions as he became symptomatic 60 days after the ascending-arch aortic repair including FET. Acute type A dissection (ATAD) as well as mega aortic syndrome (MAS) were defined according to the current literature (6, 7). In case of acute type A dissection, early expansion above 4 cm in combination with complex dissection leading to viscero-renal or peripheral malperfusion were indication for acute and/ or emergency additional TAAA repair.

Symptomatic MAS was defined as an aortic aneurysm starting in the aortic arch, extending to the viscero-renal aorta with severe, acute pain. Sepsis and shock were defined according to current guidelines and consensus papers (8, 9). As described in literature, cardiac complications included myocardial infarction, acute heart failure and ventricular tachycardia (10). Lung complications such as pneumonia and prolonged artificial ventilation requiring tracheotomy were defined according to the guidelines of the American Thoracic Society or the Belgian Society of Pneumology, respectively (11, 12). Acute kidney injury (AKI) within

48 h was defined according to the Kidney Disease Improving Global Outcomes criteria based on serum creatinine levels (13).

Follow-up

The follow-up (FU) data were obtained in the context of clinical routine controls including physical examination. Telephone or personal interviews were conducted for each patient alive during FU until November 2020. If additional information was required, the referring physicians were contacted after obtaining the patient's consent.

Surgical repair

A prosthetic replacement of the ascending aorta and aortic arch with an ET or FET extension of the arch graft inserted into the descending aorta during the first-stage operation was performed through a median sternotomy. Adequate descriptions for the ET and FET techniques can be found in literature (14, 15). An aortic cross clamping of the aorta at the level of ET and FET is possible after dissection of the aorta.

Indication for treatment for TAAA was a diameter above 6 cm or aneurysm expansion above 5 mm in 6 months. The operative protocol for open TAAA repair, which has been published in detail before, was identical in both centers with the same lead surgeon (16, 17). Briefly, it includes intubation with a double lumen endotracheal tube, cerebrospinal fluid drainage (CSFD), perioperative monitoring of motor evoked potentials (MEP), sequential aortic clamping if possible, extracorporeal circulation (ECC) with distal aortic perfusion as well as selective visceral perfusion and mild hypothermia of above 33 °C. After heparinisation (3 mg/kg; activated clotting time [ACT] approximately 450 seconds), distal aortic perfusion is established by cannulation of the left femoral vein and the femoral artery using a centrifugal

pump. An arterial line is inserted in the contralateral femoral artery. Since 2014, 4°C Custodiol® (Dr. Franz Köhler Chemie, Austria) is used for selective renal perfusion instead of blood perfusion. Thoracolaparotomy through the sixth to eighth intercostal space depending on the extent of the aneurysm is used as surgical access. The diaphragm is incised anteriorly, saving as much diaphragm muscle and phrenic nerve as possible, which could help avoiding pulomary complications as good as possible. For the proximal anastomosis, which involves the ET or FET and the aneurysm sac in the area, a teflon felt-supported suture line is used. Postoperatively, the mean arterial pressure (MAP) is adjusted based on MEPs and the intracranial pressure (ICP) is kept ≤10 mmHg during the first 72 hours for all patients. Extubation is performed as soon as possible postoperatively. In general, the aortic reconstruction is performed from proximal to distal. However, in extensive dissection cases involving the iliac arteries a reversed surgical approach is applied, because of the unpredictable changes in organ perfusion with retrograde flow through dissected iliac arteries and aorta. This is feasible if the infrarenal aorta can be cross clamped temporarily. At the end of the aortic reconstruction the heart lung machine is removed and heparin is antagonized with Protamin ® (Protaminhydrochlorid, Mylan Healthcare). According to the coagulation check using ROTEM ® (Rotationsthromboelastometrie. [ROTEM®, Tem Innovations GmbH, München] the need of fresh frozen plasma and thrombocyte concentrates is analyzed and blood products are substituted. Application of low dose heparin is started 6 hours after surgery on ICU.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median with lower and upper quartile (Q1 – Q3) in case of heavily skewed data, where the skewness of data was investigated graphically using boxplots. Categorical variables are summarized through

absolute and relative frequencies (%). The number of missing observations per variable were reported.

Kaplan-Meier curves with 95%-confidence interval (type "log") were used to illustrate the overall survival after surgery within the cohort. Median follow-up times were computed using the reverse Kaplan-Meier method. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary NC, USA) and R, version 3.6.3.

Results:

Patient characteristics

32 patients, 62.5 % male, mean age 45.0 ± 13.6, have been treated because of acute or chronic aortic pathologies between 2006 and 2019 in two European centers for aortic surgery. Patients were treated because of chronic post-type A dissection TAAA (n = 18) or MAS (n = 7), 11 patients have been treated because of acute, life-threatening aortic pathologies (acute symptomatic ATAD n = 7, symptomatic MAS n = 4) by ascending aorta and aortic arch repair including ET/FET and TAAA repair. 87.5 % (n = 28) required type II TAAA repair, 12.5 % (n = 4) received type III TAAA repair, additional infrarenal aortic or iliac vessel reconstruction because of aneurysm or dissection was necessary in 38.71 % (n =12), respectively 29.4 % (n = 10) of the patients. 14 patients (43.75 %) suffered from connective tissue disease (CTD). Details regarding the comorbidity profile can be found in table 1.

Table 1. Patient characteristics by urgency of the intervention.

Characteristic	All patients (N = 32)	Elective (N = 21)	Emergency (N = 11)
Demographics			
Age, years	45.0 ± 13.6	44.2 ± 11.7	46.5 ± 17.2
Sex (male)	20 (62.5%)	13 (61.9%)	7 (63.64%)
BMI, kg/m^2	24.5 ± 4.8	23.4 ± 4.5	26.5 ± 4.9
Smoker [a]	9 (29.03%)	3 (15%)	6 (54.55%)
Aortic pathology			
Chronic post- type A diss. aneurysm	18 (56.25%)	18 (85.71%)	0
MAS	7 (21.88%)	3 (14.29%)	4 (36.36%)
Symptomatic aneurysm (MAS or post type A dissection)	11 (34.38%)	0	11 (100%)
Maximum aneurysm diameter, mm [b]	66.5 ± 14.5	64.4 ± 11.8	69.8 ± 17.5
Type II TAAA repair	28 (87.5%)	18 (85.71%)	10 (90.91%)
Type III TAAA repair	4 (12.5%)	3 (14.29%)	1 (9.09%)
Infrarenal aortic repair [a]	12 (38.71%)	8 (40%)	4 (36.36%)
Iliac vessel treatment	10 (29. 4 %)	8 (38.1%)	2 (18.18%)
Comorbidities			
CTD	14 (43.75%)	10 (47.62%)	4 (36.36%)
COPD	3 (9.38%)	2 (9.52%)	1 (9.09%)
Hypertension	21 (65.63%)	12 (57.14%)	9 (81.82%)
Diabetes mellitus	10 (31.25)	6 (28.57)	4 (36.36)
Coronary heart disease	3 (9.38%)	2 (9.52%)	1 (9.09%)
Chronic kidney disease*	4 (12.5%)	2 (9.52%)	2 (18.18%)
History of myocardial infarction	0	0	0
History of stroke	4 (12.5%)	4 (19.05%)	0
Marker at baseline			
Hemoglobin, g/dL	12.8 ± 4.0	12.4 ± 1.9	13.2 ± 6.6
Serum creatinine, mg/dL	0.9 ± 0.4	1.0 ± 0.4	0.9 ± 0.2

Continuous data are reported as mean ± SD, categorical data as absolute and relative frequencies. [a] One missing observation. [b] Nine missing observations. BMI: Body mass index; ATAD: Acute type A dissection; MAS: Mega aortic syndrome; TAAA: Thoracoabdominal aortic aneurysm; CTD: Connective tissue disease; COPD: Chronic-obstructive pulmonary disease; according to KDIGO classification*

Procedural characteristics

13 patients received an FET, 19 received an ET, indications can be found in table 2. The median time between the first and second surgery was 85.5 days for all patients (IQR: 33-327) with a median time of 244 days (IQR: 86-453) for elective procedures and 16 days (IQR: 0-33) for emergency procedures. Three patients were treated in within 24h by open repair of the ascending aorta and arch combined with an FET followed by open type II TAAA repair because of symptomatic ATAD.

Table 2. Procedural characteristics by the urgency of the intervention.

Characteristic	All patients (N = 32)	Elective (N = 21)	Emergency (N = 11)
Previous aortic surgery			
FET	13 (40.63%)	9 (42.86%)	4 (36.36%)
ET	19 (59.38%)	12 (57.14%)	7 (63.64%)
Time between first and second surgery, days	85.5 (33 – 327)	244 (33 – 453)	16 (0 – 60)
Operation time, min [a]	395.9 ± 100.5	398.3 ± 109.4	391.7 ± 88.7
Ventilation time, min [a]	4920 (1685 - 21825)	3420 (1791 – 17280)	9611 (1685 – 33060)
Mass transfusion	15 (46.88%)	11 (52.38%)	4 (36.36%)

Continuous data are reported as mean ± SD or median (Q1 – Q3) in case of heavily skewed data, categorical data as absolute and relative frequencies. [a] *Five missing observations.*

Perioperative outcome of ascending aortic and arch repair including ET/FET:

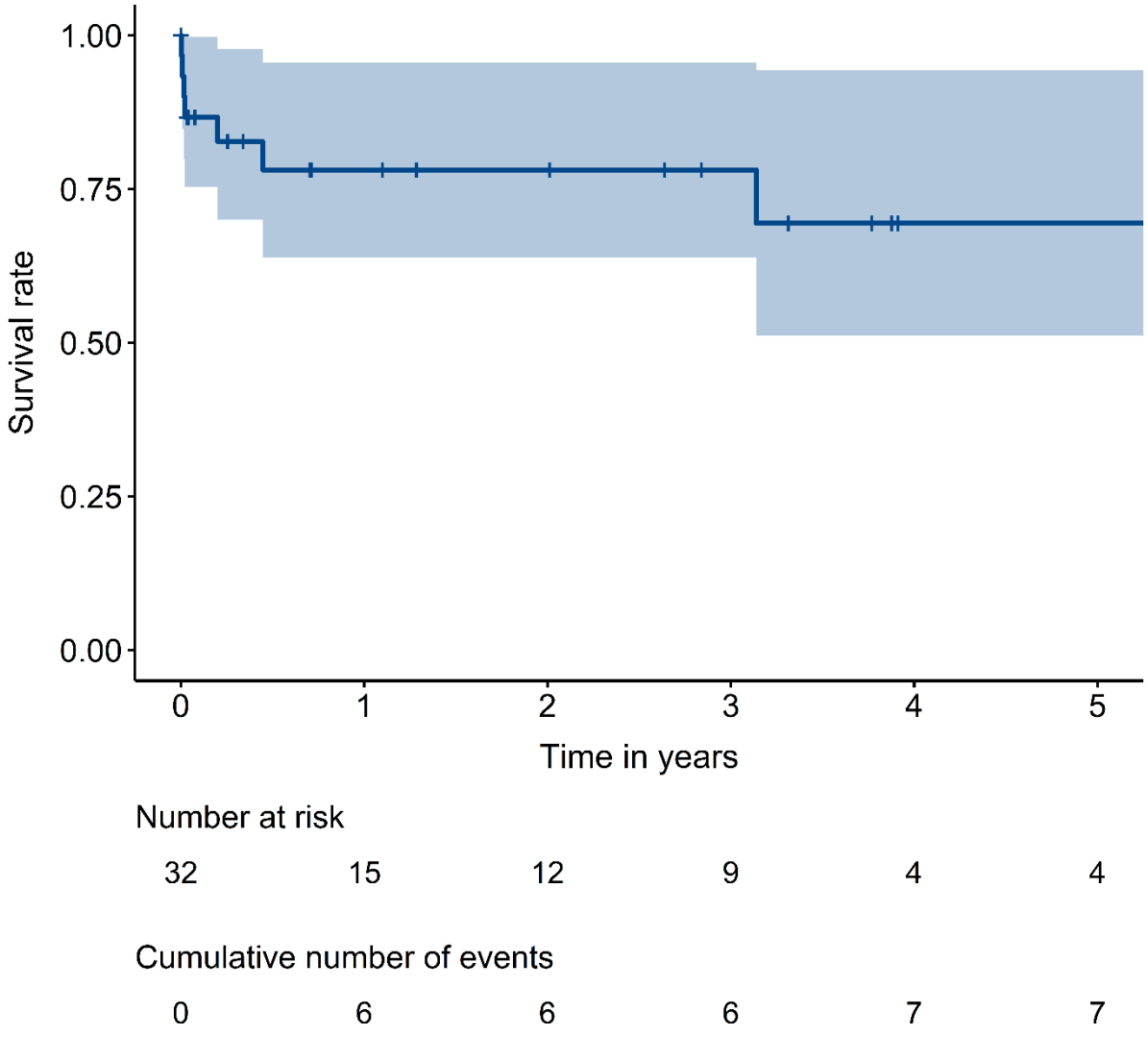
Regarding outcome after the first step of the procedure, ascending aortic and aortic arch repair including ET or FET, seven major complications were encountered: Three patients suffered from stroke, four patients required emergency re-do surgery due to hematothorax.

Post-operative survival:

The in-hospital mortality rate was 18.75 % (n = 6), with comparable findings in the elective and the emergency subgroup (elective: 14.29 % [n=3], emergency: 27.27 % [n=3]). Four patients died because of multi-organ failure (MOF) after elective (n=2) and emergency (n=2) repair, one patient died because of prosthesis infection despite antibiotic treatment and surgical revision after elective repair of a MAS. One patient died because of acute heart failure postoperatively after elective repair of a post-dissection TAAA type II.

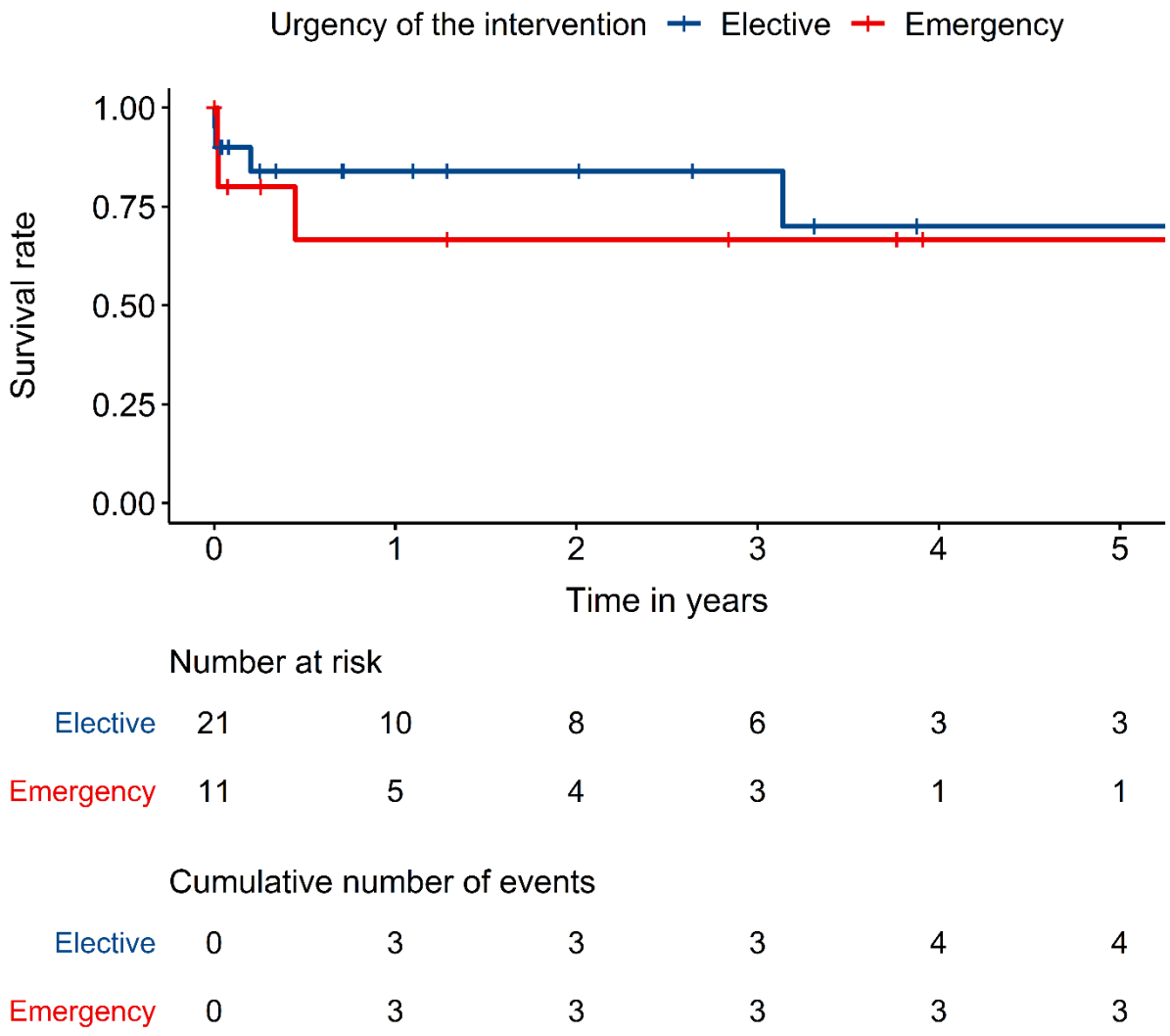
The Kaplan-Meier curve for post-operative survival within the entire cohort is shown in Figure 1. The estimated 1-year survival rate was 78.1% (95%-CI = [63.9%, 95.6%]) with a median follow-up time of 1.29 years (IQR: 0.26 – 3.88).

Figure 1: Kaplan-Meier curve for post-operative survival within the entire cohort.



The Kaplan-Meier curves of the elective and the emergency subgroup were similar (Figure 2) as well as the estimated 1-year survival rate which was 79.4% (95%-CI = [63.2%, 100%]) and 80.0% (95%-CI = [58.7%, 100%]), respectively. The median follow-up time was shorter in elective patients with 1.28 years compared to 2.84 years in emergency patients.

Figure 2: Kaplan-Meier curves for post-operative survival by the urgency of the intervention.



One aortic-related death was observed during follow-up because of graft infection, within the first year after surgical repair. No further patients died during follow up.

<u>Perioperative outcome for all procedures under elective and emergency conditions:</u>

Sepsis was observed in 35.48 % (n = 11) of all patients, mainly of pneumonic origin (54.5 %, n = 6). Four patients suffered from MOF with unclear primary origin and one patient suffered from prosthesis infection. Spinal cord function could be assessed after surgery in all patients, even those patients who died in hospital. Spinal cord ischemia (SCI) was present in two patients, both of these in the elective sub-group. One patient suffered from permanent paraplegia and one patient had paraparesis with weakness of both legs but a remaining ability to walk. Acute kidney injury (AKI), defined according to KDIGO, occurred in 41.94 % (n = 13), and in 9 (29.03 %) cases temporary renal replacement (RRT) therapy was required. One patient required permanent RRT during follow up. Surgical revision was performed in 43.75 % (n = 14) of all procedures. Ten patients required revision of a hematothorax either by re-thoracotomy or additional drain placement, three patients required groin revision because of hematoma and one patient required re-laparotomy for prosthesis infection and abscess evacuation. Twelve patients suffered from perioperative cardiac complications. Nine cases of self-limiting cardiac arrhythmia and three cases of acute heart failure, one of these was lethal, were observed. No cases of graft occlusion could be assessed in this cohort of patients. Perioperative stroke occurred in three patients, all of these were treated under emergency conditions (table 3). All patients, except those who suffered from paraplegia or paraparesis (n =2), were able to walk and to communicate, three patients required renal replacement therapy and none was ventilating via the tracheostomy.

Table 3. Postoperative outcomes by the urgency of the intervention.

Outcome	All patients (N = 32)	Elective (N = 21)	Emergency (N = 11)
In-hospital length of stay, days	28 (17.5 – 47.5)	27 (16 – 46)	35 (19 - 53)
ICU length of stay, days	12.5 (6 – 28)	12 (6 – 28)	13 (7 – 39)
In-hospital mortality	6 (18.75%)	3 (14.29%)	3 (27.27%)
Sepsis [a]	11 (35.48%)	6 (28.57%)	5 (50%)
SCI [a]	2 (6.45%)	2 (9.52%)	0
Hereof paraplegia [a]	1 (3.23 %)	1 (4.76)	0
AKI [a]	13 (41.94%)	10 (47.62%)	3 (30%)
AKI requiring RRT [a]	9 (29.03%)	6 (28.57%)	3 (30%)
Pneumonia	13 (40.63%)	8 (38.10%)	5 (45.45%)
Surgical revision	14 (43.75%)	10 (47.62%)	4 (36.36%)
Perioperative graft/ vessel occlusion	0	0	0
Tracheotomy [a]	7 (22.58%)	4 (19.05%)	3 (30%)
Perioperative cardiac complications	12 (37.5 %)	9 (42.86%)	3 (27.27%)
Perioperative myocardial infarction [a]	0	0	0
Perioperative stroke	3 (9.38%)	0	3 (27.27%)

Continuous data are reported as median (Q1 – Q3), categorical data as absolute and relative frequencies. [a] One missing observation. [b] Two missing observations. ICU: Intensive care unit; SCI: Spinal cord ischemia, AKI: Acute kidney Injury, RRT: Renal replacement therapy

Discussion:

Second-stage acute and elective TAAA repair after previous aortic arch and elephant or frozen elephant trunk implantation is related to a relevant mortality and morbidity rate, yet, as alternative therapeutic options may be absent, this therapy modality seems to be appropriate for patients with extensive thoracoabdominal aortic disease.

Treatment of a diseased aortic arch and descending aorta, regardless whether from degenerative or post-dissection origin, acute or chronic, is challenging, even as endovascular techniques are gaining ground and the indication for total endovascular aortic repair is getting more and more attention (18, 19).

Since the early 1980's, when Borst et al. reported their results for the staged elephant trunk (ET) principle to treat combined disease of aortic arch and the descending aorta, it has become a standard procedure in cardio-thoracic surgery (20).

Based on this pioneer work, Karck et al. described the concept of the frozen elephant trunk in 2003 (21). This approach allows a definitive treatment of combined aortic lesions in a one-stage procedure, which amalgamates the elephant trunk principle and an antegrade endovascular stenting of the proximal descending thoracic aorta. Based on the growing number of elective aortic procedures including the usage of ET or FET within the last decades, an increasing number of TAAA requiring repair may be expected.

The definite placement of the FET and its anchorage in an aspired landing zone are advantageous if compared with the ET and other techniques such as a clamshell approach for the repair of complex and extensive thoracic aortic pathologies (22, 23). Furthermore, new devices including an extra-anatomic bypass to the distal left subclavian artery may lead to an improved early postoperative outcome (24).

In this study we reported a relevant morbidity and mortality rate after open TAAA repair following previous aortic repair including ET and FET. Surprisingly, no relevant difference between emergency and elective TAAA repair regarding outcome could be assessed in this study, although the mortality rate after emergency repair is higher, yet not significantly. Other parameters such as sepsis may be more common and length of ventilation time may be longer, yet in general the outcome in the elective and emergency subgroup is similar. An appropriate explanation for this finding is the small number of patients included in this study, which forms a relevant confounder impeding a more complex statistical analysis leading to more generalizable findings and conclusions. The relevant total mortality rate is related to the extensive aortic repair, which has been performed in less than three weeks in one third of the patients. It is well known, that the extend of TAAA repair correlates directly with the perioperative morbidity and mortality rate(25) All patients have been assessed by experienced cardiovascular anesthesiologists pre-operatively and were evaluated as in appropriate physical condition for open repair. Regarding the perioperative morbidity and mortality, an

endovascular approach may have been favorable, yet a recently published meta-analysis questioned the peri-operative improved survival rate in case of extended repair of complex aortic aneurysm. Still, endovascular solutions have been discussed, yet have been declined in the presented cases because of the emergency setting, patients age (mean age less than 50 years) and existence of a connective tissue disease. Furthermore, even if challenging anatomy may be an issue for endovascular and open repair, in the presented cases the panel decided for open repair because of complex aortic dissection involving the viscero-renal vessels and inappropriate distal aortic landing zone.

During long-term follow-up, a favorable long-term mortality rate with only one case of aortic-related mortality and no aortic-related re-intervention were observed. According to the expertise and assessment of the interdisciplinary team including vascular and cardiac surgeons, radiologists and anesthesiologists involved in the presented cases, there was no endovascular options for the treated patients, furthermore the relevant percentage of patients suffering from CTD limits the applicability of endovascular aortic therapy (26). Considering the lack of further therapeutic options, open repair of those extensive aortic pathologies seems to be appropriate with regard to the relevant morbidity and mortality rate. Furthermore, focusing on patients' age, open repair may be favorable regarding a lower re-intervention rate and reduced frequency of CT-scans during follow-up.

Literature on outcome after open and endovascular TAAA repair following previous ET and FET is scarce, especially focusing on treatment of acute pathologies.

The articles of Roselli et al and Folkmann et al. have to be discussed as relevant contributions regarding these complex aortic reconstructions (1, 3). While Folkmann et al. observed no case of in-hospital mortality in their cohort including nine patients undergoing elective open TAAA repair after FET, several major complications were reported, such as acute renal failure requiring dialysis, severe gastrointestinal bleeding and prolonged stay on ICU.

However, no stroke or spinal cord ischemia was observed. The surgical procedure including specific organ-protective measures was comparable with the technique used by our group. Roselli et al., comparing open and endovascular repair of thoracoabdominal aortic aneurysms, observed 133 patients undergoing open TAAA repair under elective conditions. A 30-day mortality rate of 6 % was reported and major bleeding occurred in 8.9 % of all patients. Neurological complications, namely stroke and paraparesis, were observed in 4.9 % and 3.8 % respectively.

Acute respiratory failure with or without tracheotomy occurred in 12 % respectively 9% and was the most common severe complication after open TAAA repair following frozen elephant trunk procedures.

With regard to follow-up, 85 patients were available, of whom 13 required further open surgical procedures. Within the first year of follow-up, five patients after open TAAA repair following FET died in a skilled nursing facility due to their severe perioperative complications, increasing the aortic-related mortality from 6 % to nearly 10% within the first year after elective TAAA repair following FET.

In our cohort, the relevant in-hospital mortality rate of 18.75 % needs to be discussed. Extensive acute and chronic aortic aneurysm and aortic dissection cases with no option for staged repair have been included in this cohort. A recently published study of our group suggest a favorable survival rate of a staged approach if extended type II TAAA is required (27). If feasible, an endovascular approach should be considered as treatment option in extensive TAAA repair cases. The findings of Patel et al and Tsilimparis et all suggest that even complex aortic aneurysm involving the ascending aorta and the aortic arch may be treated by endovascular means with or without supraaortic debranching(28, 29). In fields of endovascular aortic aneurysm repair, a staged approach has been suggested as favorable since a couple of years, as it seems to be related to an improved survival rate and a reduced major

complication rate, namely spinal cord ischemia. Yet a relevant major stroke risk and an unfavorable relevant re-intervention rate remains. The impact of the emergency and especially the procedures performed within 24 h regarding the total-mortality and the morbidity rate seems evident. Especially the meaning of open one-stage TAAA repair due to symptomatic mega aortic syndrome or following shortly after acute type A dissection repair because of distal malperfusion have to be considered while assessing this maximal invasive aortic surgery.

Furthermore, TAAA repair was conducted as type II or III repair, accompanied by infrarenal and iliac artery reconstruction in the majority of all procedures. As the extension of aortic repair is directly related to peri-operative mortality and morbidity rates, this may be one contributing factor regarding the mortality rate in this study (30).

The negative pre-selection of this cohort, namely the missing opportunity of endovascular therapy, as assessed by the expert panel, may be of relevance regarding the mentioned mortality and morbidity rate. As the most common cause of death in this cohort, multi-organ failure, may indicate a severe disturbance of the patients' physiology peri- and postoperatively, the burden of the extensive surgical repair may play a relevant role regarding the reported in-hospital mortality rate. An extensive ischemia-reperfusion syndrome could be assumed for all assessed cases, without any option to prove this, as no reliable biomarker or parameter exists to underline severe, perioperative ischemia-reperfusion or inflammation reaction after open TAAA repair so far.

Regarding the peri-operative morbidity, pneumonia, AKI and surgical revision are the most relevant complications. Surgical revisions are linked to hematoma, especially of the thorax, the increased incidence of pneumonia following thoracotomy and one-lung ventilation are a frequent complication following thoracic aortic surgery (31). The increased incidence of acute kidney injury may be a consequence of ischemia-reperfusion damage following the aortic

cross-clamping although the kidneys have been perfused using Custodiol or selective perfusion via the heart-lung machine (32). In out setting, the TAAA repair is performed using selective organ perfusion via heart-lung machine or in advance selective Custodiol perfusion of the renal arteries. Deep Hypothermic Circulatory Arrest (DHCA) is another approach which has been described in literature to treat TAAA by open repair(33). In their manuscript Covera et al. reported an excellent morbidity and mortality rate, in particular the rate of AKI, defined according to the Society of Thoracic Surgeons, was below 5 % , which seems favorable if compared with the findings in the present study(34). The operation protocols in the Covera's study and in this study are comparable regarding organ-protective measures, suggesting that DHCA may be related to favorable results. Yet, as the extend of disease is pronounced in this study and as the percentage of emergency procedures as well as type II Repairs are higher, the findings are only comparable to a limited extent. Furthermore, if compared with the available literature, the strict assessment of AKI according to the KDIGO criteria may lead to a more accurate assessment of AKI following open TAAA repair. In general, the frequency of AKI following complex aortic procedures may be underestimated in literature, which is of relevance, as AKI is an independent predictor for perioperative outcome following aortic surgery (35, 36). The rate of spinal cord ischemia seems to be favorable, as only one case of permanent paraplegia could be observed in the elective cohort and none in the emergency cohort, despite extensive aortic repair was performed in all 32 cases. It seems reasonable to state that the neuroprotective measures such as CSFD and neuromonitoring using MEPs are related to this favorable result (17).As there are only two cases of spinal cord ischemia in the whole cohort of patients, the absence of SCI in the emergency cohort is most likely caused by chance.

During follow-up, similar as reported in literature, the re-intervention rate as well as the aortic-related mortality rate were favorably low. No re-interventions and only one case of

aortic-related mortality could be observed (26, 37). Even if the reported findings are based on a retrospective study including patients treated over more than 10 years the presented findings may indicate that open TAAA repair after ET or FET is an appropriate treatment option. In contrast, according to the meta analysis published by Antoniou et al., a relevant re-intervention rate could be observed after fenestrated and branched endovascular TAAA repair(38). As a relevant re-intervention rate was also reported for endovascular aortic arch repair, these findings may support the relevance of open repair of extensive TAAA in physically fit patients(29).

Limitations:

Relevant limitations of this retrospective observational study should be acknowledged to enable an adequate interpretation of the results. Only a rather small number of patients was enrolled due to the overall low number of TAAA procedures performed worldwide. This limits the study's ability to draw conclusions about the impact of elective or emergency procedures on perioperative outcomes such as in-hospital mortality and major complications. Due to the retrospective nature, the study is prone to selection bias. Hence, we involved at least two researchers during the data assessment. Moreover, a more homogeneous study setting regarding the treated pathologies would have been favorable from a statistical point of view. Yet, due to the rarity of the pattern of disease and its therapy, we decided to include all available cases. As the follow up data was collected retrospectively, some smaller re-interventions such as hernia repair may be missed. By that, we tried to avoid an artificial selection of patients enabling a more valid and reliable assessment of our findings. The decision for open repair because of lacking endovascular therapy option was based on the decision of an expert panel involving cardiac surgeons, vascular surgeons as well as interventional radiologists and anesthesiologists. Yet, even if the decision was guided by the

current guidelines of the ESVS and appropriate clinical experience, there is a risk for bias (39).

Conclusion:

Elective and emergency open TAAA repairs following ET or FET procedures are associated with a relevant perioperative morbidity and mortality rate. Favorable low spinal cord event rates may be realized if a dedicated neurological monitoring protocol is applied. According to this study, favorable long-term outcome with an appropriate aortic-related mortality and surgical re-intervention rate are possible.

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Chapter 3

Outcome of elective and emergency open thoracoabdominal aortic aneurysm repair in 255 cases- a retrospective single center study

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What this paper adds:

In a substantial number of patients with thoracoabdominal aortic aneurysm (TAAA) endovascular treatment is not feasible, leaving open repair as the only option. A huge variety of outcomes of open TAAA repair in elective and emergency cases has been reported in literature. Large series, including long-term follow up combined with aortic-related re-intervention rate, are scarce. By presenting this monocentric, retrospective experience, the authors would like to underline, that, regardless of the relevant peri-operative morbidity and mortality rate, short- and long-term mortality and re-intervention rate after open TAAA repair seems adequate if these procedures are performed in specialized centers.

Keywords:

TAAA, thoracoabdominal aortic aneurysm repair, spinal cord ischemia, SCI

Abstract:

Objective

This study reports on open TAAA repair comparing short- and long-term patients' outcome according to the conducted type of repair defined by the Crawford classification and elective vs. emergency repair. Endpoints were mortality, acute kidney injury (AKI), sepsis, spinal cord ischemia (SCI) and re-intervention rate.

Methods

Retrospective study reporting outcome of 255 patients (between 2006 and 2019), designed according to the STROBE criteria.

Results

The TAAA-distribution was type I 25%, type II 26%, type III 23%, type IV 18%, and type V 7%. 51 (20%) patients had an emergency procedure. 51 % of all patients had a history of aortic surgery, 58% of all patients suffered from post-dissection TAAA, 26 % of all patients had a connective-tissue disease.

In-hospital mortality rate among elective treated patients was 16 % (n = 33) vs. 35 % (n = 18) in the emergency subgroup; the total mortality rate was 20 % (n = 51). The adjusted Odds ratio for in-hospital mortality following emergency repair compared with elective repair was 2.52 (1.15; 5.48).

Temporary renal replacement therapy because of AKI was required in 29 % (n = 74) of all patients, sepsis from different cause was observed in 37 % (n = 94), SCI in 7% (n = 18, 10 patients suffering from paraplegia and 8 from paraparesis).

The mean follow-up time was 3.0 years (median 1.5, 0-12.8 years). Aortic related re-intervention rate occurred in 2.8 %. Total mortality rate during follow up was 22.5 % (n =46), 5.3 % (n =11) of all patients died due to aortic-related events.

Conclusion

Open TAAA repair is associated with a relevant morbidity and mortality rate, yet the incidence of spinal cord ischemia may be favorably low, if a neuromonitoring protocol is applied. Aortic-related re-intervention and aortic-related mortality rate during follow-up are low.

Introduction:

Although thoracoabdominal aortic aneurysms (TAAA) are treated with increasing frequency by endovascular means and post interventional mid-term results are improving, open TAAA repair remains a cornerstone of aortic surgery in elective and emergency cases (1, 2). Besides the fact that the reported reduced peri-operative mortality rate following endovascular repair of TAAA has been questioned by a recently published meta-analysis, relevant re-intervention rates following endovascular TAAA repair have been reported in several studies (3). Furthermore, the value of open TAAA repair in case of primary or secondary aortic infection and as bail-out treatment in case of complex anatomy and failed endovascular repair seems to be undisputed (4). According to the current guidelines of the European Society of Vascular and Endovascular Surgery, open treatment of TAAA in patients suffering from connective tissue disease (CTD) is strongly recommended, even if some authors discuss endovascular repair as feasible, yet high-risk option (5, 6).

Surgeon- and hospital volume of open TAAA repair decreased significantly over the last two decades, subsequently leading to increased numbers of centers offering endovascular repair (7).

The purpose of our study is to present the short- and long-term outcome of elective and emergency TAAA repair performed in one aortic center. By focusing on clinical endpoints such as mortality, AKI, sepsis, SCI as well re-intervention rate, we would like to contribute to the ongoing discussion, evaluating the status of open TAAA repair in the 21. century.

Methodology:

This retrospective, single center observational study included 255 patients treated by open TAAA repair between 2006 and 2019 in one single center. This study, approved by the local internal review board (EK004/14), was reported according to the STROBE criteria (7). All information regarding patients' outcome peri-operatively and during follow up were assessed by at least three physicians. For follow-up completion, which was performed between July 2019 and July 2020 based on telephone or personal interviews, informed consent for each surviving patient was obtained; the study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Inclusion criteria was elective or emergency open TAAA repair, classified according to the Crawford classification (8). Emergency repair was defined as treatment within 24 h because of symptomatic TAAA without signs of open rupture, namely severe back or abdominal pain with concomitant TAAA larger than 50 mm and absence of further plausible explanation for the symptoms. A clear separation between a covered rupture and a symptomatic TAAA was not possible in every case, hence only open ruptured TAAAs have been excluded in this study. Exclusion criteria were missing willingness to complete follow up by personal or telephone interview as well as age below 18 years, chronic kidney disease requiring permanent dialysis treatment, pregnancy and ongoing immunosuppressive medication therapy. Mycotic TAAA or freely ruptured TAAA were excluded likewise. A majority of 72 % of the elective as well as the emergency patients were referred by other centers, including high volume endovascular TAAA centers. Final treatment strategy was discussed and decided by a multidisciplinary team involving vascular surgeons, cardiac surgeon, interventional radiologists and anesthesiologists. In all patients, no endovascular options were deemed possible at the time of judgement because of morphological reasons. Patients suffering from connective-tissue disease related TAAA were by principle treated by open repair and included in the study.

Data collection

Physiological variables were determined based on electronic bedside flow charts.

All patients with TAAA were discussed in the multidisciplinary aortic conference, considering either endovascular or open repair as mentioned above. Elective open procedures were performed for asymptomatic TAAA larger than 60 mm or aneurysm expansion above 5 mm in 6 months. In case of connective-tissue disease, TAAA larger than 50 mm received treatment. Furthermore, any symptomatic TAAA which received open repair according to the same protocol and was treated within 24 hours was included.

Chronic kidney disease was defined according to the KDIGO criteria, chronic heart failure according to the Heart failure society of America's definition(9, 10).

Outcome measures

Acute kidney injury (AKI) within 48 h postoperatively was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria based on serum creatinine levels(11) . Severe neurological complications were defined as any stroke, cerebral bleeding, spinal cord ischemia (SCI) and critical illness polyneuropathy occurring after TAAA surgery(12-14). SCI was defined as a peri- or postoperative bilateral lower extremity motoric weakness and

sensory loss to pain and temperature modalities while preserving vibration and position sense(15). Temporary and permanent SCI was assessed and documented peri-operatively as well as each pathological finding without recovery during the intraoperative motoric evoked potential (MEP) (16). Patients were clinically assessed for neurological dysfunction during the intensive care-stay each 4-6 hours by a physician or more frequently if necessary. Critical illness polyneuropathy was defined as severe muscle weakness and failure to wean from the ventilator (17).

Any surgical re-intervention after the hospital stay and within the first 6 months after treatment was defined as surgical revision, including also surgical-site revision during hospital stay as well as afterwards. Sepsis and shock were defined according to current guidelines, so were cardiac complications, including myocardial infarction, acute heart failure and ventricular tachycardia (18-20). Lung complications were defined according to two different guidelines (21, 22). Intraoperative mass transfusion was defined as requirement of more than 10 blood transfusions intraoperatively.

Survival was assessed during the hospital stay, as well as during follow-up using phone and post.

Surgical repair

The operative protocol for open TAAA repair, which has been published in detail before, was performed by the same lead surgeon (23, 24). It includes a double lumen intubation with, cerebrospinal fluid drainage (CSFD), perioperative monitoring of MEPs, patient placement on a beanbag in a modified right lateral decubitus position. The operation table is overstretched for optimal access to the thorax cavity. Pathological changes of the MEP signals, namely a decrease of more than 50% indicates a spinal cord ischemia (16). Intercostal artery bypass (ICB) or reimplantation was applied in case of pathological intraoperative MEP findings or in case of a dominant intercostal (ICA) or lumbar artery in a cross clamped aortic segment (25).

The operative procedure includes sequential aortic clamping if possible, femoro-femoral extracorporeal circulation (ECC) with distal aortic perfusion as well as selective visceral perfusion and mild hypothermia of 32 to 33 °C. After full heparinization (3 mg/kg; activated clotting time [ACT] above 450 seconds), extra-corporal circulation (ECC) and distal aortic perfusion is established. Custodiol® (Dr. Franz Köhler Chemie, Austria) was used for renal perfusion instead of blood perfusion since 2014. Depending on the extent of the aneurysm,

thoraco-laparotomy through the sixth to eighth intercostal space was used as surgical access. The aortic reconstruction was performed from proximal to distal.

Statistical analysis:

Frequencies and proportions of categorical variables were tabulated and continuous variables were analyzed using means, medians, standard deviations and minimum, maximum values. Associations of outcomes were analyzed using logistic regression models for each outcome parameter and odds ratio estimates are presented. Independent variables of models included age (years, continuous), sex (male/female), Crawford classification (5 categories), surgery type (elective/emergency), and preoperative renal insufficiency (yes/no). Patients with complete data were used in the analyses. Kaplan-Meier survival estimates were calculated using follow-up time from surgery. Hazard ratios were estimated using Cox models adjusted for age (years), sex (male/female), Crawford classification (5 categories), surgery type (elective/emergency) and preoperative kidney insufficiency (yes/no). Short-term follow-up was defined as follow-up within the first year after surgery, long-term follow-up was defined as follow-up longer than 5 years.

Results:

Patient and operational characteristics

There were 255 patients (186 male) with open surgical procedures conducted between 2006 February and 2019 June (supplemental figure 1). The distribution of patients by Crawford classification was as follows: Type I 25%, Type II 26%, Type III 23%, Type IV 18%, and Type V 7%. 51 (20%) patients had an emergency procedure. 51 % of all patients had a history of aortic surgery, 26 % suffered from connective-tissue disease. The mean maximum pre-operative aortic diameter was 67±14 mm. All details and patient characteristics are presented in table 1. The median duration of surgery was 375 minutes, 43% of the patients received intraoperative mass transfusion. Regarding neuromonitoring, significant MEP changes occurred in 33 %, leading to implantation of ICB or re-implantation of ICA in 36% (table 1).

Table 1: Patient and operational characteristics

Patient characteristics

* Classification data missing for 5 patients

	All	Type I n= 64*	Type II n=66	Type III n=58	Type IV n=45	Type V n=17
Age (years)	56.2 (13.0)	54.8 (12.4)	55.2 (14.0)	57.7 (12.3)	61.6 (11.2)	47.9 (13.8)
Male (%)	186 (73)	42 (66)	50 (76)	37 (64)	38 (84)	14 (82)
BMI kg/m^2	26.0 (5.0)	26.6 (5.6)	25.4 (4.6)	25.2 (4.6)	26.8 (4.5)	25.3 (5.9)
Preoperative creatinine (mg/dL)	1.07 (0.60)	1.01 (0.36)	0.96 (0.43)	1.17 (0.82)	1.12 (0.46)	0.96 (0.32)
Preoperative Hb (g/dl)	13.0 (1.9)	13.4 (1.8)	12.3 (2.1)	13.5 (1.4)	12.8 (2.0)	12.8 (1.8)
Chronic Heart failure	66 (26)	14 (22)	17 (26)	12 (21)	19 (42)	3 (18)
COPD	73 (29)	19 (30)	19 (29)	20 (35)	9 (20)	6 (35)
Prior aortic operation	131 (51)	26 (41)	40 (61)	29 (50)	20 (44)	12 (71)
Aortic dissection	148 (58)	43 (67)	47 (71)	29 (50)	14 (31)	10 (59)
Connective tissue disorder	67 (26)	15 (23)	24 (36)	12 (21)	7 (16)	6 (35)
Chronic kidney disease	136 (53)	32 (50)	29 (44)	37 (64)	27 (60)	9 (53)
ASA (median; % >=3)	3 (87)	3 (90)	3 (88)	3 (83)	3 (85)	3 (94)
Diabetes	24 (9)	5 (8)	5 (8)	5 (9)	5 (11)	2 (12)
Current smoker	98 (38)	24 (38)	25 (38)	23 (40)	19 (42)	6 (35)
Hypertension	212 (83)	56 (88)	51 (77)	50 (86)	38 (84)	12 (71)
Coronary heart disease	73 (29)	18 (28)	15 (23)	14 (24)	20 (44)	3 (18)
History of stroke	24 (9)	4 (6)	6 (9)	6 (10)	7 (16)	1 (6)
Max. pre-op. Aortic diameter (mm)	67 (14)	64 (11)	71 (17)	64 (10)	70 (17)	67 (21)

Operation characteristics

	All	Type I n= 64*	Type II n=66	Type III n=58	Type IV n=45	Type V n=17
Emergency procedure	51 (20)	6 (9)	13 (20)	6 (10)	19 (42)	6 (35)
Duration of surgery (mins) median [min;max]	375 [179;935]	326 [188;524]	454 [254;935]	395 [214;664]	381 [227;653]	337 [179;513]
Duration of intraoperative ventilation (mins) median [min;max]	475 [278;1101]	419 [278;599]	554 [349;1101]	516 [302;750]	475 [308;817]	449 [299;571]
Intraoperative mass transfusion (%)	110 (43)	16 (25)	40 (61)	26 (45)	21 (47)	4 (24)
Pathological findings neuromonitoring (%)	83 (33)	18 (28)	36 (55)	19 (33)	7 (16)	2 (12)
Intercostal artery bypass (%)	91 (36)	26 (41)	38 (58)	17 (29)	4 (9)	6 (35)

* Classification data missing for 5 patients (BMI: Body Mass Index, Hb: Hemoglobin, COPD: Chronic-obstructive pulmonary disease, ASA: American Society of Anesthesiology)

In-hospital outcome

The total mortality rate was 20 %, the frequency of renal replacement therapy because of AKI was 29%. Sepsis was observed in 37 %, spinal cord ischemia in 7%. Of these 18 cases, 10 were permanent paraplegia (3.9% of all patients) and 8 patients (3.1 % of all patients) showed a paraparesis. The median length of hospital stay was 27 days. Surgical revision, was

required in n = 65 patients (25%) and was related to an increased in-hospital as well as total mortality rate during follow-up (table 2 and figure 1).

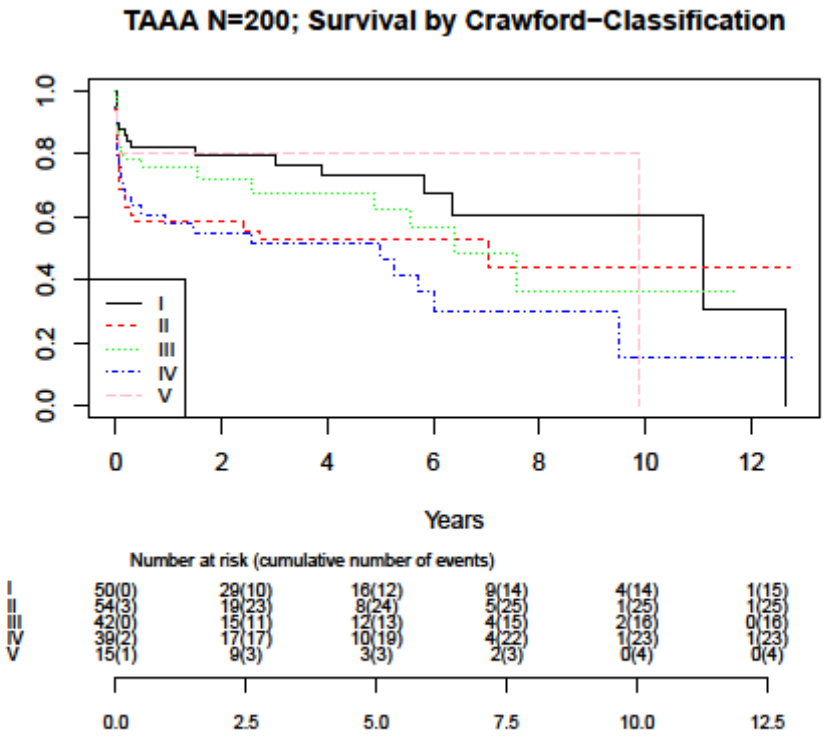


Table 2: Outcomes by type of repair and by elective/emergency surgery

Intraoperative Outcomes by type of repair according to the Crawford classification

	All	Type I n= 64*	Type II n=66	Type III n=58	Type IV n=45	Type V n=17
In-hospital mortality	51 (20)	7 (11)	20 (30)	8 (14)	12 (27)	3 (18)
Temporary renal replacement therapy	74 (29)	8 (13)	29 (44)	19 (33)	15 (33)	3 (18)
Sepsis	94 (37)	18 (28)	32 (48)	24 (41)	13 (29)	5 (29)
Spinal cord ischemia	18 (7)	4 (6)	8 (12)	4 (7)	2 (4)	0 (0)
Tracheotomy	80 (31)	13 (20)	29 (44)	23 (40)	11 (24)	2 (12)
Myocardial infarction	15 (6)	2 (3)	4 (6)	3 (5)	5 (11)	0 (0)
Length of hospital stay (days) median [min;max]	27 [1;128]	25 [4;79]	32 [1;128]	30 [8;109]	26 [1;112]	21 [1;89]
Pneumonia	141 (55)	36 (56)	40 (61)	38 (66)	20 (44)	4 (24)
Stroke	16 (6)	4 (6)	8 (12)	2 (3)	1 (2)	0 (0)
Cerebral bleeding	10 (4)	3 (5)	1 (2)	4 (7)	1 (2)	0 (0)
Critical illness polyneuropathy	10 (4)	1 (2)	4 (6)	4 (7)	1 (2)	0 (0)
Surgical revision	65 (25)	13 (20)	23 (35)	14 (24)	11 (24)	4 (24)

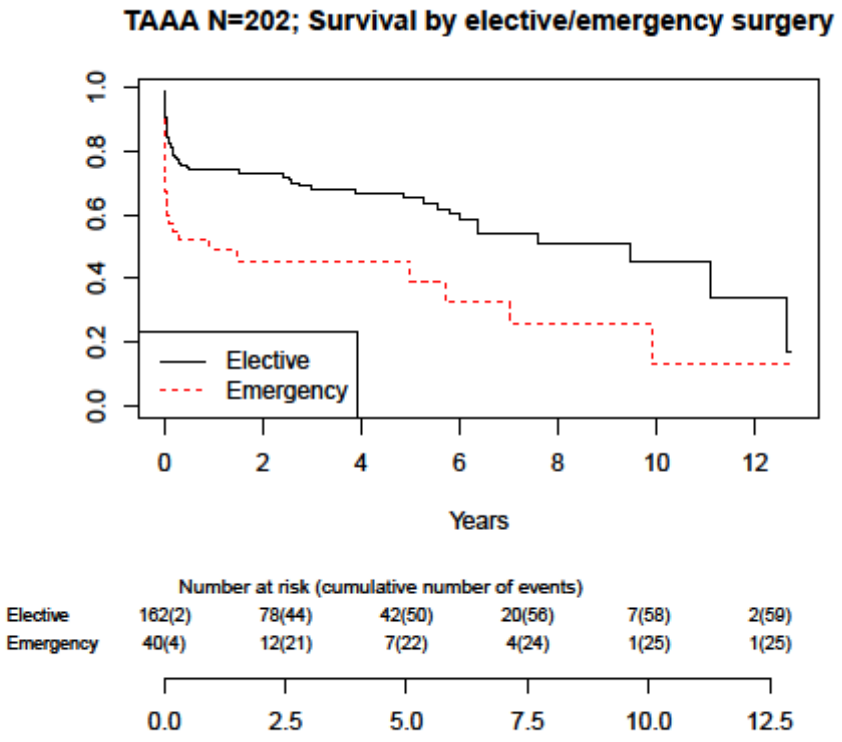
* Classification data missing for 5 patients

Outcomes by elective/emergency surgery

	All n=255	Elective n= 204	Emergency n=51	Percentage point difference (standard error)
In-hospital mortality	51 (20)	33 (16)	18 (35)	-19 (7.2)
Temporary renal replacement therapy	74 (29)	56 (28)	18 (35)	-8 (7.4)
Sepsis	94 (37)	76 (37)	18 (35)	2 (7.5)
Spinal cord ischemia	18 (7)	12 (6)	6 (12)	-6 (4.8)
Tracheotomy	80 (31)	65 (32)	15 (29)	3 (7.2)
Myocardial infarction	15 (6)	10 (5)	5 (10)	-5 (4.4)
Length of hospital stay (days) median [min;max]	27 [1;128]	28 [2;128]	26 [1;112]	2 (4.7)*
Pneumonia	141 (55)	118 (58)	23 (45)	13 (7.8)
Stroke	16 (6)	15 (7)	1 (2)	5 (2.7)
Cerebral bleeding	10 (4)	10 (5)	0 (0)	5 (1.5)
Critical illness polyneuropathy	10 (4)	7 (3)	3 (6)	-3 (3.5)
Surgical revision	65 (25)	47 (23)	18 (35)	-12 (7.3)

* Difference in medians (standard error)

Relevant differences between the elective and the emergency subgroup could be observed. The in-hospital mortality rate was 16 % (n=33) vs. 35 % (n=18), the rate of SCI was 6 (n=12) vs. 12 % (n=6), although all procedures were performed applying the same strategy to prevent SCI (table 2 and figure 2).



For postoperative outcomes, adjusted odds ratios (age, sex, pre-operative creatinine and type of TAAA) were calculated. Patients undergoing emergency surgery had an odds ratio for in-hospital mortality of 2.52 (95% confidence interval: 1.15;5.48) compared to patients with elective surgery (table 3, supplemental table I).

Table 3: Estimates of adjusted odds ratios and 95% confidence intervals from logistic models for different clinical parameters according to the type of surgery and type of repair according to the Crawford classification.

Estimates of adjusted odds ratios and 95% confidence intervals from logistic models#

	Emergency surgery*	Type I	Type II (ref.)	Type III	Type IV	Type V
In-hospital mortality	2.52 (1.15;5.48)	0.31 (0.11;0.80)	1	0.35 (0.13;0.89)	0.48 (0.18;1.23)	0.56 (0.11;2.18)
Temporary renal replacement therapy	1.38 (0.65;2.87)	0.19 (0.07;0.45)	1	0.66 (0.30;1.42)	0.51 (0.22;1.18)	0.29 (0.06;1.02)
Spinal cord ischemia	2.67 (0.82;7.99)	0.55 (0.14;1.92)	1	0.59 (0.14;2.09)	0.24 (0.03;1.11)	Not estimated
Pneumonia	0.72 (0.36;1.43)	0.79 (0.38;1.60)	1	1.18 (0.55;2.52)	0.56 (0.25;1.25)	0.20 (0.05;0.64)

Each outcome was analyzed using a separate logistic model adjusted for age (years), sex (m/f), and pre-operative creatinine (mg/dL continuous).

* Reference is elective surgery

Type III repair showed a significant lower adjusted in-hospital mortality rate (adjusted odds ratio: 0.35 0.13;0.89) (table II supplemental data). Patients who died in hospital had a significant longer operation time than patients who were discharged alive (378.10 ± 98.14, 453.0 ± 130.9). Patients who died in hospital had on average an hour and 15 minutes longer operation times (standard error: 19.6 min.) compared to patients who did not die in hospital.

The adjusted Odds Ratio for AKI requiring renal replacement therapy and mass transfusion was 1.97 (1.06;3.69), the adjusted odds ratio for mass transfusion and need for postoperative tracheotomy was 2.9 (1.51; 5.67). (supplemental table IIIa and IIIb).

Regarding an association of the type of TAAA repair and the need of tracheotomy, a significant lower frequency was observable after type I, type IV and type V repair if compared with type II repair (supplemental table IV a).

Regarding the frequency of spinal cord ischemia, no significant difference between the different types of TAAA repair was observed (supplemental table IV b).
The adjusted odds ratio for in-hospital death comparing patients with and without acute renal failure was 3.07 (95%CI: 1.45;6.59).

Outcome during Follow Up:

The mean follow-up time was 3 years (median: 1.5; min.: 0; max.: 12.8 years), available for 202 patients. In 24 patients (12 %) further aortic surgery respectively aortic related re-intervention was required. An ascending aorta and arch repair was required in 10 patients; thoracic endovascular aortic repair was required in 8 patients. Three patients (1.4 %) required re-intervention due to symptomatic stenotic anastomosis of the celiac trunk. Because of aortic graft infection three emergency graft replacement operations were required, leading to a 2.8 % aortic-re intervention rate totally. Details can be found in table 4.

The total mortality during follow up was 22.5 % (n =46), 5.3 % of all patients died due to aortic-related events, including 2.9 % (n =6) of all patients who died during postoperative rehabilitation within the first six months after discharge. Of these three patients died because of sepsis/ multiorgan failure and two patients died because of unknown reason. One patient who died required emergency re-intervention because of early aortic graft infection and

related aortic rupture. Five patients died because of proven or suspected aortic rupture during follow up. Two of these suffered from aortic graft infection. In case of uncertain death, which was the case in three patients, an aortic event was suspected. Details can be found in table 4.

Table 4: Aortic surgery/re-intervention and mortality during follow up

Total n (%)	24 (12)
Ascendending/Arch repair	10 (4.9)
Endovascular thoracic aortic repair	8 (3.9)
Interventional therapy of stenotic anastomosis	3 (1.4)
Replacement of Infected prosthesis	3 (1.4)

Mortality during follow up n (%)	46 (22.5)
Aortic-related mortality	11 (5.3)
- Postoperative/ during rehabilitation	6 (2.9)
- During follow up	5 (2.5)
Cardiovascular	25 (12.3)
Cancer-related	8 (3.9)
other	2 (0.9)

The crude hazard ratio for death comparing patients with and without peri-operative acute renal failure was 1.93 (95%CI: 1.22; 3.04). The relative adjusted hazard was 1.41 (95% CI: 0.86; 2.30).

Kaplan Meier Curves showed no evidence that survival rates were different across the types of TAAA repair during follow up, both unadjusted and adjusted for age, sex and elective/emergency surgery. Regarding emergency and elective repair, there is an estimated two-fold increase in the risk of death.

The operation time, if adjusted for age, sex and type of TAAA repair, emergency and elective repair, had a significant impact on survival during follow up, as an operation above the median time of 375 min. had a significant lower survival rate if compared with procedures, operated below the median time. (supplemental figure 2).

Discussion:

The results of this study show that open surgical repair of TAAA is related to relevant morbidity and mortality. However, in order to objectively assess the outcomes on their merits, and to determine the role of open repair in 2021, it is important to understand the characteristics of the patients.

First, the majority of patients suffered from extend I-III TAAA, requiring a two cavity (chest and abdomen) surgical approach, being a massive trauma for the patient. Next, 58% were post-dissection aneurysms, which in principle complicates the surgical procedure. In addition, more than half of the patients underwent previous aortic surgery, contributing to higher intra-operative technical challenges. Finally, the vulnerable aortic tissue in CTD patients (26%) demands specific surgical techniques to master adequate and long lasting anastomoses (26, 27). Taking all these issues into consideration, clearly indicates that we discuss a highly selective group of patients with maximal complex pathology.

The included emergency procedures in this study, which are related to a significant higher mortality and complication rate, are another relevant cause explaining unfavorable postoperative outcome, even if the surgical repair was conducted under comparable conditions. Two recently published metaanalysis emphasized that the estimated postoperative early survival advantage after endovascular TAAA repair if compared with open repair is not based on robust data, hence this controversial topic needs to be further elaborated (3, 28).

In 2021 endovascular repair of TAAA is the first line treatment for older patients with degenerative aneurysms. Despite that, as the group of Gustavo Oderich recently showed in an analysis of 430 patients with a mean age of 74 years, endovascular TAAA repair can have excellent midterm results regarding aortic-related mortality, yet the overall survival of these patients with degenerative aneurysms after 5 year is lower than 60% (28).

Obviously, the timeframe 2006-2019 implies that patients considered not suitable for endovascular repair in the earlier phase could have been treated at present by endovascular means. However, all patients included in the study were judged by a multidisciplinary team according to the technical possibilities at that specific moment. In addition, high volume endovascular TAAA centers referred patients because of absent endo options. In our practice, being an endovascular TAAA center as well, all patients will receive endovascular repair if technically feasible, except for CTD patients.

In summary, a highly selective group of patients with complex aortic pathology, deemed not suitable for endovascular repair, was treated by open surgical techniques in a high volume center. Are the 16% mortality rate among elective patients and the encountered

morbidities in elective patients acceptable enough to justify the massive invasive procedure? In order to answer one should question what the alternative could be, in absence of endo solutions.

A substantial number of patients with TAAA have morphological or complicating challenges which cannot be solved by endovascular means: absence of proximal and/or distal landing areas, complex chronic post dissection aneurysms, vascular access issues, severely diseased visceral and/renal arteries, aorto-bronchial and aorto-esophageal fistulas, endograft infections and CTD related aneurysms. The only remaining alternatives for these patients are either conservative management, which is associated with relevant mortality, or open surgical repair. Judging the surgical outcome from a positive perspective depicts a survival rate of > 80% with a relatively low re-intervention rate if compared with endovascular repair (3). Whether long term survival after open repair is associated with adequate quality of life has unfortunately never been investigated.

If open TAAA repair in young patients and in selected patients without endovascular options is an acceptable strategy, the next question is where the procedure should be performed. For open surgery of abdominal aortic aneurysms (AAA) there is compelling evidence that outcome is significantly better when performed in high volume institutions (29). A recent analysis of the International Consortium of Vascular Registries described significant increased peri-operative mortality after open repair in low-volume hospitals, which was not the case after EVAR(30). Obviously, this volume-outcome association is prominently demonstrated in more complex open AAA procedures such as juxta- renal and ruptured aneurysms (31, 32). Actually, this volume-outcome phenomenon is easy to understand when looking at the dramatic decrease of open AAA repair in the last two decades and the progressive shortfall in open aneurysm experience for vascular surgery trainees (33). Now, what is true for AAA repair certainly applies for open TAAA repair, having a much lower incidence, being technically more challenging and requiring an extensive infrastructure to perform these procedures.

In this study, pneumonia is the most frequent complication. The surgical access, the temporary one-lung ventilation as well as the severe systematic inflammation reaction following the ischemia-reperfusion damage during open TAAA surgery are well known risk factors for postoperative pneumonia and acute respiratory failure, contributing to the relevant mortality and morbidity rate (34, 35).

Spinal cord ischemia is related to an increased peri-operative and total mortality rates after complex aortic surgery (36). Based on specific preventive strategy favorably low rates have been published recently (37). In context of open TAAA repair, distal aortic perfusion seems to enable improved spinal cord blood supply, realized by the collateral network of the spinal cord (38). The usage of cerebro-spinal fluid drainage should be considered as a useful and established strategy to manage increased spinal cord pressure, in particular if combined with MEP's, allowing intraoperative assessment of spinal cord perfusion and the necessity of intercostal artery revascularization (37, 38). This "aggressive" strategy to prevent spinal cord ischemia may have contributed to the relatively low incidence of paraplegia. A favorable SCI rate could be assessed while comparing the incidence in this study cohort of type II TAAA repair (8 %) with recently published papers focusing on SCI and endovascular type II TAAA repair, in which SCI-rates between 11 and 20 % have been reported (39, 40).

Aortic-related re-interventions during follow-up after surgery may be caused by failure of the performed open TAAA repair namely stenotic target vessels or life-threatening infection of the aortic graft. Yet, as reported here, the majority of re-interventions and subsequent aortic procedures were related to progression of disease, as native aortic segments have been treated. This is in contrast to the findings after endovascular aortic repair, as a relevant re-intervention rate could be assessed here (3). During short-term follow up, an aortic-related mortality rate of 2.9 % was observed after discharge from hospital, indicating that patients treated by open TAAA repair are in a weak condition and require close surveillance in the first postoperative phase.
Yet, in general, a low aortic–related mortality rate for the total follow-up period could be observed, highlighting the favorable robust results of open TAAA repair (41, 42).

Limitations:

This retrospective observational study should be assessed against the background of the following limitations. Even if the number of included patients may be appropriate to enable certain conclusions, relevant confounders regarding some parameters of this study remain. Due to its retrospective nature, the study is prone to selection bias, although the study was reported according to the STROBE criteria and involved at least three researchers and one independent medical statistician during the data assessment. Moreover, a prospective study setting would have been favorable, even if it may have been barely realizable due to the rarity of the pattern of disease and its therapy. The decision for open repair was based on the

assessment of specialist of multiple medical disciplines, which may be, as it is not a randomized decision making process, another relevant confounder.

Conclusion:

Open surgical TAAA repair is a reliable treatment option, associated with significant morbidity and mortality even if performed in high-volume centers. A low incidence of spinal cord ischemia could be observed in this cohort of patients. During long-term follow up, the aortic-related re-intervention rate and aortic-related mortality rate are low, underlining the consistent meaning of open repair of the treatment of thoracoabdominal aortic pathologies.

Tables and Figures:

Table 1: Patient and operational characteristics

Table 2: Intraoperative Outcomes by type of repair and by elective/emergency surgery

Table 3: Estimates of adjusted odds ratios and 95% confidence intervals from logistic models for different clinical parameters according to the type of surgery, type of repair according to the Crawford classification and preoperative serum creatinine.

Table 4: Aortic surgery/re-intervention and mortality during follow up

Figure 1: Kaplan Meier Curve of Survival according to the type of TAAA repair

Figure 2: Kaplan Meier Curve of Survival according to emergency/elective surgery.

Supplemental figure 1: Number of performed open TAAA repairs per year.

Supplemental figure 2: TAAA survival by operation time.

Supplemental table I-VI: Supplemental logistic models.

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Chapter 4

Postoperatively increased bioactive adrenomedullin is related to adverse outcome after complex aortic surgery

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Abstract:

Background

Open and endovascular thoracoabdominal aortic aneurysm repair is related to major complications and increased mortality rates. Up to now, specific biomarkers for adverse outcome are scarce, although routine usage of such biomarkers could enable an earlier and more appropriate treatment of complications during the postoperative course after complex aortic aneurysm repair.

Patients and Methods

In a prospective single-center study (NCT03093857, clinicaltrials.gov). including 33 patients (51.5 % women, mean age 63.0 ± 16.2 years) undergoing elective complex open and endovascular aortic aneurysm repair, bioactive adrenomedullin (bio-ADM) was measured for 72 h perioperatively and an association with clinical endpoints, namely shock, death and the combined endpoint of the two aforementioned parameters was assessed. Furthermore, the association between bio-ADM and baseline characteristics and perioperative details including sepsis biomarkers score were assessed.

Results

51.5% (*n*=17) of patients developed postoperative acute kidney injury, 21.2 % (n=7) pneumonia or sepsis (18.2 % [n=6]). Shock was observed in 12.1 % (n=4) patients. The in-hospital mortality rate was 18.2 % (n=6), and 24.2% (n=8) of patients developed shock and/or died in hospital.

A significant correlation of bio-ADM concentrations from all available time points was observed with leucocytes (P<0.001), C-reactive protein (P<0.001) and serum creatinine levels (P<0.001).

Increased bio-ADM at 12 h, 24 h, 48 h and 72 h after admission to ICU was associated with both, in-hospital death and cardiogenic shock, with an area under the curve for the combined

endpoint of 0.598, 0.720, 0.880 and 0.967. Bio-ADM concentrations at 48 h and 72 h after admission to ICU were predictive for in-hospital death and shock (both $P<0.01$).

Conclusion

Bioactive ADM may serve as postoperative biomarker for shock and death after complex open and endovascular aortic aneurysm repair, potentially enabling an earlier and by that more adequate treatment of adverse outcome after major surgery.

Introduction:

Complications after open and endovascular repair of thoracoabdominal aortic surgery are common and related to adverse impact on patients' outcome and survival (1, 2).

In fields of open aortic repair, extensive ischemia-reperfusion damage may lead to systemic inflammatory response syndrome (SIRS), which in turn may cause multiple organ failure, shock and secondary bacterial infection as well as sepsis (3-5). Even if endovascular aortic repair seems to be favorable with regard to the early postoperative phase and a decreased rate of in-hospital mortality, major complications occur commonly (6). Few is known about the inflammatory impact of endovascular aortic repair on the endothelial-driven inflammatory reaction (7, 8). With regard to circulating biomarkers for postoperative inflammation reaction, comparable results have been observed for open and endovascular repair (9). Specific biomarkers, which could predict major adverse endpoints such as death or shock postoperatively, are scarce. Biomarkers such as lactate are well-established predictors for adverse outcome, yet they are rather nonspecific (10). Given the unique characteristics of bioactive adrenomedullin (bio-ADM), which reflects endothelial function and emerged as diagnostic and therapeutic target in life-threatening conditions such as septic shock, could render this peptide as valuable biomarker to guide interventional decision-making after aortic surgery (11, 12). Adrenomedullin (ADM), a member of the calcitonin gene-related family of peptide hormones, triggers multiple endocrine processes such a vasodilation of resistance vessels and is involved in diuretic and immunomodulatory pathways, which are of special relevance in the pathophysiology after thoracoabdominal aortic surgery. Within this, shear stress, inflammation and hypoxia may lead to an upregulation of ADM (13). So far, no study evaluated the course of bio-ADM after TAAA surgery. Therefore, the assessment of bio-ADM as predictor in fields of complex aortic surgery for adverse outcome including mortality as well as shock was our motivation for this study.

Material and Methods:

The study was approved by the internal review board, informed consent was obtained from each subject included in this study. This study was part of research project which has been pre-registered in 2017 (NCT03093857, clinicaltrials.gov).

Thirty-three patients have been enrolled in this prospective, non-randomized single-center study between January and December 2017.

Patients were eligible for inclusion if an elective open or endovascular TAAA repair was planned. TAAAs were defined according to the Crawford classification (14). Exclusion criteria were age below 18 years, pregnancy, chronic kidney disease requiring permanent dialysis treatment, ongoing immunosuppressive medication and emergency procedures (15).

Data collection

Demographics such as age, sex, body mass index, medical history including cardiovascular disease, diabetes and chronic kidney insufficiency as well as daily physiological variables and type of surgery were obtained from medical records and electronic bedside flow charts (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). Details can be found in tables 1 Plasma samples were collected before surgery, after admission to intensive care unit (ICU), and 12 h, 24 h, 48 h, and 72 h after admission to ICU. Blood samples were centrifuged ten minutes at 3000 rpm, and the resulting supernatants were stored at -80 °C until further analysis. Standard laboratory parameters (e.g. biomarkers of organ injury, such as creatinine and creatinine kinase isoenzyme MB) were routinely drawn and subsequently measured as part of the clinical routine during the postoperative ICU treatment.

Paraplegia was defined as complete loss of motor function of the lower extremity, while paraparesis means an incomplete loss of the motor function of the lower extremity(15). Pneumonia and tracheotomy were defined according to the guidelines of the American

Thoracic Society or the Belgian Society of Pneumology, respectively (16, 17). Cardiac complications included myocardial infarction, acute heart failure and ventricular tachycardia; all defined according to current guidelines (18-20).

Acute kidney injury (AKI) was defined according to the KDIGO criteria based on serum creatinine levels (21, 22). The sequential organ failure assessment (SOFA) score was used according to its definition (21, 22).

Sepsis was defined according to the guidelines of the German Sepsis Society (23): Fever above 38 ° Celsius or hypothermia below 36 ° Celsius, tachycardia with a heart rate above 90 beats per minute, tachypnea with a respiratory rate above 20 per minute or a leukocytosis (≥ 12 000/mm³) or leucopenia (≤ 4 000/mm³). Shock was defined as recommended by Vincent et al: The systolic arterial pressure was less than 90 mm Hg or the mean arterial pressure was less than 70 mm Hg with associated tachycardia (24).

Adrenomedullin measurement

Plasma levels of bio-ADM were measured using a commercially available chemiluminescence immunoassay (sphingotest® bio-ADM®, sphingotec GmbH, Hennigsdorf, Germany) as previously described. The analytical assay sensitivity was 2 pg/mL. Using this assay, the median bio-ADM concentration of healthy adults was 20.7 pg/mL, with a 99th percentile of 43.0 pg/mL. This normal value is not gender- or age-matched but refers to the study of Marino et al. (25).

Anesthesia and Surgery

Preoperative medication was continued until the day of surgery, except for metformin, ACE inhibitors and AT2 receptor antagonists. In accordance with our institutional routine and as previously described, all patients received a balanced anesthesia (26). The protocol for open

TAAA repair includes sequential aortic clamping, extracorporeal circulation using distal aortic perfusion as well as selective visceral perfusion (27-29). Custodiol® (Dr. Franz Köhler Chemie, Austria) was used for renal perfusion to avoid ischemic organ damage (30).

The endovascular procedure was performed under general anesthesia as well; the detailed procedure of fenestrated endovascular aortic aneurysm repair has been described before (31). In cases of endovascular procedure, renal perfusion was not interfered directly. Radiation and iodinated contrast solution were used conservatively to reduce the toxic effects on the kidneys. Furthermore, a reduced contrast solution dose (one fourth of the standard dose) for the selective angiography of the renal arteries was used because it has been described as protective regarding AKI (32).

Diluted contrast medium was applied to avoid functional impairment of the kidneys as good as possible, leading to a mean application of 65 ± 17 ml per endovascular procedure (32).

Endpoints

Levels of bio-ADM and their correlation with death and shock following open and endovascular aortic repair were the primary endpoints of this study.

Statistics

Values are expressed as means and standard deviations (SD), medians and interquartile ranges (IQR), or counts and percentages as appropriate. Group comparisons of continuous variables were performed using the Kruskal-Wallis test. Categorical data were compared using Pearson's Chi-squared Test for Count Data. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of bio-ADM concentration obtained at each time point and to compare their ability to predict in-hospital death, cardiogenic shock, or the combination of both. Bio-ADM was not normally distributed and was therefore log-

transformed. Logistic regression was used to derive significance and the area under the ROC curve (AUC) is given as a measure of effect size.

All statistical tests were two-tailed and a two-sided p-value of 0.05 was considered for significance. P-values were not adjusted for multiple testing. The statistical analyses were performed using R version 3.4.3 (http://www.r-project.org, library rms, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results:

Patients age was 63 ± 16.2 years, 16 patients (48.5%) were women. Endovascular TAAArepair was conducted in 19 patients (57.6%), open repair in 14 patients (42.4%). Type 1 and type II TAAA repairs were performed six times (18.2%), a type III TAAA repair seven times (21.2%), a type IV TAAA repair ten times (30.3%) and a type V TAAA repair four times (12.1%). Further demographic and procedural details can be found in table 1.

Mean ± SD operation time was 374 ± 111 min. The median (IQR) total ventilation time was 1410 (960-2505) min. Further operational characteristics can be found in table 1.

Table 1. Patient characteristics

Characteristic		All patients (n=33)	Endovascular surgery (n=19)	Open surgery (n=14)	P-value
Demographics					
	Age, years	63.0 ± 16.2	72.5 ± 7.1	50.1 ± 16.4	<0.001
	Sex (women)	16 (48.5%)	10 (52.6%)	6 (42.9%)	0.733
	BMI, kg/m^2	25.4 ± 5.0	25.0 ± 5.1	26.0 ± 5.1	0.600
Comorbidities					
	Smoker	12 (36.4%)	8 (42.1%)	4 (28.6%)	0.483
	Chronic kidney disease	5 (15.2%)	2 (10.5%)	3 (21.4%)	0.636
	Coronary heart disease	14 (42.4%)	10 (52.6%)	4 (28.6%)	0.283
	Diabetes mellitus	6 (18.2%)	4 (21.1%)	2 (14.3%)	0.685
	Hypertension	23 (69.7%)	15 (78.9%)	8 (57.1%)	0.253
	COPD	13 (39.4%)	11 (57.9%)	2 (14.3%)	0.019
	Connective tissue disease	5 (15.2%)	0 (0%)	5 (35.7%)	0.010
	PAD	4 (12.1%)	4 (21.1%)	0 (0%)	0.111
	Maximum aortic diameter, cm	6.6 ± 1.3	6.4 ± 1.2	6.9 ± 1.4	0.314
Marker at baseline					
	bioADM, pg/mL	17.3 (11.2-24.1)	21.9 (14.6-24.4)	14.3 (9.7-18.6)	0.0558
	Hemoglobin, mg/dL	10.0 ± 1.7	9.9 ± 1.7	10.2 ± 1.7	0.563
	Serum creatinine, mg/dL	1.1 (0.8-1.4)	1.2 (0.9-1.5)	0.9 (0.7-1.1)	0.071
Operational characteristics					
	Operation time, min	374 ± 111	376 ± 111	372 ± 115	0.908
	ICU ventilation time, min	835 (300-1571)	400 (0-1360)	1003 (763-3630)	0.021
	Total ventilation time, min	1410 (960-2505)	1080 (628-1947)	1786 (1316-19441)	0.017
	Stay on ICU, days	4 (3-5)	3 (2-5)	5 (4-16)	0.032
	In-hospital stay, days	26 (11-35)	15 (10-35)	27 (21-34)	0.222
	Blood transfusion, units	8 (4-15)	5 (4-13)	10 (6-27)	0.083
Type of TAAA					0.562
	TAAA 1	5 (15.2%)	2 (10.5%)	3 (21.4%)	
	TAAA 2	7 (21.2%)	3 (15.8%)	4 (28.6%)	
	TAAA 3	7 (21.2%)	4 (21.1%)	3 (21.4%)	
	TAAA 4	10 (30.3%)	8 (42.1%)	2 (14.3%)	
	TAAA 5	4 (12.1%)	2 (10.5%)	2 (14.3%)	

BMI: Body Mass Index, COPD: Chronic obstructive pulmonary disease, PAD: Peripheral artery disease, TAAA: Thoracoabdominal aortic aneurysm
Continuous data is reported as mean ± standard deviation or median (interquartile range). Categorical data is reported as absolute and relative frequencies.

Seventeen patients (51.5%) developed postoperative AKI. With regard to major complications, pneumonia occurred in seven patients (21.2%) and four patients (12.1%) required tracheotomy. Three patients (9%) developed spinal cord ischemia directly after surgical treatment. Cardiac complications were found in ten patients (30.3%) (one case of myocardial infarction, four cases of cardiogenic shock caused by acute heart failure and five cases ventricular tachycardia). Overall, six patients (18.2%) suffered from sepsis and four patients (12.1%) developed a cardiogenic shock. Shock occurred on day 7 and day 14 in two patients suffering from acute heart failure and on day 28 in two patients suffering from myocardial infarction and ventricular tachycardia. The in-hospital mortality rate was 18.2 % (6 patients) (table 2). Two patients died on day 2 because of cerebral bleeding and two

patients died on day 3 after surgery due to small intestine ischemia. Two patients suffering from pneumonic sepsis died later than 28 days post-surgery. Overall, eight patients (24.2%) developed a cardiogenic shock or died in hospital.

Course of bio-ADM following open and endovascular aortic aneurysm repair

When comparing the course of bio-ADM after open and endovascular TAAA repair, bio-ADM concentrations in the endovascular group demonstrated a rapid, more pronounced increase and bio-ADM concentrations were higher after endovascular repair compared to open repair (on admission to ICU and 12 h after admission to ICU) (supplemental data figure 1). Compared to other routinely measured biomarkers of inflammation, such as procalcitonin, bio-ADM concentrations showed a faster increase and remained significantly elevated in the observation period (data not shown).

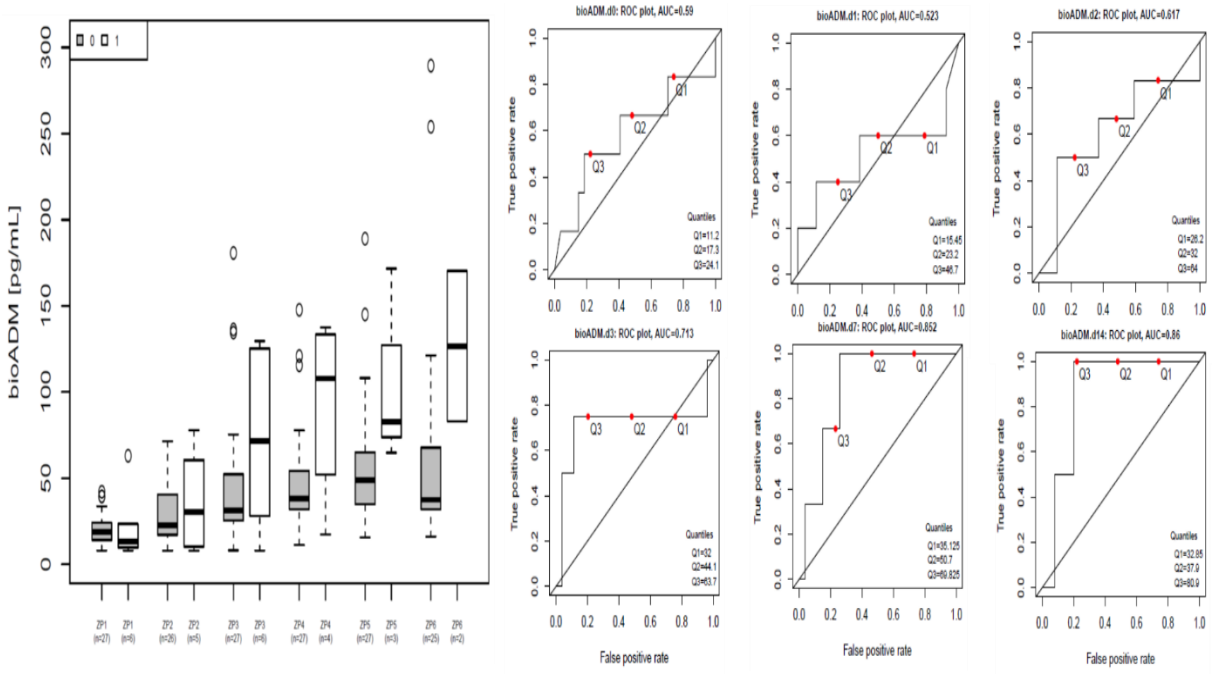


Figure 1: Course of bioactive adrenomedullin in correlation with the endpoint „in-hospital death"-for all patients after open and endovascular thoracoabdominal aortic aneurysm repair (n=6 events). Significant differences could be assessed 48 h after surgery (p=0.049) Figure 1a) Box Plot, Figure 1b) ROC-Analysis for the test accurracy of Adrenomedullin to predict in-hospital mortality.

Correlation of bio-ADM and additional perioperative variables

While evaluating associations between bio-ADM concentrations and clinically relevant parameters, significant correlations with patient age (r=0.45) and body mass index (r=0.39) were detected. Further associations were investigated and can be found in the supplemental data, table 1.

Association between bio-ADM and further biomarkers

A significant correlation could be observed between bio-ADM and leucocytes, C-reactive protein and serum creatinine (r=0.37, r=0.56 and r=0.52, respectively, P<0.001). Only a trend could be detected in association between bio-ADM and the SOFA score (r=0.18, P=0.084). At 48 h after admission to ICU, SOFA score and bio-ADM are available in n=15 patients and the correlation was r=0.59. The SOFA score was available predominantly at 12 h and 24 h after admission to ICU (n=32 and n=29, respectively). Correlation with bio-ADM at these time points was r=0.20 and r=0.14, respectively.
(supplemental data, table 2).

Table 2. Complications and mortality rate

Outcome	All patients (n=33)	Endovascular surgery 19 (57.6%)	Open surgery 14 (42.4%)	p-value
Pneumonia	7 (21.2%)	1 (5.3%)	5 (35.7%)	0.068
Tracheotomy	4 (12.1%)	0 (0%)	4 (28.6%)	0.025
Spinal cord ischemia	3 (9.09%)	1 (5.3%)	2 (14.3%)	0.177
Cardiac complications	10 (30.3%)	4 (21.1%)	6 (42.9%)	0.253
Acute kidney injury	17 (51.5%)	7 (36.8%)	10 (71.4%)	0.075
KDIGO 1	10 (58.8%)	4 (21.1%)	6 (42.9%)	
KDIGO 2	2 (11.8%)	1 (5.3%)	1 (7.1%)	
KDIGO 3	5 (29.4%)	2 (10.5%)	3 (21.4%)	
Sepsis	2 (6.1%)	0 (0%)	2 (14.3%)	0.177
Cardiogenic shock	4 (12.1%)	1 (5.3%)	3 (21.4%)	0.291
In-hospital mortality	6 (18.2%)	4 (21.1%)	2 (14.3%)	0.685
Hereof pneumonia	2 (33.3 %)	2 (10.5%)	0	
Hereof small intestine ischemia	2 (33.3%)	1 (5.2%)	1 (7.1 %)	0.82
Hereof cerebral bleeding	2 (33.3%)	1 (5.2%)	1 (7.1 %)	0.82
Cardiogenic shock or in-hospital mortality	8 (24.2%)	4 (21.1%)	4 (28.6%)	0.697
Acute kidney injury or in-hospital mortality	17 (51.5%)	7 (36.8%)	10 (71.4%)	0.075

Data is reported as absolute and relative frequencies.
KDIGO: Kidney Disease – Improving Global Outcomes. Data is reported as absolute and relative frequencies. Cardiac complications included myocardial infarction, arrhythmia and acute heart failure.

Predictive ability of bio-ADM for the endpoints death, shock and the combined endpoint death or shock

With regard to the postoperative measurements of bio-ADM an increasing test accuracy was observed for all assessed endpoints. Starting 24 h after admission to ICU a moderate to good AUC of 0.713 could be observed for the endpoint in-hospital death, which reached an AUC of 0.86 until 72 h after admission to ICU (figure 1). For the endpoint shock, bio-ADM levels at 72 h after admission to ICU reached a significant predictive accuracy (AUC 0.97, P<0.0001) (figure 2), and for the combined endpoint death or shock, 48 h and 72 h were significant (AUC 0.88 and 0.97, P=0.0066 and P<0.0001, respectively) (figure 3). A separate analysis of

the predictive accuracy for the combined endpoint in the endovascular and open surgery group showed a diagnostic accuracy of AUC 0.889 (P=0.0186) for bio-ADM 24 h after admission to ICU in the endovascular group. Regarding the open repair group an AUC of 0.933 (P=0.0064 and P=0.0040, respectively) at admission to ICU and 48 h after admission to ICU could be observed. An overview of AUCs for all time points and endpoints can be found in table 3.

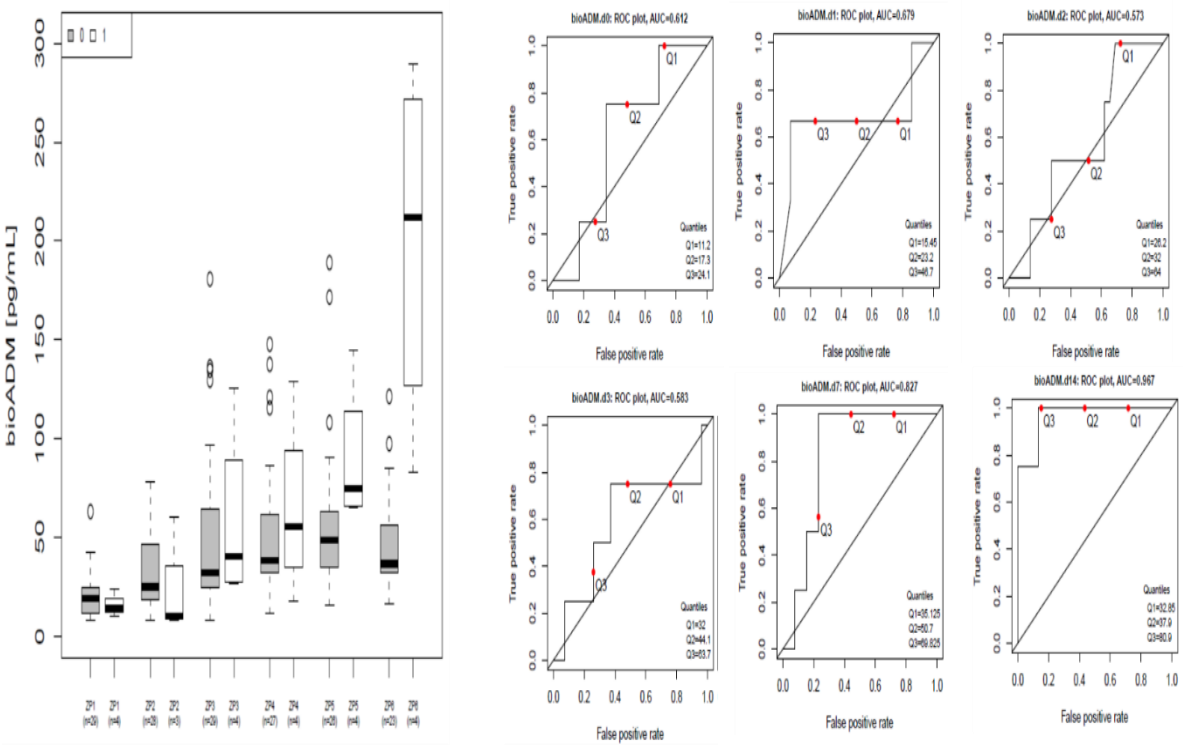


Figure 2: Course of Adrenomedullin in correlation with the endpoint „cardiogenic shock“ for all patients after open and endovascular thoracoabdominal aortic aneurysm repair (n=4 events). Significant differences could be assessed 48 h and 72 h after surgery-Figure 2a) Box Plot Figure 2b) ROC-Analysis for the test accurracy of Adrenomedullin to predict cardiogenic shock.

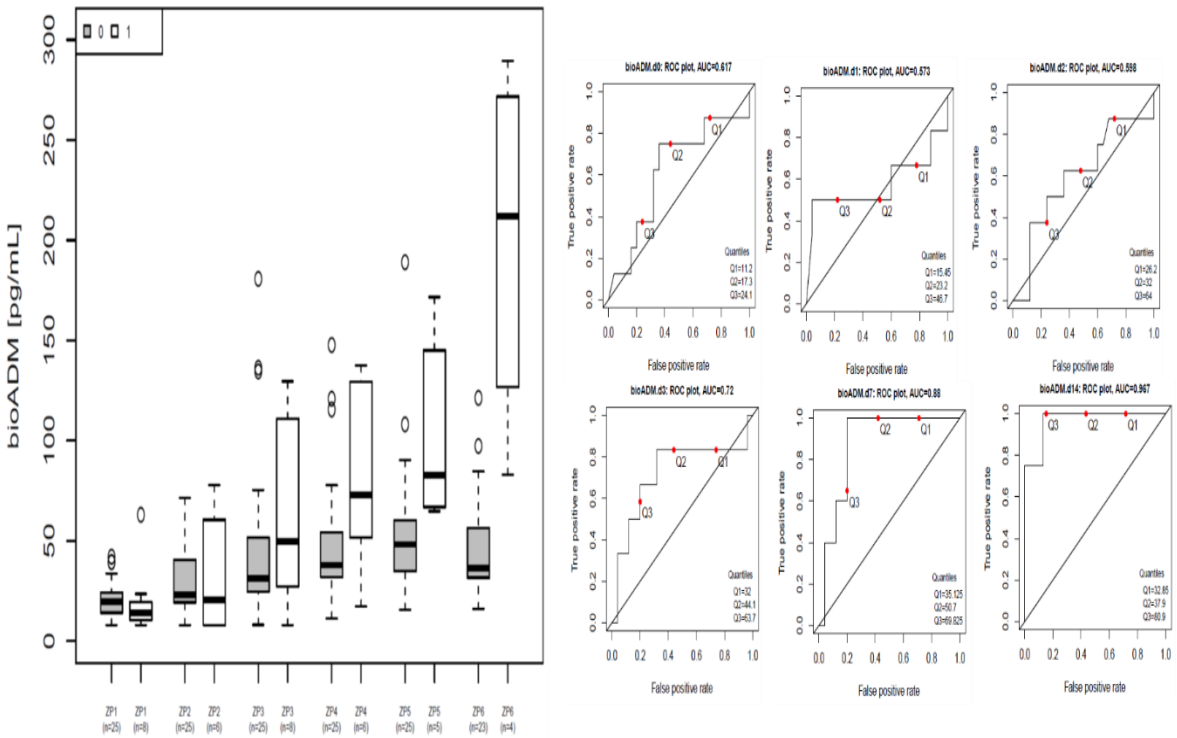


Figure 3: Course of Adrenomedullin in correlation with the combined endpoint „death or shock“ for all patients after open and endovascular thoracoabdominal aortic aneurysm repair (n=8 events). Significant differences could be assessed 48 h and 72 h after surgery.Figure 3a) Box Plot Figure 3b) ROC-Analysis for the test accurracy of Adrenomedullin to predict „death or shock“.

Table 3a. ROC analysis for the test accuracy of bio-ADM to predict the endpoints „in-hospital mortality“, „shock“ and „in-hospital mortality or shock“

	In-hospital mortality			**Shock**			**In-hospital Death or shock**		
Time point	**AUC**	**P-value**	**N (events)**	**AUC**	**P-value**	**N (events)**	**AUC**	**P-value**	**N (events)**
Baseline	0.590	0.6756	33 (6)	0.612	0.4220	33 (4)	0.617	0.4651	33 (8)
Admission to ICU	0.523	0.9595	31 (5)	0.679	0.2709	31 (3)	0.573	0.4660	31 (6)
12 h after admission to ICU	0.617	0.3618	33 (6)	0.573	0.5897	33 (4)	0.598	0.4297	33 (8)
24 h after admission to ICU	0.713	0.1128	31 (4)	0.583	0.6758	31 (4)	0.720	0.1016	31 (6)
48 h after admission to ICU	0.852	0.0513	30 (3)	0.827	0.0724	30 (4)	0.880	0.0066	30 (5)
72 h after admission to ICU	0.860	0.1070	27 (2)	0.967	<0.0001	27 (4)	0.967	<0.0001	27 (4)

AUC: area under the curve, bio-ADM: bioactive adrenomedullin, ICU: intensive care unit, ROC: receiver operating characteristic

Table 3b. ROC analysis for the test accuracy of bio-ADM to predict the endpoint „in-hospital mortality or shock“ in patients with open vs. endovascular surgery.

	endovascular surgery (n=19)			**open surgery (n=14)**		
Time point	**AUC**	**P-value**	**N (events)**	**AUC**	**P-value**	**N (events)**
Baseline	0.800	0.0283	19 (4)	0.600	0.3566	14 (4)
Admission to ICU	0.711	0.2357	18 (3)	0.933	0.0065	13 (3)
12 h after admission to ICU	0.717	0.0927	19 (4)	0.575	0.9278	14 (4)
24 h after admission to ICU	0.889	0.0186	18 (3)	0.600	0.6141	13 (3)
48 h after admission to ICU	0.867	0.0618	17 (2)	0.933	0.0040	13 (3)
72 h after admission to ICU	0.769	0.2679	14 (1)	1.000	0.0002	13 (3)

AUC: area under the curve, bio-ADM: bioactive adrenomedullin, ICU: intensive care unit, ROC: receiver operating characteristic

Discussion:

In this study, increased bio-ADM levels between 12 h and 72 h after admission to ICU were associated with in-hospital death and shock. With regard to a separate analysis of the endovascular and open repair group, we were able to observe even increased predictive accuracy for bio-ADM for the combined endpoint death and cardiogenic shock. Thus, the present findings highlight for the first time the role of perioperatively measured bio-ADM as potential biomarker for the prediction of shock and mortality in patients after either open or endovascular TAAA.

Regarding early postoperative risk assessment several trials suggest a potential integration of bio-ADM as predictor of hemodynamic support requirement and mortality during sepsis and organ failure (11, 33). In consideration of the boundary conditions of this study, namely the small cohort of patients and the non-randomized design, our findings are comparable.

As described before in literature, elevated levels of peptides derived from the proadrenomedullin precursor are linked to adverse outcome including organ failure such as AKI, SIRS, cardiogenic and septic shock (11, 33-36). A relevant rate of complications and mortality could be observed in the present cohort of patients undergoing elective open and endovascular TAAA repair, which is comparable with reported outcome in the current literature (37, 38) and thus underlines the need for reliable biomarkers for a better monitoring of the patients' recovery. With regard to the applied treatment modality, an increased release of bio-ADM could be observed after endovascular repair compared with open repair. These findings are surprising, as the major tissue trauma in combination with the ischemia-reperfusion damage during aortic cross clamping has been described to lead to a stronger inflammatory reaction than the minimal-invasive endovascular aortic repair (39). Consequently, early outcome has been described to be better and the mortality rates to be lower after endovascular TAAA repair (40). As ADM is a repair hormone for impaired vascular integrity derived from endothelial cells and vascular smooth muscle cells (41), the increased levels of bio-ADM may be a consequence of increased endothelial activation following endovascular repair (8). In fact, the plasma levels of bio-ADM after open and endovascular TAAA repair were comparable 48 h to 72 h after admission to ICU, indicating the magnitude of vascular damage for release of ADM to counteract the damage. Yet, the exact trigger for ADM release into the blood remains unknown.

No correlation was found between bio-ADM and interleukin 6, demonstrating that bio-ADM release could be a result from other factors than sterile inflammation or trauma (42).

We detected a significant association between the perioperative bio-ADM levels and measured levels of leucocytes, CRP and serum creatinine, no significant correlation with the SOFA score was observed, which could be a result of the small number of patients in a rather heterogeneous patient population. Yet, although no causative effect can be demonstrated, the

aforementioned correlations indicate the role of bio-ADM as early marker for the development of inflammatory complications and resulting organ worsening.

Notably, we detected a strong association between elevated bio-ADM levels and the occurrence of death and/or shock. Especially 48 h after admission to ICU, high bio-ADM levels showed a good prognostic accuracy for the endpoints death and shock which is in line with previous findings on the prohormone midregional pro-adrenomedullin (MR-proADM) in different groups of vascular patients (43, 44). For smaller types of vascular surgeries a correlation between MR-proADM and the development of AKI and SIRS within 30 days postoperatively (35) has been described. Up to now, a correlation between perioperative bio-ADM levels and death as well as shock in surgery patients has not yet been investigated systematically although this may draw the clinicians' attention to a clinically relevant worsening, which often remains challenging to detect.

As ADM is involved in numerous regulatory cascades that are afflicted in the first perioperative days, the difficulty to interpret increased plasma levels of bio-ADM in the context of different severe complications is comprehensible (13, 45, 46). The perioperative course of bio-ADM

was rather related to general organ failure than the development of specific organ injury, which overall may result from significant vascular injury and following dysfunction leading to hemodynamic instability and insufficient perfusion of relevant organs. Therefore, if approved in following studies, increased bio-ADM levels may draw the clinicians' awareness to an increasing risk of renal, pulmonic and associated postoperative complications.

Some relevant limitations must be taken into account while evaluating the discussed results: Defined time points were used for pre- and postoperative blood sampling, not correlating the time of sampling to the onset of clinical symptoms. A larger, prospective, multicenter study would have improved the quality of the assessed data. However, as TAAA is a rare disease, the planning of such, or even larger studies may be hard to realize. Although our analysis was

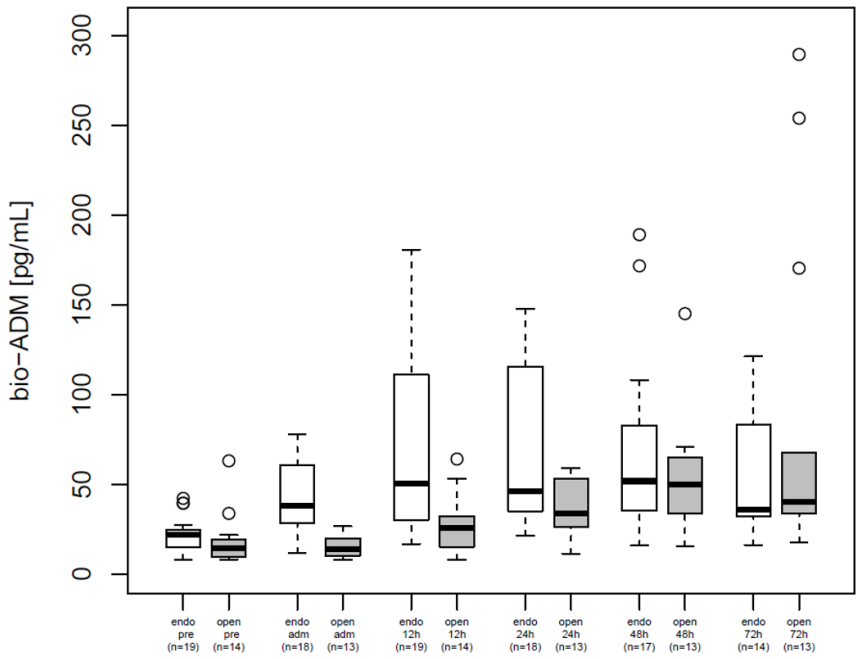
conducted in a rather small but relevant group of critically ill patients and thus has to be considered as exploratory, the results are concordant with what has been reported in the literature in other types of critically ill patients.

Conclusion:

The present findings highlight the role of postoperatively measured bio-ADM as promising biomarker for the early prediction of adverse outcomes, namely death and shock, after open and endovascular TAAA repair. Given the fast kinetics and accuracy of this emerging biomarker, it may assist the decision-making process for clinicians during the postoperative care of TAAA patients in future.

Supplemental Data

Supplemental figure 1:



Supplemental figure 1: Course of bioactive adrenomedullin (box plot) after open or endovascular thoracoabdominal aortic aneurysm repair measured before surgery, after admission to intensive care unit as well as 12 h, 24 h, 48 h, and 72 h after admission to ICU.

Table 1 supplemental data: Correlation of bio-ADM at baseline with clinical variables.

Characteristics	Median [IQR]	r (Spearman)	P-value
Age (years)	66 [57-75]	0.45	0.0086*
BMI (kg/m^2)	25.4 [21.5-28.2]	0.39	0.0234*
Ventilation time (surgery) (min)	508 [447-615]	0.05	0.7753
Ventilation time (ICU) (min)	835 [300-1571]	-0.25	0.1654
Ventilation time (re-intubation)	0 [0-0]	-0.23	0.1932
Total ventilation time (min)	1410 [960-2505]	-0.30	0.0899
Length of stay (in-hospital) (days)	26 [11-35]	-0.04	0.8107
Length of stay (ICU) (days)	4 [3-5]	-0.12	0.5089
Operation time (min)	359 [300-460]	0.18	0.3244
Blood transfusion (units)	5 [2-9]	-0.34	0.0558
Total thrombocytes transfusion (units)	2 [0-4]	-0.24	0.1749
Crystalloid transfusion during surgery (units)	5 [4-6]	-0.17	0.4827
Colloid transfusion during surgery (units)	2 [1-3]	0.06	0.7936
Maximum aortic diameter (cm)	6.2 [5.9-7.0]	0.05	0.7945

bio-ADM: bioactive adrenomedullin, BMI: Body Mass Index, ICU: intensive care unit, IQR: interquartile range

Table 2 supplemental data: Correlation of bio-ADM with SOFA score and other biomarkers from all available measurements and time points.

Characteristics	n	r (Spearman)	P-value
SOFA score	89	0.18	0.084
Leucocytes	162	0.37	<0.001
Procalcitonin	75	0.19	0.095
C-reactive protein	108	0.56	<0.001
Serum creatinine	161	0.52	<0.001

bio-ADM: bioactive adrenomedullin, SOFA: Sequential Organ Failure Assessment

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Chapter 5

Retrospective observational study evaluating zinc plasma level in patients undergoing thoracoabdominal aortic aneurysm repair and its correlation with outcome

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Abstract:

Thoracoabdominal aortic aneurysm (TAAA) repair is related to a relevant morbidity and in-hospital mortality rate. In this retrospective observational single-center study including serum zinc levels of 33 patients we investigated the relationship between zinc and patients' outcome following TAAA repair. Six patients died during the hospital stay (18%). These patients showed significantly decreased zinc levels before the intervention (zinc levels before intervention: 60.09 µg/dl [survivors] vs. 45.92 µg/dl [non-survivors]). The post-interventional intensive care SOFA-score (Sepsis-related organ failure assessment) (at day 2) as well as the SAPS (Simplified Acute Physiology Score) (at day 2) showed higher score points in case of low pre-interventional zinc levels. No significant correlation between patient comorbidities and zinc level before intervention, except for peripheral arterial disease (PAD), which was significantly correlated to reduced baseline zinc levels, was observed. Septic shock, pneumonia and urinary tract infections were not associated to reduced zinc levels preoperatively as well as during therapy. Patients with adverse outcome after TAAA repair showed reduced pre-interventional zinc levels. We speculate that decreased zinc levels before intervention may be related to a poorer outcome because of poorer physical status as well as negatively altered perioperative inflammatory response.

Keywords: zinc, aortic surgery, vascular surgery, inflammation, atherosclerosis

Introduction

Treatment of thoracoabdominal aortic aneurysms (TAAA), either by open or endovascular procedures, is related to a high risk of postoperative complications and in-hospital mortality[1]. Careful planning of the surgical approach is of utmost importance, and it requires diligent assessment of the clinical condition of the patient and the comorbidities. A reliable prognostic biomarker could be of high value in this context leading to improved preoperative care[2]. Next to familiar predisposition, age, gender and genetic factors, atherosclerosis is the most common causality for the pathogenesis of TAAA[3]. Atherosclerotic diseases are generally associated with increased systemic inflammation[4] and therefore the patients' zinc status, as a biomarker directly connected to the immune response, was examined in this study[5]. Zinc, as an essential trace element, has multiple functions such as in wound healing and the function of the immune system[6]. In humans, an overdose of zinc is rare, whereas its deficiency is a frequent state, especially in elderly people and in patients suffering from chronic diseases[7,8]. A reduced dietary intake of zinc may be one of the most common causes of deficiency[9]. One of the first symptoms of zinc deficiency is the impairment of immune function leading to an excessive release of proinflammatory cytokines and an increased susceptibility towards infections[10]. In developing countries, zinc deficiency is the fifth most common cause of increased overall disease burden, measured in disability-adjusted life years (DALYs)[11]. Physiologically lowered levels of zinc in the blood occur in the course of infections with an accompanying strong inflammatory reaction. In the context of acute phase reaction, serum zinc shifts into the liver, which is mediated by the transport protein Zrt- and Irt-like protein 14 (Zip14). Moreover, it was shown that changes in zinc levels correlated with the severity and mortality rates of sepsis[12]. Data concerning zinc levels in patients with TAAA are sparse. In order to investigate the prognostic value of zinc levels before intervention, we retrospectively analyzed the clinical data available from 33 TAAA patients undergoing treatment. The impact of altered zinc levels on patients' outcome after open and endovascular TAAA repair were assessed.

Results

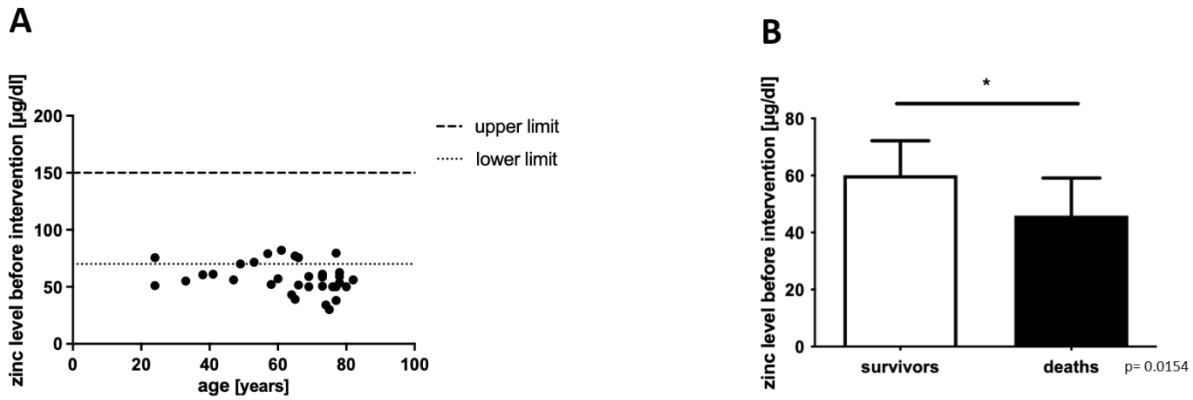
Characteristics of the recruited patient cohort

We included 33 subjects undergoing TAAA repair (see *Table 1*). Our study population consisted of 17 female and 16 male patients. The age of the participating subjects ranged from 24 to 82 years with a mean of 63 years and a median of 66 years. The average body mass index (BMI) (kg/m^2) was 25.4 (+/- 5.05; standard deviation (SD)). 14 patients underwent open surgical repair and 19 patients received endovascular intervention. Six patients suffered from connective tissue disease, namely Marfan syndrome, Loeyz-Dietz syndrome, alpha smooth muscle actin (*ACTA2*) mutation or suspected genetic aortic syndrome (GAS).

The average duration of the intervention was 374.30 minutes (+/- 111.03; SD). On average, patients remained in the hospital for 29.06 days (+/- 23.73; SD). Patients' intensive care stay was on average 7.61 days (+/- 10.99; SD)) long and during their hospital stay, patients were ventilated for 10539.03 minutes (+/- 25818.35; SD). 18.18% of all patients (n=6) died during the inpatient stay. The average zinc level before interventions was 63.15 µg/dl for patients under 60 years compared to 55.07 µg/dl for patients that were 60 years or older. Moreover,

there was a tendency for zinc levels to decrease comparably with the decline of renal function (*Supplementary Figure 1B*).

Figure 1



A) *Zinc levels of all patients (n=33) in correlation with the age before endovascular/surgical intervention are shown. The dashed lines show commonly used upper (70 µg/dl)/lower (150 µg/dl) standard values for the zinc level.* ***B)*** *The zinc levels of patients that survived endovascular/surgical intervention and were released from the hospital (n=27) were compared to patients that died during the hospital stay (n=6). Zinc level was measured before endovascular/surgical intervention. Shown is the mean and SD. Significance was determined using Student's t-test assuming significance if *p < 0.05.*

It was also shown that decreasing BMI levels tended to be associated with decreasing serum zinc levels (*Supplementary Figure 1C*; not significant). This was corroborated by the observation that decreased body weight correlated with a decrease in serum zinc levels (*Supplementary Figure 1D*; p=0.0285). Interestingly, body weight and BMI had also a significant (adverse) correlation with survival indicating that these patients were more likely suffering from a malnourished state (*Table 1*). Moreover, the duration of the intervention itself as well as the number of the administered blood products as red blood cells and platelets were significantly correlated to an increased rate of fatal outcomes (*Table 1).* We found no correlation between the zinc level and intake of different medications (e.g. antihypertensives, beta blockers, anticoagulants, diuretics or opiates) (*Supplementary Figure 2*). Moreover, we found no correlation of serum zinc level and the presence of arterial hypertension, coronary heart disease (CHD), chronic obstructive pulmonary disease (COPD), diabetes or previous aortic intervention (*Supplementary Figure 2*). In addition, we do not see any association between blood zinc concentrations and the smoking behavior, the presence of allergy, the occurrence of major adverse cardiac events (MACE) or even the occurrence of acute kidney injury (AKI) during treatment (*Supplementary Figure 3*).

Table 1: Patients characteristics and procedural details.

Characteristics	All patients (n=33)	Survivors		p-value
		Yes (n=27)	No (n=6)	
Demographics				
Age, years (mean +/- SD)	63 +/- 16.2	62.1 +/- 16.6	67.2 +/ 14.9	0.4986
Sex (female) (n)	51.52% (17)	51.85% (14)	50% (3)	0.9371
Body size, cm (mean +/- SD)	173.6 (12.6)	173.7 (12.45)	173.2 (14.46)	0.9265
Weight, kg (mean +/- SD)	76.89 (17.70)	80.61 (16.78)	60.17 (11.44)	0.0082
BMI, kg/m^2 (mean +/- SD)	25.4 (5.05)	26.6 (4.7)	20 (2.29)	0.0022
Smoking (current) (n)	36.36% (12)	33.33% (9)	50% (3)	0.4585
Pre-existing conditions				
Coronary heart disease (n)	42.42% (14)	44.44% (12)	33.33% (2)	0.6314
Peripheral arterial disease (n)	12.12% (4)	7.41% (2)	33.33% (2)	0.0829
COPD (n)	39.39% (13)	40.74% (11)	33.33% (2)	0.7465
Diabetes (n)	18.18% (6)	18.52% (5)	16.67% (1)	0.9185
GAS (N)	18.18% (6)	18.52% (5)	16.67% (1)	0.9185
Prior operations of the aorta (n)	48.49% (16)	93.75% (15)	6.25% (1)	0.0897
Trace element levels before intervention (peripheral blood)				
Zinc (mean +/- SD)	57.52 (13.28)	60.09 (12.06)	45.92 (13.20)	0.0154
Type of TAAA				
TAAA 1 (n)	15.15% (5)	18.52% (5)	0% (0)	0.2664
TAAA 2 (n)	21.21% (7)	18.52% (5)	33.33% (2)	0.4379
TAAA 3 (n)	21.21% (7)	22.22% (6)	16.67% (1)	0.7721
TAAA 4 (n)	30.30% (10)	25.93% (7)	50% (3)	0.2595
TAAA 5 (n)	12.12% (4)	14.81% (4)	0% (0)	0.3298
Procedure				
Open intervention (n)	42.42% (14)	70.59% (12)	33.33% (2)	0.6314
Overall stay in hospital, days (mean +/- SD)	29.06 (23.73)	31 (22.08)	22.5 (31.73)	0.4629
Duration of the intervention, min (mean +/- SD)	374.30 (111.03)	356.52 (107.71)	454.33 (95.80)	0.0492
Total ventilation time, min (mean +/- SD)	10539.03 (25818.35)	7807 (21592.99)	22831 (40249.42)	0.2021
Complications				
Infections (n)	45.45% (15)	44.44% (12)	50% (3)	0.8120
Tracheotomy (n)	12.12% (4)	11.11% (3)	16.67 (1)	0.4910
MACE (n)	33.33% (11)	29.62% (8)	37.5% (3)	0.3539
AKI (n)	51.52% (17)	44.44% (12)	83.33% (5)	0.0897
Total number of red blood cell transfusions (mean +/- SD)	13.76 (17.69)	9.44 (10.68)	33.17 (29.29)	0.0017
Total number of platelet transfusions (mean +/- SD)	2.88 (3.71)	2.04 (2.81)	6.67 (5.09)	0.0039

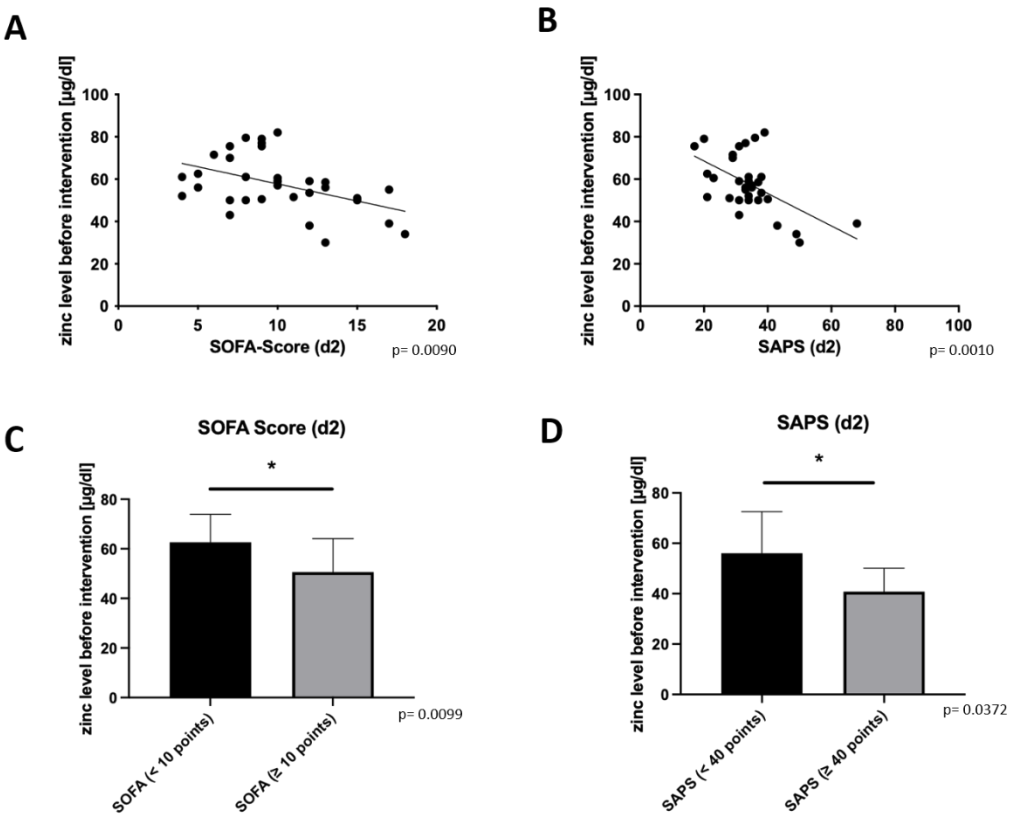
Table 1: Patients characteristics concerning demographics, pre-existing conditions, type of aneurysm, chosen procedure and complications. Shown are mean values +/- standard

*deviation (SD) or percentage with total number in brackets. Significance was determined using Student's t-test assuming significance if *p < 0.05. (COPD: Chronic-obstructive pulmonary disease; TAAA: Thoracoabdominal aortic aneurysm; MACE: Major Adverse Cardiac Event; AKI: Acute Kidney Injury; GAS: Genetic Aortic Syndromes)*

Low serum zinc levels before intervention correlate with increased mortality

A large proportion of patients showed a serum zinc level below 70 µg/dl, which was used as cut-off value for incipient zinc deficiency (*Figure 1A*)[13]. As reference values, we used 70 µg/dl zinc as the lower limit and 150 µg/dl as the upper limit. These limit values are frequently reported as part of routine diagnostics[13]. Patients who had decreased zinc levels before intervention showed an increased in-hospital mortality rate (*Figure 1B*; p=0.0154). At the time before intervention, there was no correlation between the value of C-reactive protein (CRP) compared with the measured zinc levels. Patients with an initial CRP value under 10 mg/l (n=11) had a mean zinc level before the intervention of 59.26 µg/dl compared to 53.91 µg/dl in patients with an CRP value above 10 mg/l (n=21) (not significant; p=0.2934). The prediction of worse outcome by the zinc level before intervention is strengthened by the fact that lowered zinc levels were associated with worse intensive care risk scores, namely SOFA-Score and SAPS. Patients with decreased zinc levels at baseline showed a higher ranking in SOFA-Score on day 2 (Figure 2A) as well as in SAPS on day 2 (Figure 2B). Patients with a SOFA-Score on day 2 above 10 points (n=13) had an average zinc level of 50.58 µg/dl before the intervention compared to 62.66 µg/dl for patients that showed lower values in the SOFA-Score (n=19) (Figure 2C; p=0.0099). It is particularly interesting that we see this correlation for zinc levels before the respective intervention. For the SAPS score, comparable findings were observed. Patients with a SAPS above 40 points on day 2 had an initial zinc level about 40.83 µg/dl (n=6) compared to 56.12 µg/dl (n=26) in patients with a better prognostic assessment using SAPS (Figure 2D; p=0.0372).

Figure 2



A) *The SOFA-Score (Sepsis-related organ failure assessment) of 32 patients after two days is shown. For one patient no SOFA-score could be obtained after two days. Accompanying the regression line (p=0.0090).* ***B)*** *The SAPS (Simplified Acute Physiology Score) for 32 patients after two days is demonstrated. For one patient no SAPS could be obtained on day two. Also shown is the regression line (p=0.0010).* ***C)*** *Demonstrated is the SOFA score after two days according to the point value <10 points (n=19) or ≥10 points (n=13) (0.0099).* ***D)*** *Shown is the SAPS score divided into patients with a score <40 points (n=26) or patients with ≥40 points (n=6) (p=0.0372).*

Zinc level and the acute phase reaction

It has previously been shown that zinc levels in serum decrease during the acute phase reaction due to a physiological zinc shift. In our study we could not find a significant correlation between zinc levels and inflammatory parameters. Zinc levels 24 hours (*Supplementary Figure 4A*) and 7 days (*Supplementary Figure 4B*) after intervention showed no significant correlation with CRP levels on the same day, respectively. Even immediately after intervention or 24 hours after, the comparison of zinc levels with the CRP levels of the coming days, showed no significant correlation. Nevertheless, a tendency of decreased zinc levels concomitantly to an increase of the CRP values over the course of the following days was observed. We demonstrate that patients with higher zinc levels after intervention tended to have lower CRP values on day 2 (*Supplementary Figure 4C*; p=0.0642). Comparable results were observed for the zinc level after 24 hours and the CRP level on day 3

(*Supplementary Figure 4D*; p=0.1179). Since it is only possible to detect an increase in the CRP after several hours[14] and only one measurement per day was possible, we consider the approach to be justified. Although the correlation between zinc level and inflammation has been described previously by various publications[15–18], we could not demonstrate a correlation in our patient collective. Likewise, we did not observe any correlation with other infection parameters such as procalcitonin (PCT) or interleukin (IL)-6 (data not shown).

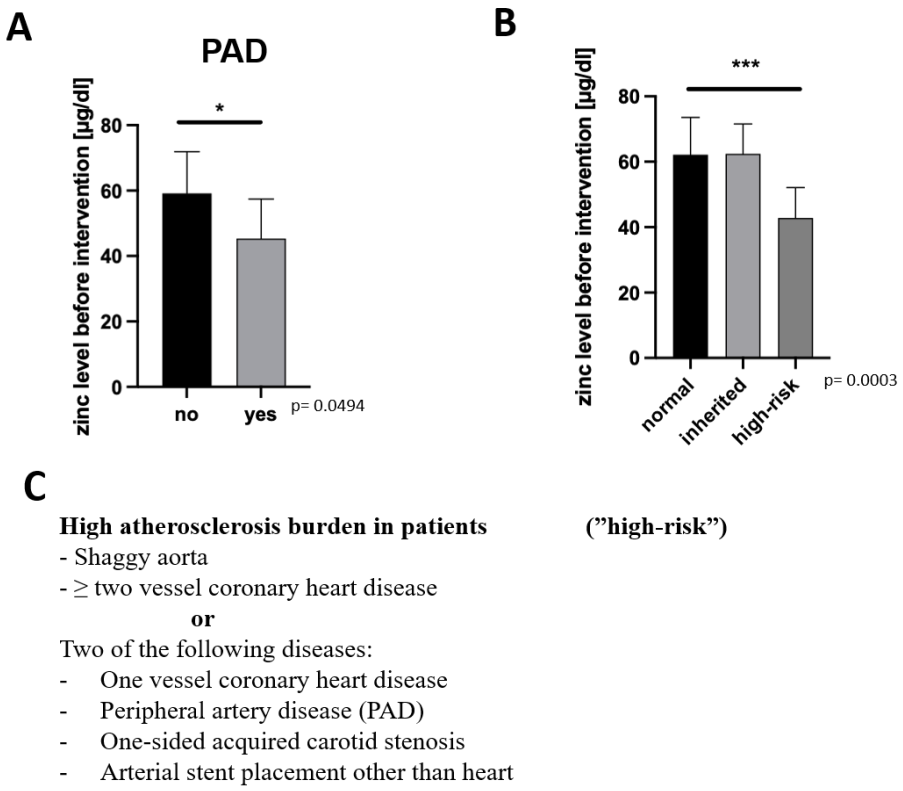
Serum zinc level and infection

Zinc levels 12 hours after intervention were not significantly decreased in patients who experienced septic shock during the course of treatment (*Supplementary Figure 5A*). The same is true for the difference of zinc (delta zinc level) 12 hours after intervention compared to the zinc level at the time of inpatient admission (*Supplementary Figure 5B*). Patients who developed pneumonia, urinary tract infection (UTI) or both during the course of the study did not show lower zinc levels after 12 hours compared to patients for whom no specific infection was documented (*Supplementary Figure 5C*). In addition, for patients with pneumonia, UTI or both we could not observe an augmented serum hypozincemia 12 hours after the intervention (*Supplementary Figure 5D*).

Chronic inflammation and atherosclerosis

The correlation of zinc deficiency with atherosclerosis is subject of an ongoing debate[19–22]. We did not observe a correlation between coronary artery disease and zinc level in our patient population. However, we showed that patients with PAD had significantly lower zinc levels at the time of admission (*Figure 3A*). Since PAD is typically caused by atherosclerotic plaques, a correlation of the serum zinc level with this atherosclerotic disease is likely. This was the motivation to classify patients based on their degree of atherosclerosis-related diseases. Furthermore, due to the fact that both the consecutive occurrence of new atherosclerotic lesions[23] and the number of manifestation sites in the human body partially reflect the burden of atherosclerosis[24], we have graded the severity of atherosclerosis in our patients based on the available data. In the presence of a shaggy aorta or in the case of atherosclerosis at two different sites, the patients were classified into a "high-risk" group. The high-risk group represents patients with elevated risk for atherosclerosis-related life-threatening complications (*Figure 3C*)[24]. We did not observe a difference between patients with a normal-risk score (n=19) and patients who have a congenital cause for aneurysms (n=6) (*Figure 3B*). Our patient cohort included patients with a familial predisposition to aneurysm formation (n=6). Three patients were found to have Marfan syndrome, one patient had Loeyz-Dietz syndrome, one patient had an *alpha smooth muscle actin* (*ACTA2*) mutation and one patient showed a suspected congenital disorder because of various aneurysms in the patient's medical history. Patients that we classified as high atherosclerotic burden ("high risk"; n=8) showed significantly decreased zinc levels at the time of hospital admission (*Figure 3B*). This is in line with the decreased zinc level in patients with PAD (*Figure 3A*) and supports the assumption of lowered serum zinc levels in patients with degenerative vascular diseases.

Figure 3



A) *Zinc levels before intervention of patients who had or did not have peripheral arterial disease ("PAD"; n=4; p=0.0494).* ***B)*** *Mean zinc level of our patient cohort before the respective intervention depending on whether we assigned the patients to the group with proven (n=5) or high suspicion (n=1) of a genetic predisposition ("inherited"; n=6; p=0.9601) or to the group with high atherosclerosis burden ("high-risk"; n=8; p=0.0003).* ***C)*** *Representation of the criteria that we used to categorize patients to the high-risk group ("high-risk") or not ("normal"). Shown is the mean and SD. Significance was determined using Student's t-test assuming significance if *p < 0.05, **p < 0.01 and ***p < 0.001.*

Discussion

In our study, decreased serum zinc levels in patients undergoing TAAA repair were found to be related to lower survival rates (*Figure 1B*) and correspondingly worsened intensive care prognostic scores two days after intervention (*Figure 2A and 2B*). Our study cohort of 33 patients, although all have TAAA, presents itself as quite small and heterogeneous group (*Table 1*). Impressively, a large proportion of patients showed decreased serum zinc levels before surgery, which can be interpreted as a concomitant effect of pre-existing diseases and an altered nutritional status (*Figure 1A*). Moreover, there was no association observed between preoperative zinc levels and most patients' comorbidities (like hypertension, coronary heart disease, COPD, diabetes mellitus), medication (antihypertensives, beta-blockers, anticoagulants, diuretics and opiates) or prior operations of the aorta (*Supplementary Figure 2*). We were able to confirm previous observations, such as the negative (not significant) association of serum zinc level with age[25] (*Supplementary Figure 1A*) as well as the negative (not significant) correlation with renal dysfunction[26] (*Supplementary Figure 1B*). However, some publications do not indicate a correlation of zinc level with age and they explain the differences in serum zinc levels by the existence of other comorbidities[27]. By the fact that our patients' body weight and BMI correlates significantly with survival, we believe that a large proportion of patients with severe zinc deficiency are malnourished and have a nutrition-induced zinc deficiency[28,29]. Nevertheless, the preoperative low zinc level had an impact on mortality, severity of the post-interventional intensive care scores, as well as the prevalence of atherosclerosis-related diseases in TAAA patients. A large proportion of the patients studied showed serological zinc deficiency which is consistent with the suggestion that even in the western world many people are affected by zinc deficiency with predominance of older people and patients with chronic diseases among those affected. So far, a clear relationship between atherosclerosis and zinc deficiency has not been proven with certainty[22,30], but other studies suggested a correlation between low zinc levels and atherosclerotic diseases such as an association between zinc deficiency and coronary heart disease[31]. Previously published studies underlined that zinc deficiency appears to promote the production of proinflammatory cytokines such as IL-1β, tumor necrosis factor alpha (TNFα), and IL-6 by myeloid cells and activated monocytes/macrophages[32,33]. Zinc supplementation of elderly people suffering from zinc deficiency was able to improve immune cell function and abnormal cytokine expression[32,33]. Despite the described correlation of a low zinc level with the acute phase response[33], we were not able to show this relationship in our cohort (*Supplementary Figure 4*). We also observed comparable effects on patient prognosis. Furthermore, it was previously shown that zinc deficiency is associated with increased number of proinflammatory cytokines and increased activation of proinflammatory signaling cascades[33,34]. At least 50% of our patients who died had concomitant infections as diagnosis and at least one out of six persons who died had a clinically relevant impairment of wound healing. It should be pointed out once again that zinc has an significant impact on both immune function[35] as well as wound healing[36]. Since there are studies showing that zinc supplementation can reduce the excessive release of proinflammatory cytokines in elderly individuals, the possible supplementation of the trace element zinc could be considered[37]. As a perspective, further studies should clarify whether zinc supplementation can reduce the number of inflammatory cytokines, the progression of atherosclerosis and the occurrence of deadly adverse events in patients with TAAA. We hypothesize that patients who enter treatment with already markedly

decreased zinc levels suffer from worse zinc deficiency in the peripheral blood due to acute phase reaction during therapy leading to increased patient mortality.

The heterogeneous character of the assessed, rather small cohort of patients in this retrospective study is a relevant risk of bias and has to be mentioned as a relevant limitation. In addition, the data evaluation of zinc levels after intervention is problematic because critically ill patients also received artificial diets containing zinc chloride as part of intensive care treatment. Unfortunately, it is not possible to retrospectively work out the dose of zinc received in each case. This is most likely another reason why we could not observe a correlation between follow-up zinc level as well as zinc shift concerning infectious complication, changes in inflammatory values and wound healing disorders. We would like to emphasize the observational character of our study, but at the same time we underlined potential importance of zinc levels in TAAA patients. We wish that our study will give impetus to new mechanistic studies regarding the micronutrient zinc and its impact on the survival of patients with atherosclerosis-related diseases.

Conclusion

Patients with fatal outcome after TAAA repair showed reduced pre-interventional zinc levels. Zinc deficiency represents a potential phenomenon of chronic inflammation especially in patients with severe atherosclerosis, which is a common comorbidity of TAAA patients. We confirm decreased serum zinc in patients with atherosclerotic PAD and also in patients with a high burden of atherosclerosis. We speculate that decreased zinc levels before intervention may be related to a poorer outcome because of negatively altered perioperative inflammation reaction. Therefore, a preoperative screening of the zinc status could be considered for patients undergoing TAAA repair.

Materials and Methods

Study population

In this retrospective observational study 33 patients suffering from TAAA requiring treatment because of a diameter above 5.5 cm or in case of connective-tissue disease, above 5.0 cm. Patients were included between the 11th of January and the 20th of December 2017. All patients were treated at the RWTH Aachen University Hospital. Patients that underwent an open or endovascular TAAA repair wereevaluated. Exclusion criteria were the following: age below eighteen years, pregnancy, patients with immunosuppressive medication and patients with pre-existing need for renal replacement therapy. Moreover, no emergency interventions were included.

Relevant comorbidities such as chronic-obstructive pulmonary disease (COPD), coronary heart disease and diabetes, all defined according to current guidelines[38–40], pre-existing medication included antihypertensives, beta blockers, anticoagulants, diuretics or opiates were correlated to patients' zinc levels. Genetic Aortic Syndromes (GAS), namely Marfan syndrome, Loeyz-Dietz syndrome, alpha smooth muscle actin (*ACTA2*) mutation and suspected genetic aortic syndrome were assessed. All patients with peripheral arterial disease (PAD) showed disease stage II b or higher according to the Fontaine classification[41].

Extensive atherosclerosis or extensive thrombus load of the aorta, also called "shaggy aorta", has been described to be an impressive sign of systemic atherosclerosis[42].

Physiological parameters and patients' medical history namely c-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 were taken from the electronic medical records (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). The sequential organ failure assessment (SOFA)[43] and the simplified acute physiology score (SAPS) were assessed at different time points[44]. During the course of therapy, the SOFA and the SAPS were performed on days 1 (SOFA: n=14; SAPS: n=5), 2 (SOFA: n=32; SAPS: n=32), 3 (SOFA: n=29; SAPS: n=29), 7 (SOFA: n=15; SAPS: n=15), 14 (SOFA: n=8; SAPS: n=8) and 28 (SOFA: n=4; SAPS: n=4). For both scores, an increased amount of score points indicate a reduced general condition of the patient as well as a worse prognosis with higher probability of in-hospital mortality. Acute kidney injury (AKI) within 48 hours postoperatively was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria[45].

Major cardiovascular events (MACE) included acute heart failure, myocardial infarction and ventricular tachycardia were defined according to current guidelines[46–48]. Sepsis was defined according to the German Sepsis Society[49]. The category "infections" included pneumonia, urinary tract infection and surgical site infection [50–52]. All patients underwent informed consent and agreed to participate in the research project. The study protocol was approved by the ethic committee of the University Hospital RWTH Aachen, Germany (EK 004/14) and was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. Prior to this, other studies were also conducted based on this patient cohort[53–55].

Acquisition of the patient material

Serum tubes (SARSTEDT S monovette) were collected from the patients at different time points. Samples were collected before intervention, after admission to the intensive care unit (ICU) (direct after intervention), as well as during ICU follow-up (12 hours, 24 hours, 48 hours, and 7 days). All patients had a study-related blood draw before the intervention (n=33). Blood sampling as postoperative follow-up did not occur in all patients at respective time points. Acquisition of patient blood serum occurred immediately after the intervention in n=30 patients, after 12 hours in n=32, after 24 hours in n=31 patients, after 48 hours in n=30 patients, and after 7 days in n=24 patients. The material was preserved at -20°C until further processing. Although the Sarstedt tubes used are not specially designed for the measurement of trace elements like zinc, both external work[56] as well as internal validations in comparison to special sample tubes designed for trace element measurements show that same zinc levels were detected independently of the used test tube (data not shown).

Measurement of the total serum zinc level

The serum zinc concentration was determined by flame Atomic Absorption Spectrometry (AAS) using an AAnalyst 800 (Perkin-Elmer, Waltham, United States). For measurement serum samples were diluted as described elsewhere[57,58].

Surgical intervention

As published before, the protocol for open TAAA repair included aortic cross-clamping, extracorporal circulation with distal aortic perfusion, and visceral perfusion using selective perfusion catheters[59,60]. To avoid acute renal failure, contrast agent was used carefully, leading to a mean application of 65 ±17 ml per endovascular procedure. Furthermore, we applied one fourth of the standard dose for kidney angiography[61].

Classification of atherosclerosis severity

In the presence of atherosclerosis-related diseases, we classified patients into a high-risk category as soon as they had more than one severe manifestation of atherosclerosis (*see Figure 3C*). By doing so, we invoke data suggesting that respective disease severity and the amount of manifestations likely reflect the level of atherosclerotic disease burden[23,24]. As an exception, we consider the presence of a shaggy aorta as a high-risk criteria because itself represents an extensive, severe, and rather rare arteriosclerosis-related disease of the whole aorta[42].

Statistics

Statistical significances were calculated by Student's t test using GraphPad Prism software (version 5.01). Significances are indicated by: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. For linear regression we calculated the F test and we assume a significant deviation from zero if *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Author Contributions:

Conceptualization: BR, AG, PD
Methodology: BR, AG, LR
Validation: BR, IW, MV
Formal analysis: IW
Investigation: FB
Resources: BR, AG
Data curation: FB
Writing—original draft preparation: BR, AG
Writing—review and editing: DK, MJ, PD
Visualization: BR, AG
Supervision: LR
Project administration: AG
All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the University Hospital RWTH Aachen EK010/19, 21th May 2019.

Informed Consent Statement:

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement:

Data available on request from the authors.

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Conflicts of Interest:

The authors declare no conflict of interest.

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Chapter 6

In-hospital mortality and organ failure after open and endovascular thoracoabdominal aortic surgery can be predicted by increased levels of circulating dipeptidyl peptidase 3

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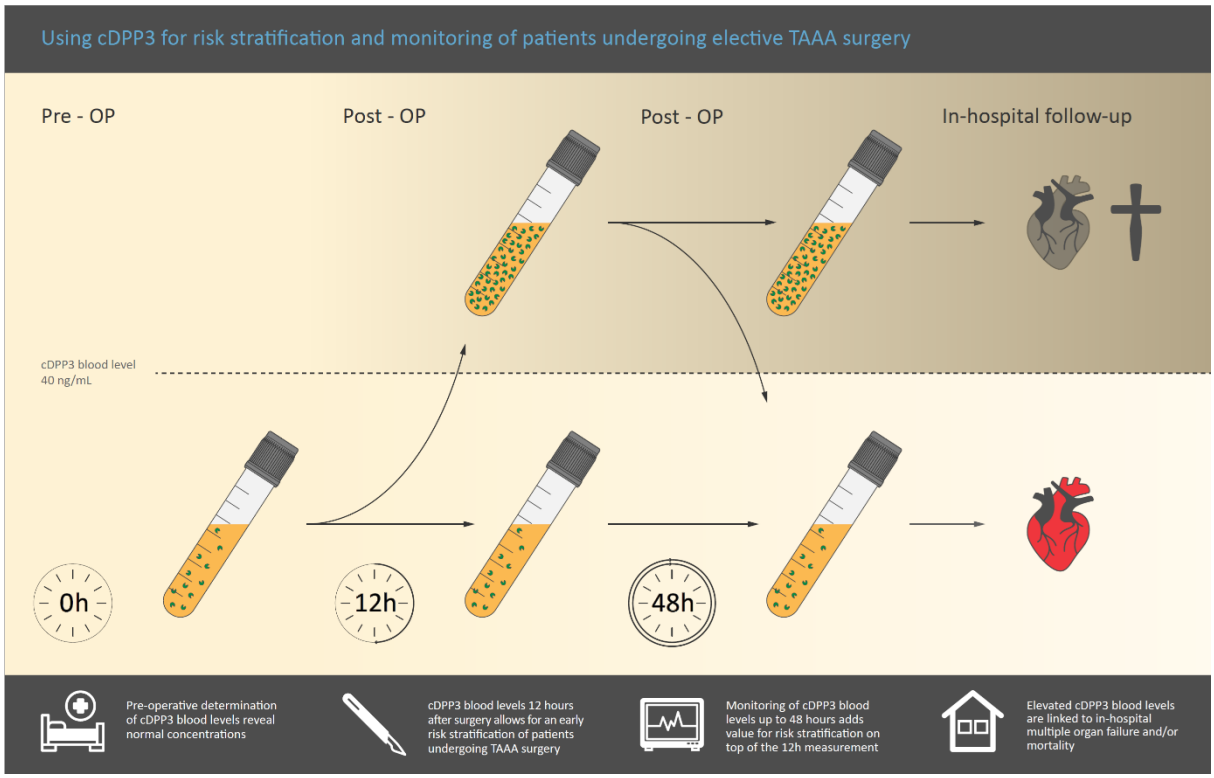
Using cDPP3 for risk stratification and monitoring of patients undergoing elective TAAA surgery
Pre - OP
Post - OP
Post - OP
In-hospital follow-up
cDPP3 blood level
40 ng/mL
0h
12h
48h
Pre-operative determination of cDPP3 blood levels reveal normal concentrations
cDPP3 blood levels 12 hours after surgery allows for an early risk stratification of patients undergoing TAAA surgery
Monitoring of cDPP3 blood levels up to 48 hours adds value for risk stratification on top of the 12h measurement
Elevated cDPP3 blood levels are linked to in-hospital multiple organ failure and/or mortality

Abstract

Background:

Endovascular and open thoracoabdominal aortic aneurysm (TAAA) repair is associated with relevant complications and mortality rates. Circulating dipeptidyl peptidase 3 (cDPP3) is a novel biomarker which shows a strong association with organ failure and fatality and has not been assessed in surgical settings. Therefore, the objective of this study is to assess the prognostic abilities of cDPP3 for patients' survival and organ failure following open and endovascular TAAA repair.

Methods:

Thirty-three patients undergoing TAAA repair have been assessed in this prospective observational single-center study. cDPP3 levels were serially measured perioperatively until 72h after admission to the intensive care unit (ICU). In-hospital-mortality and any organ failure were the clinical endpoints.

Results:

Postoperative organ failure was detected in 17 patients (51.5%) and six patients died after surgery (18.2%). At 12h after admission to the ICU, cDPP3 levels were significantly increased in patients who died or developed organ failure ($P<0.001$). cDPP3 levels after surgery demonstrated a remarkable predictive accuracy for in-hospital mortality (12h-AUC: 0.907 [$P<0.001$], 24h-AUC: 0.815 [$P=0.016$], 48h-AUC: 0.914 [$P=0.003$]) and development of organ failure (12h-AUC 0.882 [$P<0.001$], 24h-AUC: 0.850 [$P<0.001$] 48h-AUC 0.846 [$P<0.001$]). Additionally, a significant correlation between cDPP3 and SOFA score, procalcitonin as well C-reactive protein and interleukin-6 ($P<0.001$, $P<0.001$, $P=0.011$, $P=0.007$ respectively) from all available measurements and time points was assessed.

Conclusion:

The present findings highlight the role of cDPP3 as an early, highly specific post-operative biomarker for in-hospital mortality and organ failure after TAAA repair.

Key words:

cDPP3, biomarker, thoracoabdominal aortic aneurysm, TAAA.

Abbreviations and Acronyms

AKI – Acute kidney injury

AUC – Area under the curve

cDPP3 – Circulating dipeptidyl peptidase 3

CSFD – Cerebrospinal fluid drainage

DPP3-LIA – DPP3 luminescence immunoassay

ECC – Extracorporeal circulation

fEVAR – Fenestrated endovascular aortic aneurysm repair

ICP – Intracranial pressure

ICU – Intensive care unit

IQR – Interquartile ranges

MAP – Mean arterial pressure

MEP – Motor evoked potential

NGAL – Neutrophil gelatinase associated lipocalin

ROC – Receiver operating characteristic

SD – Standard deviations

SIRS – Systemic inflammatory response syndrome

SOFA – Sequential organ failure assessment

TAAA – Thoracoabdominal aortic aneurysm

Introduction

Patients after thoracoabdominal aortic aneurysm (TAAA) surgery frequently develop organ dysfunctions, which ultimately lead to high morbidity and mortality rates, regardless if performed by open or endovascular means(1, 2).

Extensive ischemia-reperfusion damage during and after open TAAA triggers systemic inflammatory response syndrome (SIRS), which often further contributes to the development of multiple organ failure, shock and sepsis (3, 4). To allow a timely and specific treatment for complications following open and endovascular TAAA repair, the use of readily accessible specific biomarkers for the detection of adverse outcomes, such as organ failure and in-hospital mortality, would be favorable to improve patients' treatment. Lactate represents one of the hitherto frequently used and established biomarkers for the detection of disease aggravation and adverse outcomes in critically ill patients. However, it is characterized neither by adequate sensitivity nor specific for worsening of any type of single organ dysfunction. In addition, increase in lactate levels occurs too late for an adequate use as predictive biomarker in the clinical practice(5). Recently, circulating dipeptidyl peptidase 3 (cDPP3), an amino dipeptidyl peptidase involved in the degradation process of angiotensin II and enkephalins, has been described as biomarker for in-hospital mortality and short-term outcome. Critically ill patients with hemodynamic instability and suffering from cardiogenic shock showed increased levels of cDPP3(6-8). Patients after TAAA repair frequently demonstrate hemodynamic instability and surgery per se represents an ideal model to study the kinetics and clinical significance of cDPP3 in a clinical model of predictable ischemia/reperfusion and inflammation. We, therefore, evaluated the clinical relevance of cDPP3 in patients undergoing open and endovascular TAAA repair with special focus on the development of organ dysfunctions and in-hospital mortality.

Methods:

Between January and December 2017, 33 patients have been included in this prospective, observational single-center study upon written informed consent. The study has been approved by the local internal review board (EK004/14). Each patient gave informed consent prior to the inclusion in the study and the study was conducted in accordance to the principles outlined in the Declaration of Helsinki.

Patients have been included if an endovascular or open TAAA repair was conducted. TAAA was defined according to the Crawford classification(9). Exclusion criteria were age below 18 years, pregnancy, chronic kidney disease requiring permanent dialysis treatment as well as emergency procedures and ongoing immunosuppressive medication.

Data collection

Daily physiological variables and demographics (age, sex, body mass index, medical history including cardiovascular disease, diabetes and chronic kidney insufficiency) and type of surgery were obtained from medical records and electronic bedside flow charts (Tables 1 and 2). Before surgery, after admission to intensive care unit (ICU), and 12 h, 24 h, 48 h, and 72 h after admission to the ICU, blood samples were collected and centrifuged ten minutes at 3000 g and the resulting plasma supernatants were stored at -80 °C until further analysis. Standard laboratory parameters (e.g. biomarkers of organ injury, such as creatinine and creatinine kinase isoenzyme MB) were measured as part of the clinical routine during the postoperative ICU treatment.

In this study, the following definitions were used to define specific organ failure. All of these complications have been summarized as “any organ failure”. Sepsis was defined according to the guidelines of the German Sepsis Society(10): Fever above 38° Celsius or hypothermia below 36° Celsius, tachycardia with a heart rate above 90 beats per minute, tachypnea with a respiratory rate above 20 per minute or a leukocytosis ($\geq$ 12 000/mm³) or leucopenia ($\leq$ 4 000/mm³). Shock was defined as recommended by Vincent et al: The systolic arterial pressure was less than 90 mmHg or the mean arterial pressure was less than 70 mmHg with associated tachycardia(11).

Cardiac complications included myocardial infarction, acute heart failure and ventricular tachycardia; all defined according to current guidelines(12). Pneumonia and tracheotomy were defined according to the guidelines of the American Thoracic Society or the Belgian Society of Pneumology, respectively (13, 14). Acute kidney injury (AKI) within 48 h was defined according to the Kidney Disease Improving Global Outcomes criteria based on serum creatinine levels (15). The sequential organ failure assessment (SOFA) score was used according to its definition (16).

cDPP3 measurement

Human plasma samples were measured using the recently published DPP3 luminescence immunoassay (DPP3-LIA)(17). Briefly, twenty microliters of samples or calibrators were pipetted into antibody-coated microtiter plates. After adding anti-DPP3 tracer antibody, the microtiter plates were incubated for 3h at room temperature and 600 rpm. Unbound tracer was removed by washing 4 times (350 µL per well). Remaining chemiluminescence was measured for 1s per well by use of the Centro LB 960 microtiter plate luminometer (Berthold Technologies GmbH & Co. KG). The concentration of DPP3 was determined with a 6-point calibration curve [0 (def. 0.01)–200 ng/mL]. Calibrators and samples were run in duplicate.

Surgery

Open and endovascular surgery

The operative protocol for open TAAA repair has been previously published in detail (18). It includes double lumen endotracheal tube intubation, cerebrospinal fluid drainage (CSFD), perioperative monitoring of motor evoked potentials (MEP), sequential aortic clamping if possible, extracorporeal circulation (ECC) with distal aortic perfusion as well as selective visceral perfusion and mild hypothermia of 32 to 33°C(19). 4°C Custodiol® (Dr. Franz Köhler Chemie, Bensheim, Germany) was used for renal perfusion instead of blood perfusion. This maneuver has been described to protect the kidneys from ischemic organ damage. Thoracolaparotomy through the fifth to eighth intercostal space depending on the extent of the aneurysm was applied as surgical access. Postoperatively, the mean arterial pressure (MAP) was adjusted based on MEPs and the intracranial pressure (ICP) was kept ≤10

mmHg during the first 72h for all patients. Extubation was performed as soon as possible postoperatively.

Endovascular TAAA repair was conducted under general anesthesia; neuro-monitoring was identical with open TAAA repair. The detailed procedure of fenestrated endovascular aortic aneurysm repair (fEVAR) has been described before(20). In case of endovascular procedure, the renal perfusion was not directly interrupted by aortic clamping. Contrast medium was applied to avoid functional impairment of the kidneys, leading to a mean application of 65 ± 17 mL per endovascular procedure.

Statistics

The primary endpoint was to evaluate the clinical significance of cDPP3 levels for the prediction of organ failure and in-hospital mortality following open and endovascular aortic repair.

Values are expressed as means and standard deviations (SD), medians and interquartile ranges (IQR), or counts and percentages, as appropriate. Group comparisons of continuous variables were performed using the Kruskal-Wallis test. Categorical data were compared using Pearson's Chi-squared Test for count data. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of cDPP3 concentration obtained at each time point and to compare their ability to predict organ failure or in-hospital mortality. cDPP3 was not normally distributed and was, therefore, log-transformed. Logistic regression was used to derive significance and the area under the ROC curve (AUC) is given as a measure of effect size.

All statistical tests were two-tailed and a two-sided p-value of 0.05 was considered for significance. P-values were not adjusted for multiple testing. The statistical analyses were performed using R version 3.4.3 (http://www.r-project.org, library rms, Hmisc, ROCR) and Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results:

1. Patients` characteristics, intra- and postoperative data

33 patients were enrolled in 12 months and followed up until hospital discharge. Mean patient age was 63 (± 16.2) years, 16 patients (48.5%) were women. Endovascular TAAA repair was conducted in 19 patients (57.6%), open repair in 14 patients (42.4%). Mean operation time was 374 ± 111 min, the mean total ventilation time was 1410 (960-2505) min. All details about surgery related data could be found in table 1.

Table 1. Patient characteristics

Characteristic		All patients (n=33)	Endovascular surgery (n=19)	Open surgery (n=14)	p-value
Demographics					
	Age, years	63.0 ± 16.2	72.5 ± 7.1	50.1 ± 16.4	<0.001
	Sex (women)	16 (48.5%)	10 (52.6%)	6 (42.9%)	0.733
	BMI, kg/m^2	25.4 ± 5.0	25.0 ± 5.1	26.0 ± 5.1	0.600
Comorbidities					
	Smoker	12 (36.4%)	8 (42.1%)	4 (28.6%)	0.483
	Chronic kidney disease	5 (15.2%)	2 (10.5%)	3 (21.4%)	0.636
	Coronary heart disease	14 (42.4%)	10 (52.6%)	4 (28.6%)	0.283
	Diabetes mellitus	6 (18.2%)	4 (21.1%)	2 (14.3%)	0.685
	Hypertension	23 (69.7%)	15 (78.9%)	8 (57.1%)	0.253
	COPD	13 (39.4%)	11 (57.9%)	2 (14.3%)	0.019
	Connective tissue disease	5 (15.2%)	0 (0%)	5 (35.7%)	0.010
	PAD	4 (12.1%)	4 (21.1%)	0 (0%)	0.111
	Maximum aortic diameter, cm	6.6 ± 1.3	6.4 ± 1.2	6.9 ± 1.4	0.314
Marker at baseline					
	DPP3, ng/mL	15.1 [12.2-18.9]	14.2 [12.2-17.0]	16.2 [9.7-22.1]	0.572
	Hemoglobin, mg/dL	10.0 ± 1.7	9.9 ± 1.7	10.2 ± 1.7	0.563
	Serum creatinine, mg/dL	1.1 [0.8-1.4]	1.2 [0.9-1.5]	0.9 [0.7-1.1]	0.071
Operational characteristics					
	Operation time, min	374 ± 111	376 ± 111	372 ± 115	0.908
	ICU ventilation time, min	835 (300 – 1571)	400 (0 – 1360)	1003 (763 – 3630)	0.021
	Total ventilation time, min	1410 (960 – 2505)	1080 (628 – 1947)	1786 (1316 – 19441)	0.017
	Stay on ICU, days	4 (3 – 5)	3 (2 – 5)	5 (4 – 16)	0.032
	In-hospital stay, days	26 (11 – 35)	15 (10 – 35)	27 (21 – 34)	0.222
	Blood transfusion, units	8 (4 – 15)	5 (4 – 13)	10 (6 – 27)	0.083
Type of TAAA					0.562
	TAAA 1	5 (15.2%)	2 (10.5%)	3 (21.4%)	

TAAA 2	7 (21.2%)	3 (15.8%)	4 (28.6%)
TAAA 3	7 (21.2%)	4 (21.1%)	3 (21.4%)
TAAA 4	10 (30.3%)	8 (42.1%)	2 (14.3%)
TAAA 5	4 (12.1%)	2 (10.5%)	2 (14.3%)

BMI: Body Mass Index, COPD: Chronic obstructive pulmonary disease, PAD: Peripheral artery disease, TAAA: Thoracoabdominal aortic aneurysm
Continuous data is reported as mean ± standard deviation. Categorical data is reported as absolute and relative frequencies.

Postoperative organ failure could be observed in 17 patients (51.5%). AKI occurred in 51.5 %, pneumonia in seven patients (21.2%) and four patients (12.1%) required tracheotomy because of weaning failure. Cardiac complications were found in 10 patients (30.3%) (one case of myocardial infarction, four cases of cardiogenic shock caused by acute heart failure and five cases of ventricular tachycardia). Sepsis was found in two patients (6.1%). The in-hospital mortality rate was 18.2 % (n=6). Postoperative outcomes are demonstrated in table 2.

Table 2. Complications and mortality rate

Outcome	All patients (n=33)	Endovascular surgery 19 (57.6%)	Open surgery 14 (42.4%)	p-value
Pneumonia	7 (21.2%)	1 (5.3%)	5 (35.7%)	0.068
Tracheotomy	4 (12.1%)	0 (0%)	4 (28.6%)	0.025
Spinal cord ischemia	3 (9.09%)	1 (5.3%)	2 (14.3%)	0.177
Acute kidney injury	17 (51.5%)	7 (36.8%)	10 (71.4%)	0.075
KDIGO 1	10 (58.8%)	4 (21.1%)	6 (42.9%)	
KDIGO 2	2 (11.8%)	1 (5.3%)	1 (7.1%)	
KDIGO 3	5 (29.4%)	2 (10.5%)	3 (21.4%)	
Sepsis	2 (6.1%)	0 (0%)	2 (14.3%)	0.177
Cardiac complications	10 (30.3%)	4 (21.1%)	6 (42.9%)	0.253
Cardiogenic shock	4 (12.1%)	1 (5.3%)	3 (21.4%)	0.291
In-hospital mortality	6 (18.2%)	4 (21.1%)	2 (14.3%)	0.685
Hereof pneumonia	2 (33.3 %)	2 (10.5%)	0	
Hereof small intestine ischemia	2 (33.3%)	1 (5.2%)	1 (7.1 %)	0.82
Hereof cerebral bleeding	2 (33.3%)	1 (5.2%)	1 (7.1 %)	0.82
Cardiogenic shock or in-hospital mortality	8 (24.2%)	4 (21.1%)	4 (28.6%)	0.697
Organ failure	17 (51.5%)	7 (36.8%)	10 (71.4%)	0.107

Data is reported as absolute and relative frequencies. KDIGO: Kidney Disease – Improving Global Outcomes. Data is reported as absolute and relative frequencies. Cardiac complications included myocardial infarction, arrhythmia and acute heart failure.

1. Time course of cDPP3 levels following open and endovascular aortic aneurysm repair

The perioperative time course of cDPP3 levels increased immediately on admission to the ICU, peaking as early as 12h post admission, followed by normalization towards 72h post-surgery (P<0.001). Patients with endovascular and open TAAA repair showed similar dynamics and characteristics, with the remarkable exception that cDPP3 levels peaked earlier in patients with open TAAA repair (cDDP3 in open vs. endovascular immediately after surgery: AUC 0.91, P<0.001; P>0.2 for all other time points) (figure 1).

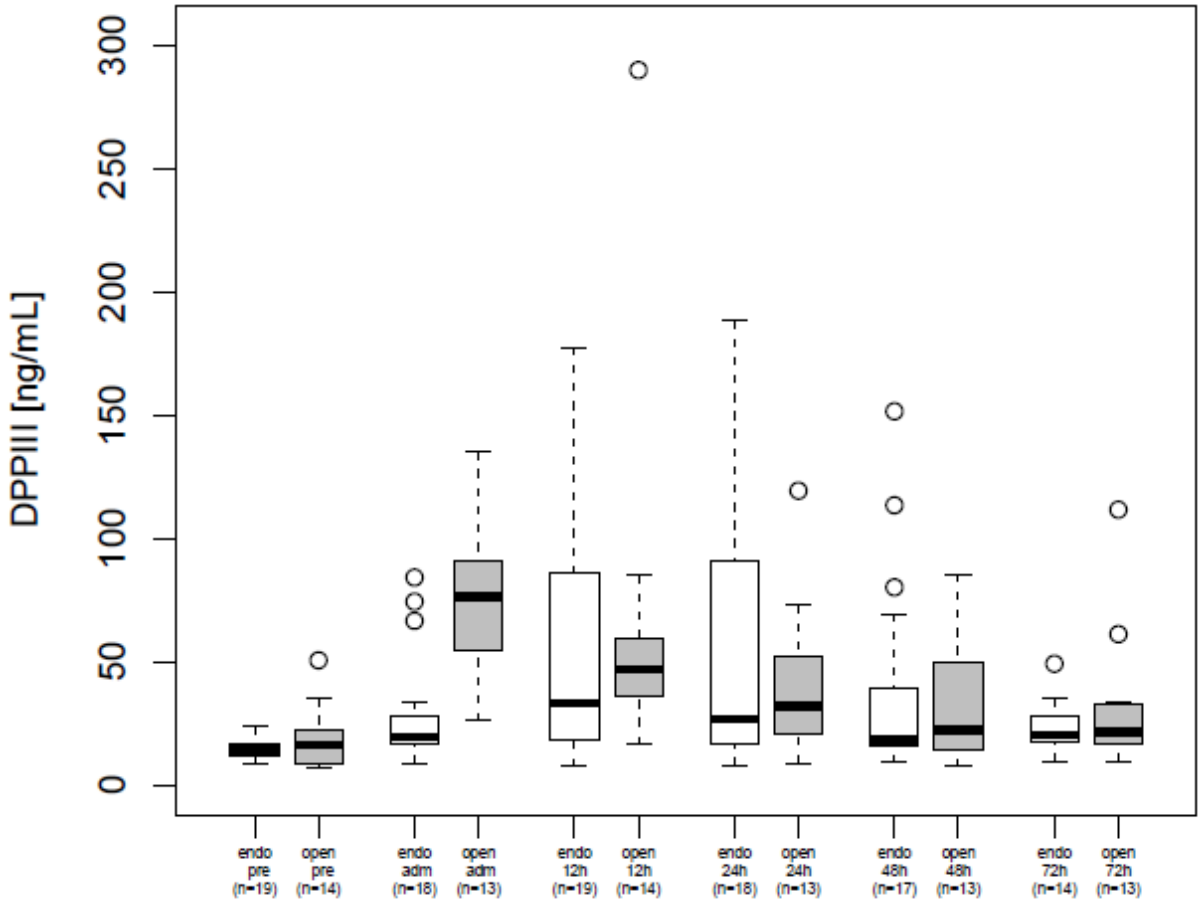

Figure 1: Box plot of course of circulating Dipeptidyl peptidase 3 (cDPP3) in patients undergoing open or endovascular thoracoabdominal aortic aneurysm repair (n=33).

2. Clinical significance of perioperative cDPP3 levels

No significant correlation with preoperative clinical variables and baseline cDPP3 levels could be observed. Significant correlations were assessed between in- and postoperative cDPP3 levels and the postoperatively assessed SOFA score, procalcitonin levels as well as blood levels of c-reactive protein, interleukin-6 and serum creatinine ($P<0.001$, $P<0.001$, $P=0.011$, $P=0.007$, $P=0.024$ respectively) at all measured time points (table 3 and 4). No significant correlation could be detected between either IL6 or CRP levels or the SOFA score.

Table 3: Correlation of DPP3 at baseline with clinical variables.

BMI: Body Mass Index, ICU: intensive care unit

Characteristics	Median [IQR]	r (Spearman)	P-value
Age (years)	66 [57-75]	−0.1	0.589
BMI (kg/m^2)	25.4 [21.5-28.2]	−0.34	0.053
Ventilation time (surgery) (min)	508 [447-615]	0.18	0.326
Ventilation time (ICU) (min)	835 [300-1571]	0.06	0.753
Ventilation time (re-intubation)	0 [0-0]	−0.08	0.656
Total ventilation time (min)	1410 [960-2505]	0.05	0.780
Length of stay (in-hospital) (days)	26 [11-35]	0.04	0.840
Length of stay (ICU) (days)	4 [3-5]	0.07	0.705
Operation time (min)	359 [300-460]	0.16	0.373
Blood transfusion (units)	5 [2-9]	0.16	0.385
Total thrombocytes transfusion (units)	2 [0-4]	0.26	0.152
Crystalloid transfusion during surgery (units)	5 [4-6]	−0.07	0.771
Colloid transfusion during surgery (units)	2 [1-3]	−0.18	0.454
Maximum aortic diameter (cm)	6.2 [5.9-7.0]	−0.23	0.197

Table 4. Correlation of cDPP3 with SOFA score and other biomarkers from all available measurements and time points.

Characteristics	n	r (Spearman)	P-value
SOFA score	89	0.67	<0.001
Leucocytes	162	0.08	0.325
Procalcitonin	75	0.68	<0.001
C-reactive protein	108	0.24	0.011
Interleukin 6	38	0.43	0.007
Serum creatinine	161	0.40	0.024

SOFA: Sequential Organ Failure Assessment

3. Predictive accuracy of cDPP3 for the development of postoperative organ dysfunctions and mortality

Early postoperatively, at 12 h after ICU admission, cDPP3 levels were significantly increased in patients who died or developed organ failure (P<0.001) during ICU stay. At this time point, elevated blood levels of cDPP3 demonstrated a highly significant predictive adequacy for the development of organ failure (12h-AUC 0.882 [P<0.001], 24h- AUC: 0.850 [P<0.001] 48h-AUC 0.846 [P<0.001]) and for the prediction of in-hospital mortality (12h-AUC: 0.907 [P<0.001], 24h-AUC: 0.815 [P=0.016], 48h-AUC: 0.914 [P=0.003]). All details can be found in figures 2 a, b and 3 a, b, as well as table 5.

Figure 2a

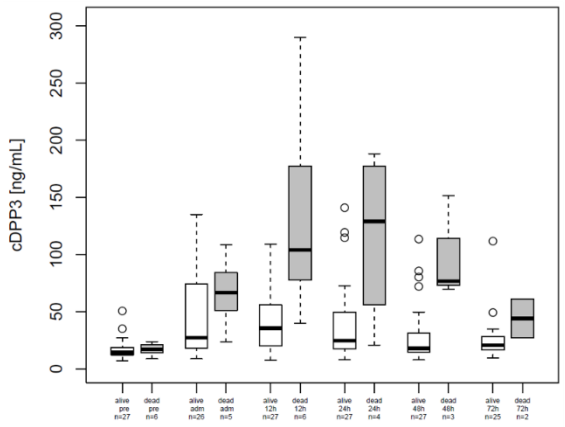

Figure 2b

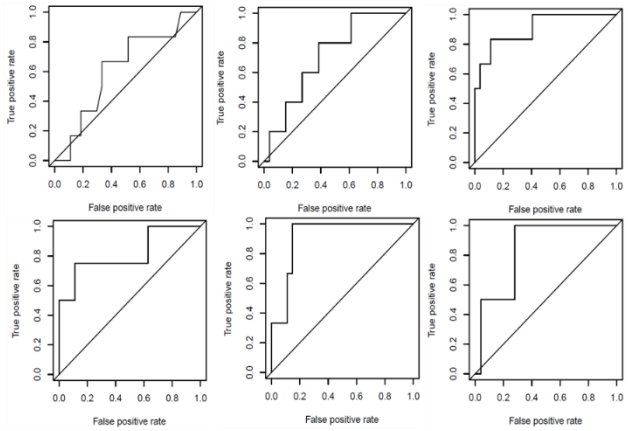

Figure 2: Course of circulating Dipeptidyl peptidase 3 in correlation with the endpoint "in-hospital mortality" for all patients (n=33). Figure 2a) Box Plot, Figure 2b) ROC Plots, endpoint "in-hospital mortality" (n=6 events).

Figure 3a

Figure 3b

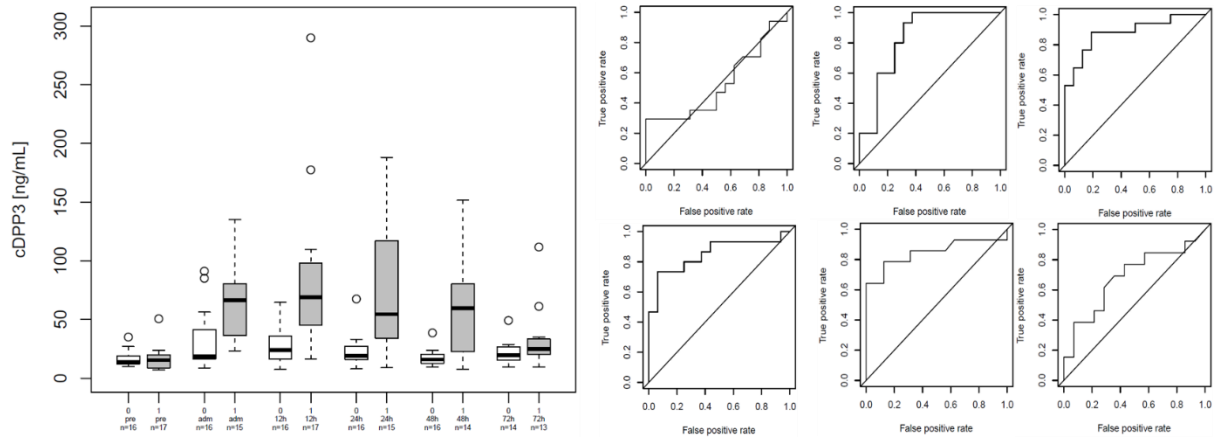

Figure 3: Course of circulating Dipeptidyl peptidase 3 in correlation with the endpoint "organ failure" for all patients (n=33, 0=no organ failure, 1=organ failure). Figure 3a) Box Plot, Figure 3b) ROC Plots, endpoint "organ failure" (n=17 events).

Table 5. ROC analysis for the test accuracy of DPP3 to predict the endpoints „in-hospital mortality" and „Organ Dysfunction". All in-hospital deaths also had organ dysfunction.

	In-hospital mortality			**Organ Failure**		
Time point	**AUC**	**P-value**	**N (events)**	**AUC**	**P-value**	**N (events)**
Baseline	0.611	0.682	33 (6)	0.522	0.620	33 (17)
Admission to ICU	0.708	0.105	31 (5)	0.833	<0.001	31 (15)
12h after admission to ICU	0.907	<0.001	33 (6)	0.882	<0.001	33 (17)
24 h after admission to ICU	0.815	0.016	31 (4)	0.850	<0.001	31 (15)
48 h after admission to ICU	0.914	0.003	30 (3)	0.846	<0.001	30 (14)
72 h after admission to ICU	0.840	0.148	27 (2)	0.681	0.098	27 (13)

AUC: area under the curve, ICU: intensive care unit, ROC: receiver operating characteristic

Discussion:

Patients undergoing open and endovascular TAAA repair frequently show a relevant rate of severe postoperative complications leading to organ failure and in-hospital mortality. Present findings confirmed that among these, AKI and pneumonia were the most frequent complications (21, 22). It is widely known that high organ failure incidence ultimately results in prolonged ICU stay. In this scenario, the use of biomarkers for risk assessment may have a positive impact in early patient treatment and potentially reduce patient severity and ICU hospitalization.

DPP3 is an intracellular amino dipeptidyl peptidase and, thereby, cleaves dipeptides from the N-terminus of bio-active substrates(6). DPP3 also activates the Keap1-Nrf2 antioxidant pathway and it has been shown to be overexpressed under oxidative stress in a model of severe heart failure in mice(8). In addition, Menale et al. showed in a mice DPP3-knockout model that lack of DPP3 results in sustained oxidative stress and impacts bone homeostasis(23).

The known substrates of DPP3 are extracellular peptides and include angiotensins, endorphins and enkephalins(6). Following this logic, intravenous injections of DPP3 induce myocardial depression, suggesting that the angiotensin metabolism might be affected by high DPP3 blood levels(8). Furthermore, high cDPP3 blood levels at admission in cardiogenic shock patients were associated with severe organ dysfunction, refractory shock and high short-term mortality, while reduction of cDPP3 levels within 24h of admission was associated with improved outcome (8, 24, 25).

The current hypothesis suggests that DPP3 is released into the bloodstream upon massive cell death, as it happens during invasive surgeries that are followed by systemic inflammation. However, the time course of cDPP3 levels and its clinical significance in postoperative outcomes, especially after TAAA repair, has not yet been addressed. In this respect, this is the first study assessing perioperative cDPP3 levels and its significance in patients' outcomes.

cDPP3 levels were characterized by a rapid postoperative increase, followed by a decrease until 72h after surgery. Notably, postoperative elevated cDPP3 levels were closely associated with poor outcomes, as assessed by the patients' SOFA score after surgery. The significant correlation between cDPP3 levels and procalcitonin and IL-6 further supports its relevance as a potential biomarker in this

setting, which is associated with the extent of surgery-related inflammation and severity of the underlying disease (26).

The present findings further demonstrate that elevated cDPP3 levels, measured early postoperatively after ICU admission, showed a remarkable predictive accuracy for the development of organ failure and ultimately in-hospital mortality of the included patients. The distinctive AUC values reported in this study underline the potential role of postoperatively assessed cDPP3 levels as a promising predictive biomarker for the detection of organ dysfunction in patients undergoing either open or endovascular TAAA repair, which are both associated with a relevant risk for severe complications (27).

The prediction of adverse outcomes after these extensive surgical procedures is difficult but of clinical relevance. The postoperative course after major surgical procedures, like TAAA, is characterized by an overwhelming release of markers of inflammation and associated with complications during the first 48h after surgery. It is, therefore, extremely challenging to identify adequate biomarkers in this time window. In this context, the use of biomarkers for risk assessment and outcome prediction, namely in-hospital mortality and organ failure, would be a desirable tool for an adequate initiation of treatment bundles leading to improvement in patients` outcome.

Until now, only few rather unspecific biomarkers for the detection of postoperative mortality have been investigated in the setting of TAAA surgery. In general, lactate and inflammation biomarkers, such as C-reactive protein and procalcitonin, may indicate adverse outcomes regardless if a bacterial infection exists or not. In the context of TAAA surgery, urinary neutrophil gelatinase associated lipocalin (NGAL) and bio-adrenomedullin (bio-ADM) have been described as potential biomarkers of adverse outcomes in small, monocentric studies (28). It is nevertheless important to highlight that specific biomarkers, which are able to predict adverse outcomes in the early postoperative phase after TAAA surgery, are absent so far. In general, the identification of new blood-based biomarkers might enable their integration into the postoperative surveillance, leading to an ameliorated diagnostic routine.

While comparing the findings of this study with trials focusing on critically ill patients, similar results are recognizable especially considering the boundary conditions of this study, namely the small cohort of patients and the non-randomized design (8, 24).

Relevant limitations of this prospective observational study should be acknowledged for proper interpretation of the results. Only a small number of patients was enrolled due to the overall low number of the yearly-performed TAAA procedures worldwide. Even if the results of our study are promising and the test quality is appropriate, the hypothesis-generating character of this study has to be emphasized. The category "any organ failure" is rather non-specific and does not enable specific measures to prevent organ failure, yet it could be used as a predictive surrogate parameter for patients' risk assessment and outcome. For validation, further clinical studies are required to verify the clinical relevance of cDPP3 as a biomarker for early detection of in-hospital mortality and organ failure after open and endovascular TAAA surgery.

Conclusion:

The present findings demonstrate for the first time the perioperative kinetics of the novel cDPP3 biomarker and highlights early postoperative cDPP3 levels to be predictive, with high adequacy, for the development of organ dysfunctions and in-hospital mortality after open and endovascular TAAA repair. If confirmed in other settings of major cardiovascular surgical procedures, the measurement of cDPP3 could improve current clinical risk prediction models and thus be a helpful decision-making tool for clinicians.

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Chapter 7

Urine neutrophil gelatinase– associated lipocalin predicts outcome and renal failure in open and endovascular thoracic abdominal aortic aneurysm surgery

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Urine neutrophil gelatinase–associated lipocalin (uNGAL) has been evaluated as an biomarker for AKI detection and adverse outcome in open and endovascular thoracoabdominal aortic aneurysm surgery context.

This observational, retrospective study included 52 patients. UNGAL was measured peri-operatively (48h) and correlated with AKI requiring dialysis, tracheotomy and adverse outcome.

Mean patients' age was 64.5 years. A total of 26.9% (*n*= 14) developed AKI, and 21.1% (*n*= 11) required dialysis, tracheotomy rate was 19.2% (*n*= 10) and in-hospital mortality rate was 7.6% (*n* = 4). uNGAL levels were related to AKI requiring dialysis at ICU (*p*= .0002), need for tracheotomy at baseline and admission on ICU (*p*= .0222, *p*= .0028, respectively), as well as adverse discharge modality (*p*= .0051, *p*= 0.0048, respectively). Diagnostic quality was good for uNGAL levels at admission to ICU regarding AKI requiring dialysis (sensitivity: 81.8% [48.2–97.7]; specificity:87.8% [73.8–95.9]; area under the curve (AUC):0.874 [0.752–0.949]). The diagnostic quality of uNGAL was favorable for the prediction of tracheotomy (sensitivity:70.0% [34.8–93.3]; specificity:83.3% [68.6–93.0]; AUC:0.807 [0.674–0.903] and adverse discharge (sensitivity:77.8% [40.0–97.2]; specificity:83.7% [69.3–93.2]; AUC:0.817 [0.685–0.910]).

uNGAL may be valuable as an post-operative predictor of AKI and adverse outcome after open and endovascular TAAA repair.

Introduction:

Open and endovascular repair of thoracoabdominal aortic aneurysms (TAAAs) is associated with a high risk of complications such as acute kidney injury[1,2]. Moreover, postoperative AKI is related to increased mortality rates after open, endovascular and emergency aortic aneurysm repair[3,4-6].

In daily clinical routine, AKI detection is based on urine volume and serum creatinine levels. With renal function limitation of more than 50%, increased serum creatinine is detectable, which may be associated with oliguria or polyuria, yet these levels are not specific for impaired renal function[2,7,8]. Early biomarkers for AKI detection in fields related to cardiovascular surgery are not clinically established, and a late diagnosis resulting in delayed treatment may be a consequence[9,10,11]. Hence, a biomarker facilitating early diagnosis of AKI after TAAA repair would be useful in facilitating early therapeutic intervention and guiding pharmaceutical treatment[12].

NGAL, a 25 kDa protein that binds covalently to neutrophil gelatinase, has been reported as a potential marker of angiogenesis and in particular as an early marker of AKI after cardiac and abdominal aortic surgery[13-15,16]. Levels of NGAL may be influenced by different factors such as systemic infection and inflammation[17]. Elevated levels have been identified after impairment of kidney function, and NGAL seems to have a protective effect on cells that may be related to its ability to scavenge iron and to induce cell growth[18],[19],[20]. In murine models, NGAL is the most rapidly induced protein of nephrotoxic and ischemic AKI and is detectable 3 h following an initial kidney injury[21],[22]. Furthermore NGAL has been described as biomarker of adverse outcome in different cardiovascular settings[23,24].

Here we investigated the intriguing potential of urine NGAL (uNGAL) as a marker of AKI and adverse outcome in the context of complex endovascular and open TAAA surgery.

Results:

Patient demographics

Fifty-two patients, of whom 25 % (*n* = 13) were women, were included between May 2014 and November 2015. Mean age was 64.5 ± 10.4 years (range, 43–85 years). Patients were treated for TAAA by open surgical 55.7% (*n* = 29) or endovascular 44.3% (*n* = 23) approach; 40.3% (*n* = 21) had type II TAAA, 4% (*n* = 2) type III, and 55.7% (*n* = 29) type IV (Table 1).

TABLE 1. Patient Characteristics According to favorable and adverse discharge

Mean + SD or median +range	All patients (N = 52)	favorable discharge (normal ward) (N=43 [82.7%])	Adverse discharge (weaning, death within 30 days after surgery) (N=9 [17.3%])	p-value (discharge modality comparison)
Patients characteristics and treatment				
Age	64.5 ± 10.4 (43;85)	64.14 ± 10.8 (43; 85)	66.22 ± 8.7 (52; 77)	P=0.5902
Gender (male)	39 (75.0)	32 (74.4)	7 (77.8)	P=1.0000 OR=0.83 (0.07; 5.36)
Open surgery	29 (55.8)	23 (53.5)	6 (66.7)	P=0.7124 OR=0.57 (0.08; 3.16)
Endovascular surgery	33 (44.2)	20 (46.5)	3 (33.3)	P=0.7124 OR = 1.74 (0.32; 12.03)
BMI	27.1 ± 3.9 (18.2; 37.5)	27.5 ± 4.0 (18.2; 37.5)	25.6 ± 3.5 (19.8; 32.1)	P=0.2006
Smoker	22 (42.3)	19 (44.2)	3 (33.3)	P=0.7167 OR=1.58 (0.29; 11.00)
Diabetes	6 (11.54)	6 (14.0)	0	P=0.5745 OR=Infty (0.32, Infty)
Chronic kidney disease	7 (13.5)	4 (9.3)	3 (33.3)	P=0.0900 OR=0.21 (0.03; 1.82)
Coronary heart disease	21 (40.4)	18 (41.9)	3 (33.3)	P=0.7236 OR=1.44 (0.26; 10.02)
Arterial hypertension	47 (90.4)	38 (88.4)	9 (100)	P=0.5726 OR=0.00 (0.00; 4.04)
Operation characteristics				
Operation time	401.3 ± 99.0 (195; 600) N=51	388.0 ± 95.9 (195;600)	472.5 ± 88.6 (330;600) N=8	P=0.0250*

Total ventilation time	980 (Q1:570; Q3: 1980) (Min:275; Max:53805) N=50	840 (Q1:525; Q3: 1410) (Min:275; Max:6660)	43320 (Q1:21615; Q3:48660) (Min:15675; Max: 53805) N=7	P=0.0001*
In- hospital stay	21 (Q1: 11; Q3:32) (Min: 6; Max: 119) N=51	18 (Q1: 10; Q3: 28) (Min: 6; Max: 45)	60 (Q1: 41.5; Q3: 68.5) (Min: 31; Max: 119)	P < 0.0001*
Stay on ICU	3 (Q1: 1; Q3:7) (Min:0; Max: 42) N=51	2 (Q1: 1; Q3: 5) (Min: 0; Max: 32)	21.5 (Q1: 18.5; Q3: 32.5) (Min: 7; Max: 42) N=8	P < 0.0001*
Complications and mortality				
AKI	14 (26.2 %)	7 (16.3)	7 (77.8)	P=0.0007* OR= 18.0 (2.5; 196.0)
AKI req. temporary Dialysis	11 (21.2%)	4 (9.3)	7 (77.8)	P < 0.0001* OR=0.029 (0.003; 0.245)
Pneumonia	10 (19.2)	1 (2.3)	9 (100)	P < 0.0001* OR=0.00 (0.00;0.03)
Tracheotomy	10 (19.2)	1 (2.3)	9 (100)	P < 0.0001* OR=0.00 (0.00;0.03)
Spinal cord ischemia	2 (3.8)	0	2 (22.2)	P=0.0271* OR=0.00 (0.00; 0.68)
Myocardial infarction	0	0	0	-
Sepsis	7 (13.4)	3 (6.9)	4 (44.4)	P=0.0125* OR=0.09 (0.01: 0.78)
Surgical revisions	6 (11.5)	3 (6.9)	3 (33.3)	P=0.5678 OR = 0.15 (0.02; 1.45)
In – hospital Mortality	4 (7.6)	3 (6.9)	1 (11.1)	P=0.5441 OR = 0.60 (0.04; 35.39)
Total mortality	5 (9.6)	3 (7.0)	2 (22.2)	P=0.2023 OR= 0.26 (0.03; 3.80)
Hereof pneumonia	2 (3.8)	2 (4.6)	0	P=1.0000 OR= Infty (0.06; Infty)
Hereof hemorraghic	1 (1.9)	0	1 (11.1)	P=0.1731 OR = 0.00 (0.00; 3.98)
Hereof small intestine ischemia	1 (1.9)	0	1 (11.1)	P=0.1731 OR = 0.00 (0.00; 3.98)
Hereof cerebral bleeding	1 (1.9)	1 (2.3)	0	P=1.0000 OR = Infty (0.01; Infty)

Examination of different patient characteristics separated by the discharge modality “favorable” and “adverse.” If data were missing, the sample size included is reported for the corresponding parameter. All variables are described as absolute frequencies n (%), mean ± SD or median (Q1, Q3), and ranges. ORs with 95% CIs are reported for dichotomous variables together with the *p* value.

Complications and mortality

A total of 26.9% (*n* = 14) of patients developed AKI, 21.1% (*n* = 11) required temporary dialysis treatment, and 5.7% (*n* = 3) needed permanent dialysis after discharge from the hospital. No occluded renal artery stents or bypasses could be observed. Of the overall group, 19.2% (10/52) developed pneumonia, 23% (*n* = 12) needed re-intubation, and 19.2% (*n* = 10) received a tracheotomy.

Of the 13.4% (*n* = 7) who developed sepsis, six cases were related to pneumonia and one case to small intestine ischemia following embolization during open type III repair. Two patients (3.8%) developed spinal cord ischemia, one after an endovascular type 2 TAAA repair and one patient after an open type 3 TAAA repair.

Six patients (11.5%) underwent surgical revisions: 5.7% (*n* = 3) because of access-related wound complications and 3.8% (*n* = 2) for hemothorax. One patient (2%) needed multiple revisions, including bowl resection, because of small intestine ischemia.

The in-hospital mortality rate was 7.6% (*n* = 4), and the total mortality rate during the follow-up (mean follow-up, 13.2 months (±5.3, [2–20 months]) was 9.6% (*n* = 5). Of the latter, there were two cases of pneumonic sepsis, one each of cerebral bleeding and small intestine ischemia associated with pancreas necrosis and peritonitis, and one thoracic aortic rupture at 19 post-operative weeks after type IV repair.

Correlation of uNGAL and biomarkers, clinical scoring systems, and outcome parameters

We observed an increasing correlation between uNGAL and serum creatinine over time. Furthermore, a significant correlation between uNGAL and the APACHE-II score was observed for all time points (ϱ = 0.457 admission to ICU; ϱ = 0.364 24 h after admission to ICU; ϱ = 0.439 48 h after admission to ICU). Additionally, a significant correlation for all time points of uNGAL and urine output could be assessed (ϱ = -0.320 admission to ICU; ϱ = -0.349 24 h after admission to ICU; ϱ = - 0.559 48 h after admission to ICU).

Looking at a correlation of non-repeated factors with uNGAL at ICU, a significant correlation was found for length of ICU stay ($\varrho = 0.390$; $p = .0046$), as well as for the duration of dialysis (uNGAL: $\varrho = 0.543$; $p < .0001$). The ventilation time and in-hospital stay showed low to moderate correlations with uNGAL but were not significant (uNGAL: ventilation, $\varrho = 0.272$; $p = .0557$; in-hospital stay: $\varrho = 0.265$; $p = .0598$) (Table 2).

TABLE 2. Correlation of uNGAL with different biomarkers and scoring systems

	Serum creatinine	Urine creatinine	Urea	Lactate	Urine excretion	APACHE II	AST	ALT
Baseline								
uNGAL	-0.065	-	-	-	-	-	-	-
Admission on ICU								
uNGAL	0.216 (N=50)	0.119 (N=24)	0.108 (N=50)	-	-0.320*	0.457* (N=48)	-0.004 (N=49)	-0.118 (N=48)
12h after ICU								
uNGAL	-	-0.333 (N=28)	-	-	-	-	-	-
24h after admission on ICU								
uNGAL	0.454*	-0.025 (N=22)	0.364*	0.413* (N=48)	-0.349* (N=51)	0.364* (N=50)	0.333*	0.301* (N=50)
48h after admission on ICU								
uNGAL	0.608* (N=43)	-0.277 (N=19)	0.443* (N=44)	0.254 (N=37)	-0.559* (N=38)	0.439* (N=35)	0.538* (N=39)	0.443* (N=39)

Correlation of uNGAL with different biomarkers using a Spearman's correlation at 5 time points. *: $p < .05$. If less than the 52 patients were included in the analysis, the corresponding sample size is given below the correlation value (AST: aspartate aminotransferase; ALT: alanine aminotransferase).

Correlation of uNGAL levels and AKI requiring dialysis

Starting with admission to ICU, uNGAL levels differed significantly among patients suffering from AKI requiring dialysis and those with non-impaired renal function. With the exception of baseline uNGAL levels, each time point showed a significant correlation. A ROC analysis for uNGAL with respect to AKI requiring dialysis treatment showed a good diagnostic quality

for the time points ‘baseline’ and ‘ICU’ (baseline: Se = 81.8% [48.2–97.7], Sp = 48.8% [32.9–64.9], AUC = 0.661 [0.516–0.786], respectively; ICU: Se = 81.8% [48.2–97.7], Sp = 87.8% [73.8–95.9], AUC = 0.874 [0.752–0.949]). An uNGAL cut-off of 10.43 ng/ml for the time point ‘admission to ICU’ had the best predictive power for the development of an AKI requiring dialysis, with an AUC of 0.874. Furthermore, Wilcoxon tests revealed a significant difference of uNGAL between patients with and without AKI requiring dialysis for all time points except baseline (details in Figure 1 and supplemental table 1–2).

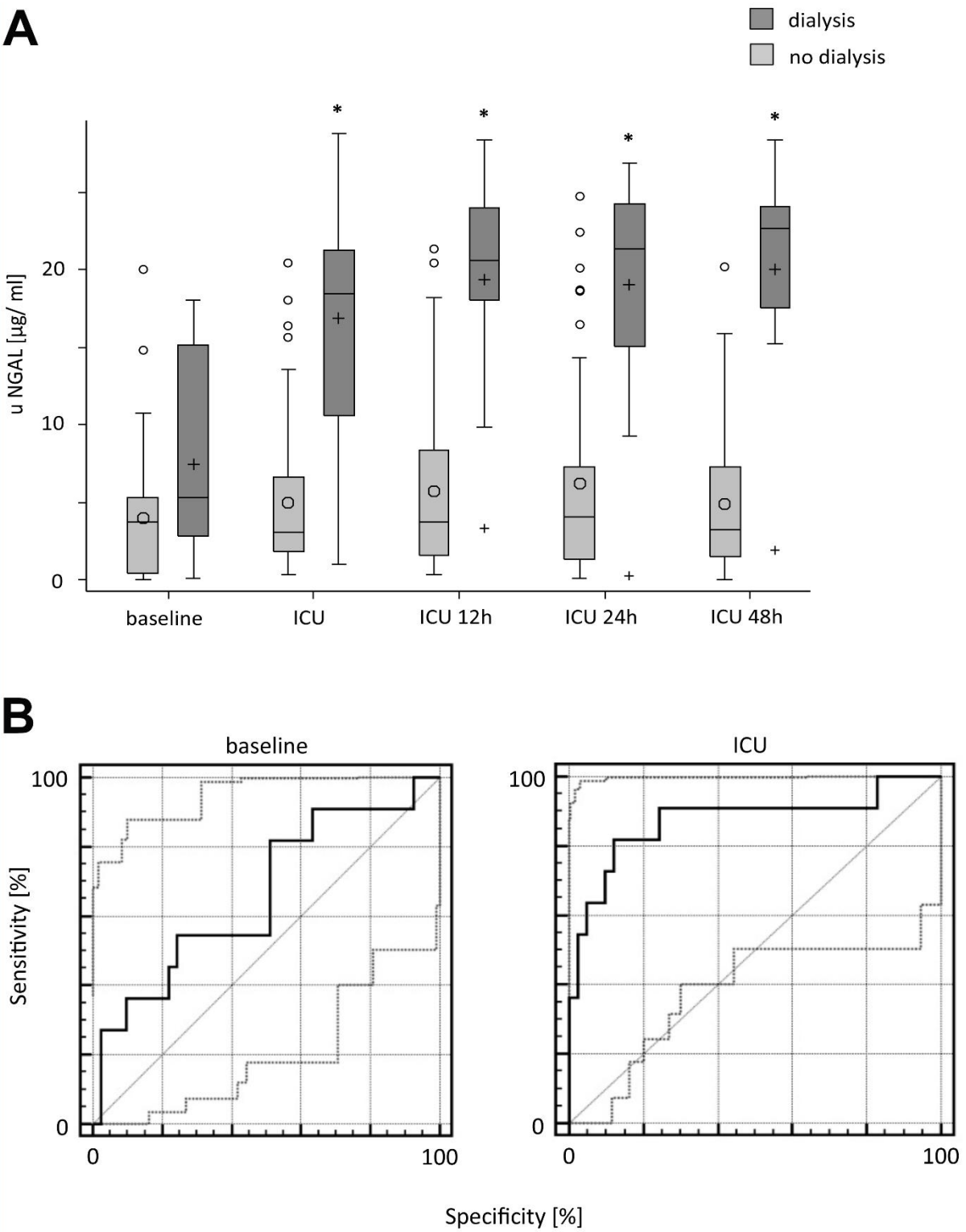


*Figure 1 Relationship between uNGAL and patients suffering from AKI requiring dialysis analyzed for every time point (mean ± SD respectively median [Q1, Q3] if data were skewed. Tests: t-test or Wilcoxon rank-sum test. * p < .05. Baseline comparison corresponds to patients in need of dialysis. ROC analysis for uNGAL with respect to AKI and the need for dialysis treatment. 95% CIs of ROC*

*curve indicated by dotted lines. For Se, Sp, and AUC, 95% CIs also are reported. *Good–moderate diagnostic quality:*
*LR+ > 3; LR- < 0.3. **: Excellent diagnostic quality: LR+ > 10; LR- > 0.1.*

Correlation of uNGAL-levels and need for a tracheotomy

Considering the need for a tracheotomy, uNGAL levels differed significantly between the tracheotomy and non-tracheotomy patients for each time point. A ROC analysis for uNGAL with respect to needing a tracheotomy showed a good diagnostic quality for the time points 'baseline' and 'ICU' (baseline: Se = 70.0% [34.8–93.3], Sp = 78.6% [63.2–89.7], AUC = 0.736 [0.595–0.848], respectively; ICU: Se = 70.0% [34.8–93.3], Sp = 83.3% [68.6–93.0], AUC = 0.807 [0.674–0.903]). Similar to AKI, uNGAL cut-offs of 5.27 ng/ml and 10.43 ng/ml for the time points 'baseline' and 'admission to ICU' had the best predictive power for the need for a tracheotomy. Additionally, when we compared uNGAL between patients with and without tracheotomy, we observed a significant difference at all time points, including at baseline (details in Figure 2 and supplemental table 3–4).

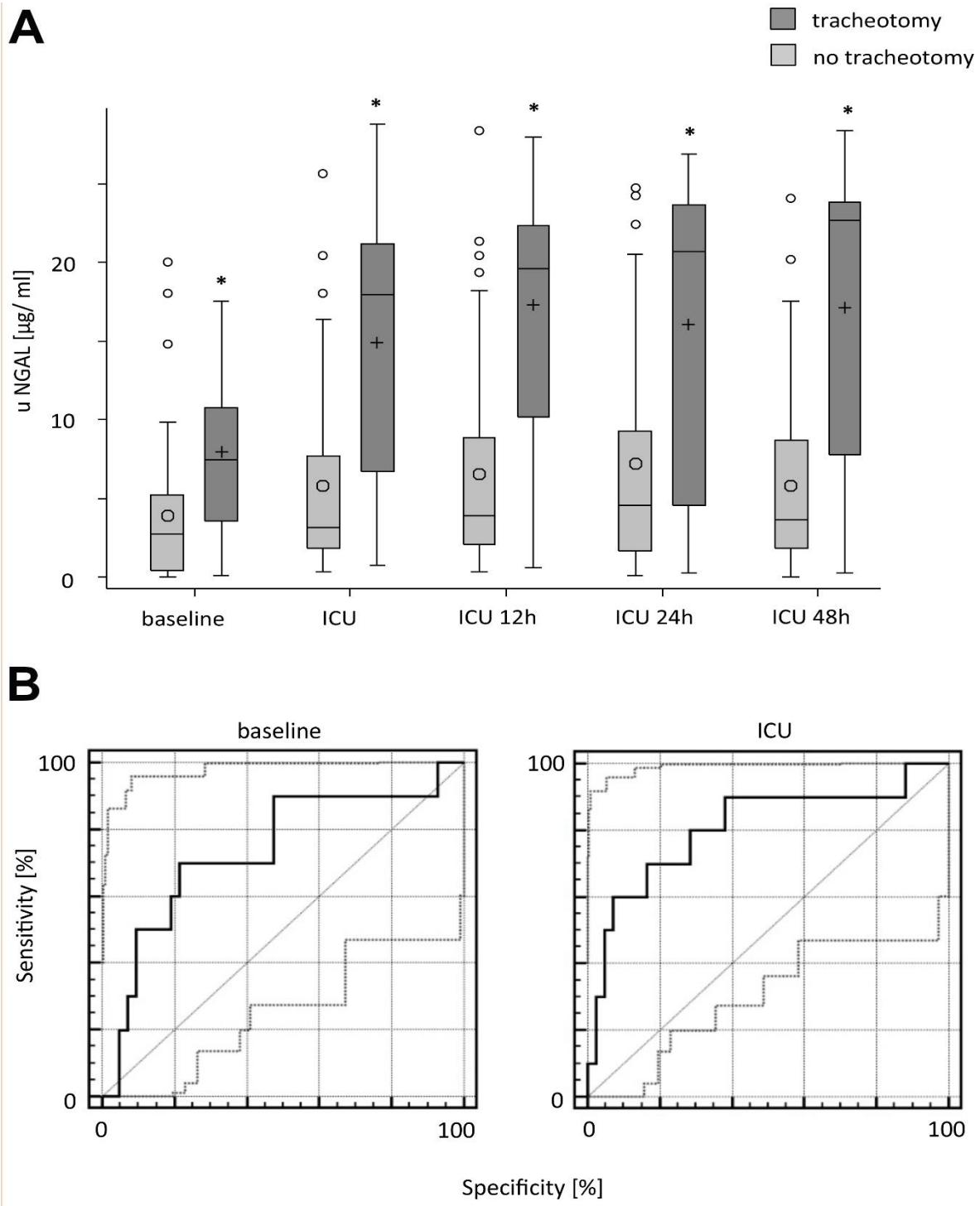


*Figure 2: Relationship between uNGAL and tracheotomy dialysis analyzed for every time point (mean ± SD respectively median [Q1, Q3] if data were skewed. Tests: t-tests or the Wilcoxon rank-sum test. * p < .05. Baseline comparison corresponds to patients in need of dialysis. ROC analysis for uNGAL with respect to AKI and the need for dialysis treatment. 95% CIs of ROC curve indicated by dotted lines. For Se, Sp, and AUC, 95% CIs also are reported. *Good–moderate diagnostic quality: LR+ > 3; LR- < 0.3. **: Excellent diagnostic quality: LR+ > 10; LR- > 0.1.*

Correlation of uNGAL levels and adverse discharge modality

ROC analysis revealed that uNGAL predicted an adverse discharge modality, namely discharge via weaning or death, for each time point. With regard to the uNGAL measurement after 48 h, a good diagnostic quality could be assessed (Se = 71.4% [29.0–96.3], Sp = 97.6% [87.1–99.9]). Overall, the preoperative uNGAL levels had good predictive quality with an AUC value of 0.814. At the baseline measurement, a cut-off of 5.27 ng/ml (LR+ = 3.72 > 3 and LR- = 0.28 < 0.3) showed the best predictive power for an adverse discharge modality. Similarly, the cut-off value of >10.43 ng/ml after admission to ICU showed almost identical diagnostic quality with an increased specificity of 83.7%. A specificity of 97.6% 48 h after admission to ICU could be observed, resulting in a positive likelihood ratio of 29.29 > 10. Investigating uNGAL at each time point, we identified a significant difference between patients with a favorable and an adverse discharge modality at all time points (details in Figure 3 and supplemental table 5–6).

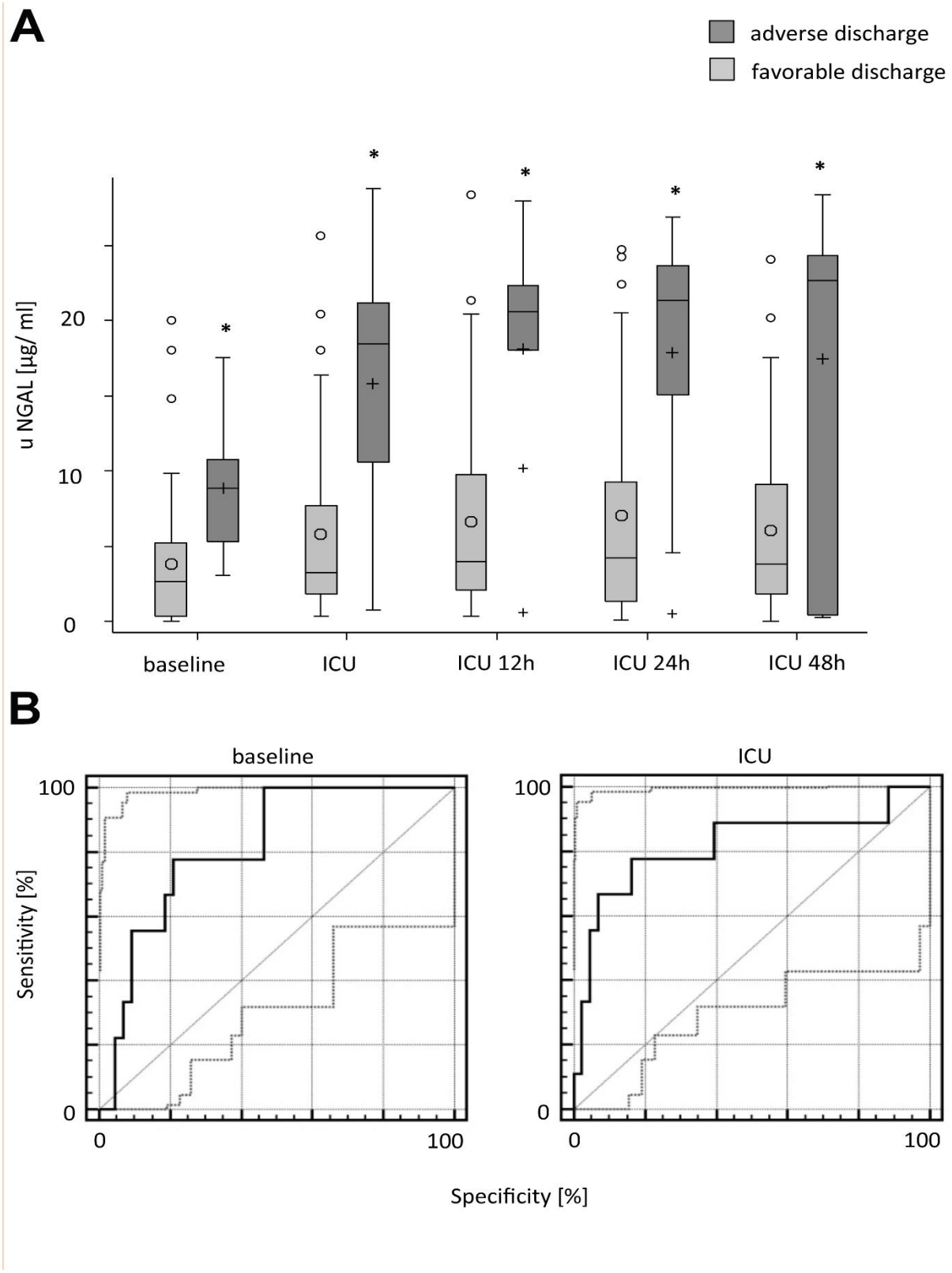


*Figure 3: Relationship between uNGAL and adverse discharge modality (weaning ward or death) analyzed for every time point (mean ± SD respectively median [Q1, Q3] if data were skewed. Tests: t-tests or Wilcoxon rank-sum test. * p < .05. Baseline comparison corresponds to patients in need of dialysis. ROC analysis for uNGAL with respect to AKI and the need for dialysis treatment. 95% CIs of ROC curve indicated by dotted lines. For Se, Sp, and AUC, 95% CIs also are reported. *Good–moderate diagnostic quality:*

*LR+ > 3; LR- < 0.3. **: Excellent diagnostic quality: LR+ > 10; LR- > 0.1.*

Coincidence of AKI requiring dialysis, tracheotomy, and discharge modality

The probability of an adverse discharge in dependence of a tracheotomy and AKI requiring dialysis was assessed to evaluate treatment dependencies in association with patient outcomes. We observed a complete or quasi-complete separation of the patients (p = .0242, p = .0012, Table 3), i.e., an adverse discharge modality was observed only if a tracheotomy was performed. Only one patient who had a tracheotomy had a favorable outcome, and this patient also had suffered from AKI requiring dialysis. Of those patients with adverse outcome, two died without requiring dialysis, and their uNGAL levels decreased within 48 h (details in Table 3 and supplemental table 7).

TABLE 3. Association between tracheotomy and discharge modality with regard to patients suffering from AKI with and without required dialysis.

Discharge modality depending on tracheotomy and AKI req. dialysis

		AKI req. dialysis and Tracheotomy			No AKI and Tracheotomy		
		Yes	**No**	**Total**	**Yes**	**No**	**Total**
discharge mortality	**favorable**	1	3	4	0	39	39
	adverse	7	0	7	2	0	2
	Total	8	3	11	2	39	41
p (Fisher Test)		**P=0.0242***			**P=0.0012***		

Data listed in each of the combination cell corresponds to absolute frequency. *: p < .05.

Repeated measures analysis of uNGAL

In a longitudinal analysis, a significant impact of the baseline value of uNGAL on the post-surgical measured levels of uNGAL within the first 48 h after admission to ICU (Table 4; base: p = .0011) was observed. Overall, uNGAL did not change significantly over time (base: p = .6935). The repeated factors urea, urine, and serum creatinine were also identified as

significantly related to uNGAL (Table 4; urea: $p = .0462$; urine: $p = .0044$; serum creatinine: $p = .0018$). Nevertheless, the slope estimate of urine was close to zero.

Analyzing patients and surgery characteristics in the single parameter model, sex influenced uNGAL levels significantly ($p = .0337$) while age did not show a significant effect ($p = .2754$). Judging from the estimates, the highest regression slopes were related to the sex (0.7529 ± 0.3445), chronic kidney disease (CKD) (0.5158 ± 0.4384), and serum creatinine levels (0.4636 ± 0.1451). Although the CKD impact was not significant, it had a large impact on data variation. Furthermore, the multivariable model confirmed a significant influence of baseline uNGAL value, serum creatinine, and sex on uNGAL (baseline uNGAL: $p = .0010$; serum creatinine: $p = .0153$; sex: $p = .0365$), but uNGAL levels still did not differ significantly over time (time: $p = .8628$). For the multivariable model, the largest slope estimate still corresponded to sex (0.7149 ± 0.3324) and serum creatinine (0.3548 ± 0.1440).

Looking at the estimate for sex, men showed increased uNGAL values in contrast to women. Nevertheless, discharge modalities did not differ significantly between women and men ($p = 1.0000$; Table 1).

TABLE 4. Repeated measures analysis of uNGAL in a single parameter and Multivariable analysis of the longitudinal model

Linear mixed model for log(uNGAL)					
Covariables	**Estimate**	**SEM (Estimate)**	**DF resp. Num DF/Den DF**	**t- resp. F-value**	**p-value**
Base model					
Intercept	0.9606	0.2228	71.5	4.31	<.0001*
Baseline (uNGAL)	0.1031	0.0297	50.1	3.47	0.0011*
Time Point (overall)			3/113	0.48	0.6935
ICU (Reference)	0	.	.	.	.
12h after ICU	0.1154	0.1487	127	0.78	0.4390
24h after ICU	-0.0152	0.1496	118	-0.10	0.9192
48h after ICU	-0.0608	0.1568	122	-0.39	0.6989
Single parameter analysis of the longitudinal model					
Continuous covariables					
Age	0.01583	0.01435	48.7	1.10	0.2754
BMI	-0.06411	0.03861	48.8	-1.66	0.1032
Operation time	0.00291	0.00149	47.6	1.95	0.0573
Total ventilation time	0.00002	0.00001	47.8	1.99	0.0525
Serum creatinine (repeated)	0.4636	0.1451	112	3.20	0.0018*
Urea	0.01383	0.00686	116	2.02	0.0462*
Urine	-0.00024	0.00008	114	-2.90	0.0044*
Categorial covariable					
Gender (Male)	0.7529	0.3445	48.9	-2.19	0.0337*
Diabetes	0.3283	0.4682	48.6	0.70	0.4865
Stroke	0.1274	0.4453	48.3	0.29	0.7761
Endovascular procedure	0.2969	0.2974	48.9	1.00	0.3230
Chronic kidney disease	0.5158	0.4384	49.3	1.18	0.2450
Arterial hypertension	0.1481	0.5105	48.7	0.29	0.7730
Multivariable analysis of longitudinal model					
Intercept	-0.0086	0.3750	60.6	-0.02	0.9819
Baseline (uNGAL)	0.1045	0.0300	52.5	3.48	0.0010*
Serum Creatinine	0.3548	0.1440	108	2.46	0.0153*
Gender (Male)	0.7149	0.3324	48.4	-2.15	0.0365*
Time Point (overall)			2/64.3	0.15	0.8628
ICU (Reference)	0	.	.	.	.
24h after ICU	-0.03958	0.1920	52	-0.21	0.8375
48h after ICU	-0.09956	0.1850	76.6	-0.54	0.5920

Target variable: Log-transformed uNGAL values from admission to ICU (reference category of time measurement). The base model was included in all analyses subsequently. Test parameters of the base model are reported only for the plain base model and in the multivariable case, not for the single parameter ('univariate') analysis. In the multivariable model, the time point 12 h after ICU could not be considered because serum creatinine was not measured at this time point. *: $p < .05$.

Discussion

Based on the results of this study, we could confirm that post-operatively measured uNGAL could be used as a postoperative biomarker for AKI requiring dialysis and as a predictive biomarker for needing tracheotomy and for adverse discharge modality (namely discharge from weaning ward or death) after open and endovascular TAAA repair. Furthermore, pre-interventional measured uNGAL levels correlated significantly with the need for tracheotomy and an adverse discharge modality. To our knowledge, no study to date has evaluated uNGAL as a biomarker for patient outcome and prolonged ventilation after cardiovascular surgery.

NGAL has been successfully used in different settings for the diagnosis and prognosis of AKI. Although several studies have investigated the value of NGAL to predict AKI in pediatrics, nephrology, and heart surgery, only limited evidence exists for its predictive abilities after TAAA surgery[25-27,28]. Chang et al. described the use of new biomarkers such as NGAL for AKI detection after endovascular aortic repair[29]. Kalimeris et al. emphasized the value of elevated NGAL levels as predictors of AKI after repair of abdominal aortic aneurysm[30]. These findings support our results on the predictive ability of uNGAL baseline measurement.

Comparing the value of serum NGAL (sNGAL) and uNGAL in the aortic aneurysm setting, Kokot et al. identified an increased predictive value for uNGAL compared with sNGAL regarding the probability of AKI[31]. In the present study, we observed a significant correlation of uNGAL with serum creatinine levels and a significant negative correlation with urine volume. These findings underline a direct relation of uNGAL and impaired renal function after TAAA repair. Of interest, two of the patients with elevated uNGAL levels who died post-operatively did not develop AKI. In both cases, uNGAL dropped within 48 h to a normal level. The pre-operative elevation of uNGAL in these patients may be a consequence of an assumed function of NGAL as a stress response protein, indicating a reduced general condition, which could influence postoperative outcomes[20]. Furthermore the course of

uNGAL in these cases may correlate with a reported decreased specificity of uNGAL as biomarker for AKI[32]. Only a study including a larger cohort of patients favorably in a multicentric setting could answer this question appropriately.

The function and meaning of NGAL as a stress response protein cannot clearly be discriminated in the setting of this study from its relevance as an early biomarker of AKI. In this context, Abella et al. described a regulatory role for NGAL in the innate immune response. NGAL is involved in a multitude of physiological and pathophysiological processes, such as apoptosis, infection and inflammation[33]. According to Kjeldsen et al, NGAL secretion by neutrophils, induced by tumor necrosis factor (TNF) and lipopolysaccharide (LPS), is activated by inflammation and infection[34]. Thus elevated baseline levels of NGAL as in the current study may be related to an altered immune status of the patients, which may be in turn related to adverse outcome [35]. Accordingly, the results regarding the predictive role of baseline uNGAL in this study may be consistent with the results of Lindberg et al., who described Plasma-NGAL as independent predictor of all-cause mortality and major adverse cardiovascular event in general population [36].

Nevertheless, while an increasing correlation between uNGAL and serum creatinine levels as well as urinary extraction could be assessed within the first 48 h after open and endovascular TAAA surgery, the initial assumption of uNGAL as a predictor of AKI still stands.

The multivariate analysis of uNGAL highlighted the influence of the baseline level of uNGAL and serum creatinine with post-surgical measurements of uNGAL, which strengthens the predictive power of uNGAL regarding postoperative AKI with the need for dialysis. With regard to the sex-specific differences of uNGAL, our results support the findings of Thrailkill et al., who identified significantly increased uNGAL levels in male patients with diabetes[37]. These results together may indicate a different applicability of uNGAL for men and women. Even if the outcome for men and women had not differed significantly in the present study,

this finding seems important regarding a potential implementation of uNGAL as a urinary biomarker.

uNGAL levels and adverse discharge correlated significantly, and every patient who died or was discharged via the weaning ward showed already elevated uNGAL levels at baseline. Furthermore, the trend increased after admission to ICU. In agreement, Siew et al. found uNGAL as a prognostic biomarker for outcome of patients suffering from AKI[38]. The observed correlation of elevated uNGAL levels with the APACHE II score also underscores its ability to predict patient outcome after complex aortic surgery, and an association of post-surgical AKI and the APACHE II score has been described before[39,40]. Post-operative AKI after complex aortic surgery is directly related to adverse outcome, so the consistent results regarding the predictive power of uNGAL seem conclusive[4,39].

Tracheotomy was conducted in this study if a prolonged artificial respiration was required. With regard to the correlation of elevated uNGAL levels and tracheotomy or adverse discharge, an almost complete concordance was found. Except for one patient who suffered from post-operative AKI and tracheotomy but was not discharged via the weaning ward, all patients who received a tracheotomy had an adverse discharge modality. In agreement, other authors also have described a correlation of tracheotomy with postoperative AKI and adverse outcome[41,42].

In terms of the elevated NGAL-levels and their correlation with patients' outcome, an early detection of elevated NGAL-levels by use of rapid testing kit may enable early medical and interventional treatment options such as dialysis therapy[43].

Certain limitations of the present study must be taken into account while evaluating the discussed results: A prospective, multicenter study including more homogenous patients treated by open or endovascular means would have improved the quality of the assessed data. Otherwise, TAAA is a rare disease, and few vascular surgery centers perform open and endovascular treatment regularly. A discrimination of the function of NGAL as a stress

response protein vs. its function as a kidney injury marker would be helpful. Currently, no panel of biomarkers associated with inflammation or kidney injury is available, which would enable a clear separation of uNGAL as a biomarker for inflammation or AKI in the described setting. An exclusion of patients with pre-existing chronic renal failure would be useful regarding the homogeneity of this patient cohort. Still, although no significant influence regarding pre-existing CKD could be observed, the presented findings emphasize the relevance of uNGAL as a postoperative biomarker of AKI and predictor of adverse outcomes after open and endovascular TAAA repair. Although our analysis was conducted in a hypothesis-generating manner, the results are concordant with what has been reported in the literature.

Methods

Conflict of Interest: The authors declare no conflict of interest.

Ethics approval and consent to participate: The local ethics committee approved this study (University Hospital Aachen EK004/14). This study was performed in accordance with the Declaration of Helsinki in its actual form. Written informed consent was obtained preoperatively from all subjects.

The datasets supporting the conclusions of this article are included within the article and its additional files.

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author upon request.

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Inclusion and exclusion criteria

Patients suffering from TAAA larger than 6 cm, defined according to the Crawford classification, were eligible for inclusion[44]. Exclusion criteria were age below 18 years, pregnancy, chronic kidney disease requiring permanent dialysis treatment. No emergency procedures were included.

Clinical and laboratory data collection

In this retrospective study, data on demographics, medical history, and admission diagnosis as well as daily physiological variables, surgical interventions, need for dialysis, and any kind of adverse event were collected from medical records and electronic bedside flow charts (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). During the surgical treatment, according to standardized and consistent operations, blood and urine samples were collected from patients at five predefined time intervals: pre-interventionally, at admission to the intensive care unit (ICU), and at 12 h, 24 h, and 48 h after admission to ICU. AKI was defined according to the Kidney Disease Improving Global

Outcomes (KDIGO) criteria[45]. In this study the serum creatinine criteria were used to define AKI, whereat urine volume was not applied for AKI determination. Baseline creatinine was the lowest pre-intervention value. AKI was defined by a reduction in kidney function with an increase in serum creatinine (>26.4 µmol/L) or percentage increase of serum creatinine above 50%, as recommended in current guidelines[46]. In this pilot study we focused on AKI requiring dialysis.

Biomarkers

Plasma and urine samples were incubated (3G and 4°C) for 10 min. The samples were stored at -20°C for less than 2 weeks and afterwards stored at -80°C until further processing by enzyme-linked immunosorbent assay (ELISA), performed according to the manufacturer's instructions (Human Lipocalin 2/NGAL ELISA BioVendor, Brno, Czech Republic). The ELISA kits were of the highest reagent grade and used without further purification. Laboratory investigations were blinded to the clinical status of the patients throughout the study. Following the recommendations of the manufacturer, the normal and pathological reference ranges were established based on the control sample in our laboratory. Based on the results of a previous study, we focused on the assessment of uNGAL.

Surgical protocol

Open surgery

The operative protocol for open TAAA repair has been published in detail before[47,48]. Briefly, it includes intubation with a double lumen endotracheal tube, cerebrospinal fluid drainage, perioperative monitoring of Motor Evoked potentials (MEP), sequential aortic clamping if possible, extracorporeal circulation with distal aortic perfusion, selective visceral perfusion and mild hypothermia of 32°C to 33°C[49]. Custodiol® (Dr. Franz Köhler Chemie, Austria) at 44°C was used for renal perfusion instead of blood perfusion. This approach had been

described to protect the kidneys from ischemic organ damage[50]. Thoracolaparotomy through the fifth to eighth intercostal spaces depending on the extent of the aneurysm was used for surgical access as well as a groin cut-down to the femoral vessels for placement of the extracorporeal circulation cannulas.

Endovascular surgery

The procedure was performed under general anesthesia, and the same protocol was used for the neurological monitoring. The detailed procedure of fenestrated endovascular aortic aneurysm repair has been described before[51]. In cases of endovascular procedure, renal perfusion was not interfered with directly. Radiation and iodinated contrast solution were used conservatively to reduce the toxic effects on the kidneys. Furthermore, a reduced contrast solution dose (one fourth of the standard dose) for the selective angiography of the renal arteries was used because it has been described as protective regarding acute kidney failure[52].

Endpoints

Levels of uNGAL and their correlation with AKI following open and endovascular aortic repair was the primary endpoint. Correlations of uNGAL levels and tracheotomy as well as adverse discharge modality were assessed as secondary endpoints as these dichotomous events are related to poor outcome. Furthermore, correlations with different parameters and scoring systems such as APACHE II and Lactate have been observed.

Tracheotomy was indicated in case of extended artificial ventilation. In our hospital, a tracheotomy is performed after a minimum 96 h of ventilation. Adverse discharge modality was defined as discharge via weaning ward or death. As no patient was discharged from ICU, this modality of discharge was not defined as adverse discharge modality.. All patients who survived the first 30 days were contacted between December 2015 and January 2016.

Statistical analyses

For description, all continuous variables are expressed as mean values ± standard deviation (SD) or 95% confidence intervals (CIs). For heavily skewed distributions, the median, the 0.25–0.50 quantile (Q1), and the 0.75 quantile (Q3) were used instead. Categorical variables are expressed as absolute frequencies and percentages. Correlations between continuous parameters were assessed by the Spearman correlation coefficient for each time point ("rho", ϱ). Some measurements in the data are missing completely at random; a systematic bias could not be detected.

The uNGAL level distribution was skewed, so exact Wilcoxon signed-rank tests were chosen to compare uNGAL for different factors between single time points. Comparisons between frequencies were conducted using a χ^2 test or Fisher's exact test in cases of small cell frequencies (≤5). For patient characteristics with respect to their discharge, exact odds ratios (ORs), corresponding 95% (penalized) likelihood Cis, and *p* values are given in Table 1.

Linear models with repeated measures were applied to evaluate the impact of certain metabolic factors on uNGAL. As a base model, we considered the fixed time effect (repeated factor), preoperative NGAL value (baseline value), and a random intercept. All further linear models are extensions of the base model. The response parameter uNGAL was log transformed to meet the model requirements. A Kenward–Rogers adjustment was used to account for the small sample size. For the covariance, an AR(1) structure was assumed. Model fit was evaluated with the help of residual plots.

Given the small sample size, we focused on analyzing the influence of single parameters (univariate analysis). Therefore, we considered the clinical factors of age and sex and all metabolic factors that showed interesting correlations or were considered relevant according to literature and experience, separately as additional covariables for the base model. Furthermore, we performed a multivariable analysis for uNGAL modeling in addition to the base model for the impact of sex, creatinine levels in the serum, and AKI/dialysis

simultaneously because they were the most promising predictors within the univariate analysis. Considering these parameters simultaneously improved the model according to the AIC (Akaike's information criterion) value. At first, tracheotomy also was considered, but because it worsened the model fit (AIC) and did not have a significant influence, we omitted it from the model. We report for all fixed-effect covariables the (slope) estimate, its standard error, degrees of freedom (DF), the value of the test statistic (t-value), and the *p* value. Because we considered more than two time points, the *p* value of the overall F-Test (type 3) is reported for the overall time effect together with the two DFs (Num DF/Den DF) and the value of the F-statistic (F-value). We assessed any effect in the statistical models as significant if the corresponding *p* value fell below the 5% margin. No alpha adjustment was carried out because this study was considered exploratory.

A receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic property of uNGAL with respect to each patient's direct discharge category (favorable/adverse), tracheotomy, and AKI requiring dialysis. Sensitivity (Se), specificity (Sp), likelihood ratios (LR ±), area under the curve (AUC), and the Youden-optimal cut-off (maximize Se+Sp-1) are reported together with the plotted ROC curves.

Boxplots were chosen to present the data distribution of uNGAL for selected patient characteristics over time. Spaghetti plots were used to illustrate the concrete course of uNGAL over time for patients with adverse direct discharge.

Statistical analysis was performed using SAS for Windows, Version 9.4 (SAS Institute, Cary, NC, USA), and "Proc Mixed" was used for the repeated measure analysis. ROC analysis was performed using MedCalc for Windows, version 12.7.7.0 (MedCalc Software, Ostend, Belgium).

Conclusion

uNGAL may be used as a postoperative biomarker of AKI requiring dialysis, tracheotomy and adverse discharge modality after open and endovascular TAAA repair.

Supplement

AKI req. dialysis			
	Yes (n=11)	**No (n=41)**	**p-value**
uNGAL			
Baseline*	5.31 (2.85; 15.11)	3.69 (0.43; 5.27)	0.1067
ICU	18.41 (10.62; 21.28)	3.05 (1.85; 6.65)	0.0002*
12h on ICU	20.55 (18.02; 24.01)	3.77 (1.58; 8.39)	< 0.0001*
24h on ICU	21.36 (15.08; 24.21)	4.10 (1.33; 7.29)	0.0003*
48h on ICU	22.67 (17.52; 24.07)	3.27 (1.52; 7.32)	0.0001*

Supplement 1: Relationship between uNGAL and patients with AKI and dialysis analyzed for every time point (median [Q1, Q3], since data was skewed). Tests: Wilcoxon ranked sum test. * p <0.05. Baseline comparison corresponds to patients in need of dialysis.

Time Point	**Youden**					**AUC**
	Optimal-Cut-Off	**Sensitivity [%]**	**Specificity [%]**	**LQ+**	**LQ-**	
Baseline	>2.69	81.8 [48.2-97.7]	48.8 [32.9-64.9]	3.72*	0.28*	0.661 [0.516-0.786]
ICU	>10.43	81.8 [48.2-97.7]	87.8 [73.8-95.9]	6.71*	0.21*	0.874 [0.752-0.949]

Supplement 2: For Se, Sp and AUC the 95% confidence intervals are reported additionally. *Good-moderate diagnostic quality: LQ+ >3; LQ- <0.3. **: Excellent diagnostic quality: LQ+ >10; LQ- >0.1.

Tracheotomy (at ICU)			
	Yes (n=10)	**No (n=42)**	**p-value**
uNGAL			
Baseline*	7.43 (3.60; 10.76)	**2.77 (0.43; 5.21)**	0.0222*
ICU	17.93 (6.74; 21.14)	**3.14 (1.85; 7.70)**	0.0028*
12h on ICU	19.62 (10.15; 22.31)	**3.86 (2.09; 8.84)**	0.0014*
24h on ICU	20.69 (4.55; 23.67)	**4.56 (1.63; 9.24)**	0.0399*
48h on ICU	22.66 (7.80; 23.82)	**3.66 (1.83; 8.69)**	0.0320*

Supplement 3: Correlation between uNGAL and tracheotomy analyzed for every time point (median [Q1, Q3], since data was skewed). Tests: Wilcoxon ranked sum test. * p <0.05. Baseline comparison corresponds to patients in need of tracheotomy.

Time Point	**Youden**					**AUC**
	Optimal-Cut-Off	**Sensitivity [%]**	**Specificity [%]**	**LQ+**	**LQ-**	
Baseline	>5.27	70.0 [34.8-93.3]	78.6 [63.2-89.7]	3.27*	0.38	0.736 [0.595-0.848]
ICU	>10.43	70.0 [34.8-93.3]	83.3 [68.6-93.0]	4.20*	0.36	0.807 [0.674-0.903]

Supplement 4: For Se, Sp and AUC the 95% confidence intervals are reported additionally. *Good-moderate diagnostic quality: LQ+ >3; LQ- <0.3. **: Excellent diagnostic quality: LQ+ >10; LQ- >0.1.

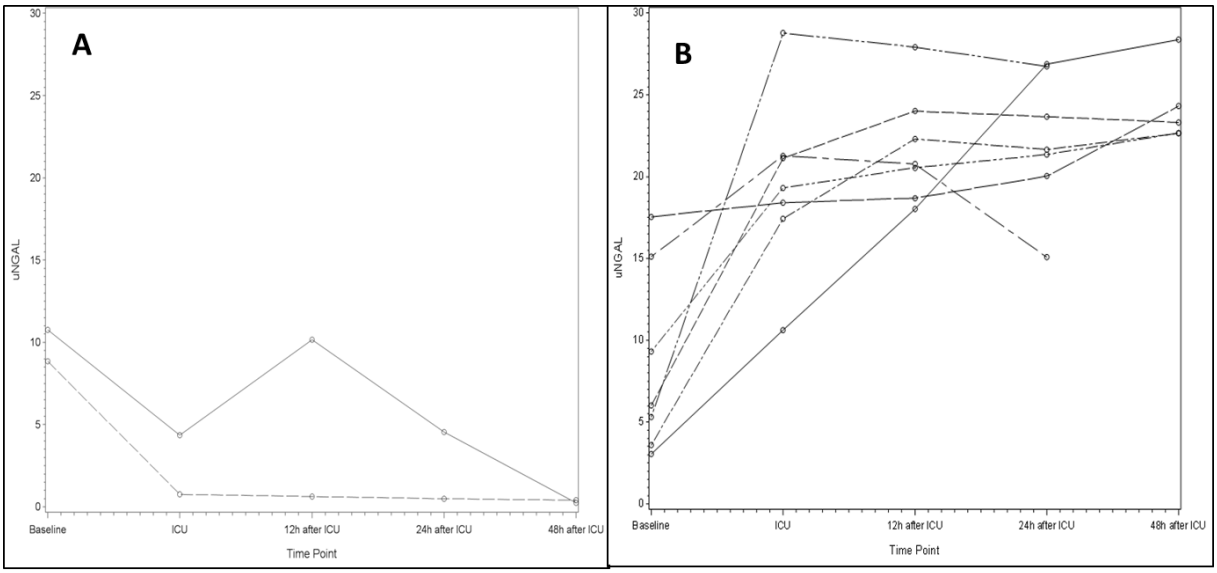
Direct discharge modalities			
	favorable discharge (normal ward) N=43	Adverse discharge (weaning, death) N=9	p-Value
uNGAL			
Baseline*	2.69 (0.38; 5.21)	8.84 (5.31;10.76)	0.0051*
ICU	3.23 (1.85; 7.70)	18.41 (10.62; 21.14)	0.0048*
12h on ICU	3.95 (2.09; 9.79)	20.55 (18.02; 22.31)	0.0030*
24h on ICU	4.27 (1.33; 9.24)	21.36 (15.08; 23.67)	0.0092*
48h on ICU	3.81 (1.86; 9.11)	22.67 (0.40; 24.31)	0.0720*

Supplement 5: uNGAL levels compared between discharge modalities analyzed for every time point (median [Q1, Q3], since data was skewed). Tests: Wilcoxon ranked sum test. * p <0.05.

Time Point	**Youden**					**AUC**
	Optimal-Cut-Off	**Sensitivity [%]**	**Specificity [%]**	**LQ+**	**LQ-**	

Baseline	>5.27	77.8 [40.0-97.2]	79.1 [64.0-90.0]	3.72*	0.28*	0.814 [0.682-0.908]
ICU	>10.43	77.8 [40.0-97.2]	83.7 [69.3-93.2]	4.18*	0.27*	0.817 [0.685-0.910]
12h after ICU	>9.81	88.9 [51.8-99.8]	79.1 [64.0-90.0]	4.25*	0.14*	0.835 [0.706-0.923]
24h after ICU	>14.29	77.8 [40.0-97.2]	81.4 [66.6-91.6]	4.18*	0.27*	0.791 [0.655-0.891]
48h after ICU	>20.19	71.4 [29.0-96.3]	97.6 [87.1-99.9]	29.29**	0.29*	0.721 [0.573-0.841]

Supplement 6: Roc-Analysis for uNGAL with respect to direct discharge. For Se, Sp and AUC with 95% confidence intervals are reported additionally. *Good-moderate diagnostic quality: LQ+ >3; LQ- <0.3. **: Excellent diagnostic quality: LQ+ >10; LQ- >0.



Supplement 7: Course of uNGAL in case of "adverse discharge" without AKI and the need for dialysis (A) and with AKI requiring dialysis.

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Chapter 8

The role of Ribonuclease 1 and Ribonuclease Inhibitor 1 in Acute Kidney Injury after Open and Endovascular Thoracoabdominal Aortic Aneurysm Repair

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Abstract: Acute kidney injury (AKI) is one of the most common post-operative complications and is closely associated with increased mortality after open and endovascular thoracoabdominal aortic aneurysm (TAAA) repair. Ribonuclease (RNase) 1 belongs to the group of antimicrobial peptides elevated in septic patients and indicates the prediction of two or more organ failures. The role of RNase 1 and its antagonist RNase inhibitor 1 (RNH1) after TAAA repair is unknown. In this study, we analyzed RNase 1 and RNH1 serum levels in patients undergoing open (n = 14) or endovascular (n = 19) TAAA repair. The role of RNase 1 and RNH1 in post-operative AKI and resulting in-hospital mortality was assessed. Increased RNH1 serum levels after open TAAA repair compared to endovascular TAAA repair immediately after surgery and 12, 48 and 72 h after surgery (all $p < 0.05$) were observed. Additionally, elevated RNase 1 and RNH1 serum levels 12, 24 and 48 h after surgery were shown to be significantly associated with AKI (all $p < 0.05$). RNH1 serum levels before and RNase 1 serum levels 12 h after TAAA repair were significantly correlated with in-hospital mortality (both $p < 0.05$). Based on these findings, RNase 1 and RNH1 may be therapeutically relevant and may represent biomarkers for post-operative AKI and in-hospital mortality.

Keywords: thoracoabdominal aortic aneurysm, ribonuclease, ribonuclease inhibitor 1, biomarker, complex aortic surgery, acute kidney injury

Introduction:

Thoracoabdominal aortic surgery is associated with several post-operative complications and increased mortality [1]. Mortality is 8.3% 30 days after open surgery and 5.8% after endovascular surgery [2]. Multiple organ failure is one of the dreaded post-operative complications after open and endovascular surgical treatment of thoracoabdominal aortic aneurysms (TAAA). Further, acute kidney injury (AKI) is one of the most common organ failures after TAAA repair with an incidence between 13 and 42%. In addition to cardiovascular morbidity, AKI is associated with increased mortality [3-5]. AKI is diagnosed according to the KDIGO criteria. Based on KDIGO, AKI is diagnosed when serum creatinine increases by ≥ 0.3 mg/dl (26.5 µmol/l) within 48 h or ≥ 1.5-fold or when there is a reduction in urine volume to < 0.5 ml/kg/h over 6 h. The three stages of AKI (I-III) are based on the aforementioned criteria: In addition to urinary excretion, diagnosis of AKI is based on patient serum creatinine levels. However, serum creatinine is a controversial biomarker for the detection of impaired renal function due to its delayed increases and low sensitivity [6-8]. Therefore, the establishment of new clinically available and reliable biomarkers and therapeutic approaches for the treatment of AKI is necessary [9].

Ribonuclease (RNase) 1 is a host defense peptide of the innate immune system. The primary function of RNase 1 is the degradation of circulating double and single stranded RNAs [10]. In a previous study, we observed increased RNase 1 serum levels in septic patients compared to healthy subjects. Furthermore, we demonstrated that RNase 1 serum concentrations indicate a prediction of dysfunction of two or more organs in septic patients [11]. RNase inhibitor 1 (RNH1) is an antagonist of RNase 1 that inhibits its activity by direct binding. In a recent study, we detected increased RNH1 serum levels in septic patients, as well as elevated extracellular RNA [12]. However, the role of RNase 1 and RNH1 as biomarkers of AKI and in-hospital mortality in the setting of TAAA repair has not yet been investigated.

The aim of this study was to evaluate the role of RNase 1 and RNH1 as potential biomarkers, as well as therapeutic strategies for the prediction of post-operative AKI and in-hospital mortality in patients undergoing complex open and endovascular TAAA repair.

Experimental Section:

2.1. Study approval and design

All serum samples were collected in 2017 between January and December after approval by the internal ethics committee of the University Hospital Aachen (EK004/14). Written informed consent was obtained preoperatively from all subjects. After screening and exclusion of patients who met the exclusion criteria, including emergency procedures, age below 18 years, pregnancy, chronic kidney disease requiring permanent dialysis treatment and ongoing immunosuppressive drugs, 33 patients were included in this study. Of these, 14 patients underwent open and 19 patients underwent endovascular TAAA repair. Demographic data, medical history and daily physiological variables were obtained from patient records and electronic flowcharts at the bedside (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). Based on serum creatinine levels and 24-hour urine output detection during the first 72 h after surgery, AKI was defined according to the KDIGO criteria [9].

2.2. Surgery

During open TAAA repair, different techniques were used to reduce intraoperative organ ischemia. Sequential aortic clamping, extracorporal circulation using distal aortic perfusion and selective visceral perfusion are established methods to reduce organ damage during surgery [13,14] [15]. Renal perfusion was achieved using 4°C tempered Custodiol® (Dr. Franz Köhler Chemie, Austria) [16]. In case of endovascular TAAA repair, a contrast agent was carefully applied to avoid kidney failure, leading to a mean application of 65 ± 17 ml per endovascular procedure [17].

2.3. Serum sampling

Serum samples were collected after patients were enrolled in the study at six different time points (before surgery, after admission to the intensive care unit (ICU) and 12, 24, 48, and 72 h after surgery). Serum samples were centrifuged at 3000 rpm for 10 min at room temperature after 10 min of coagulation. Samples were stored at -80°C until RNase 1 and RNH1 serum levels were measured.

2.4. Human RNH1 enzyme-linked immunosorbent assay

RNH1 serum levels were assessed using an ELISA designed by our research group. A 96-well plate was coated with 100 µl of diluted (2.5 µg/ml in PBS) capture antibody (#ABIN1342154; Abnova, Taipeh, Taiwan) and incubated at 4°C overnight. After washing with 0.05% Tween in PBS for 3 min three times, the plate was blocked in blocking buffer containing 5% fat free milk and 10% HS for two h at room temperature. A standard series was prepared in a range from 0.78 ng/ml to 100 ng/ml using a recombinant RNH1 Protein (#ABIN1318405; Abnova). After repeating the wash step, 100 µl of standard and samples were added to each well and incubated for 2 h at room temperature. Wells were subsequently aspirated and washed five

times, followed by addition of 100 µl of diluted detection antibody with a working concentration of 1 µg/ml to each well. Next, the wells were aspirated, washed and incubated for 1 hour in 100 µl of an HRP-conjugated goat anti-rabbit antibody (#31460; Thermo Fisher Scientific, Massachusetts, USA). The wash step was repeated, and the TMB substrate solution was added and incubated for 5 to 20 min at room temperature protected from light before the reaction was stopped with 2 N sulfuric acid. The optical density was determined at a wavelength of 450 nm using a plate reader (Tecan Group, Männedorf, Switzerland). For statistical analysis, GraphPad 7 (GraphPad Inc., California, USA) was used.

2.5. Human RNase 1 Enzyme-Linked Immunosorbent Assay

Levels of RNase 1 in human serum were determined using a commercial ELISA kit (#SEK13468; Sino Biological Inc., Peking, China) according to the manufacturer's instructions. For analysis, the optical density was measured at 450 nm using a microplate reader (Tecan).

2.6. Endpoints

To investigate the role of RNase 1 and RNH1 in AKI after TAAA repair, especially to analyze differences between open and endovascular TAAA repair, we examined preoperative renal function in relation to RNase 1 and RNH1 serum levels. To analyze only preoperative kidney function patients with preexisting kidney disease (defined as preoperative serum creatinine > 1.25 mg/dl according to the cut-off used in the Cleveland Clinic foundation score [18]) were excluded. Furthermore, we investigated the relationship between RNase 1 and RNH1 serum levels and in-hospital mortality in patients undergoing open and endovascular TAAA repair, as well as the differences in these two surgical techniques.

The association of serum levels of RNase 1 and RNH1 was also investigated with post-operative endpoints, such as ICU stay, sepsis and inflammatory markers CRP, PCT, and IL-6. Sepsis is defined as a life-threatening organ dysfunction that is identified by a 2 point increase in SOFA score (Sequential [Sepsis-related] Organ Failure Assessment) [19].

2.7. Statistical analysis

Continuous data are presented as medians with lower and upper quartiles (Q3-Q1). Categorical data are presented as absolute frequencies and percentages. RNase 1 and RNH1 serum levels are visualized over time in boxplots. To compare patient characteristics between open TAAA repair and endovascular TAAA repair, unpaired t-tests were used. For each point in time, a univariable logistic regression model was applied to assess the association between the outcome variables AKI (yes/no) and in-hospital mortality (died/survived) with RNase 1 and RNH1 serum levels.

The diagnostic quality of RNase 1 and RNH1 serum levels with respect to AKI and in-hospital mortality was evaluated for each point in time using the receiver operating characteristic (ROC) analysis. Sensitivity (Se), specificity (Sp), positive and negative likelihood ratio (LR+ and LR-), area under the curve (AUC), and the optimal cut-off value according to the Youden Index are given in addition to the ROC curves for each time point and the respective diagnostic variable.

A monotone correlation between RNase 1 and RNH1 and perioperative variables was evaluated using Spearman correlation coefficient. For all Spearman correlation coefficients statistically significant different from 0, the respective *p*-value is stated. The level of significance was set at 5%. No adjustments were made for multiple comparisons due to the exploratory nature of this study. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary NC, USA) and R, version 3.6.1. ROC analysis was performed using MedCalc, version 19.2.5.

Results:

3.1. Study population

The median age of patients undergoing endovascular TAAA repair (74 (78-69)) was significantly higher than those undergoing open TAAA repair (51 (65-37)) ($p < 0.001$; Table 1). There were eight male patients in the open TAAA group (57.1 %) and nine (47.4 %) in the endovascular group (Table 1). The median Body Mass Index (BMI) was 25.7 (30.6-20.6) in patients undergoing open vs 25.4 (27.1-21.5) in patients undergoing endovascular TAAA repair (Table 1). Ten patients undergoing open (71.4 %) and seven (36.8%) undergoing endovascular TAAA repair developed post-operative AKI as diagnosed according to the KDIGO classification criteria (Table 1).

Table 1. Patient characteristics

	open TAAA repair (n = 14)	endovascular TAAA repair (n = 19)	*p*-value
Age (year) (IQR)	51 (65-37)	74 (78-69)	< 0.001
Male sex (%)	8 (57.1)	9 (47.4)	0.593
BMI (kg/m^2) (IQR)	25.7 (30.6-20.6)	25.4 (27.1-21.5)	0.600
Diabetes mellitus (%)	2 (14.3)	4 (21.1)	0.685
Smoker (%)	4 (28.6)	8 (42.1)	0.440
Operation time (min) (IQR)	312.5 (474.5-295.5)	392.0 (460.0-280.0)	0.908
LOS ICU (days) (IQR)	5 (21-4)	3 (6-2)	0.075
LOS In-hospital (days) (IQR)	27 (38-19)	15 (35-9)	0.190
In-hospital mortality (%)	2 (14.3)	4 (21.1)	0.631
Acute kidney injury (%)	10 (71.4)	7 (36.8)	0.051

Data are presented as n (%) or median (IQR). Unpaired t-test (two-tailed) was used for statistical analysis. IQR: interquartile ranges (Q3-Q1); BMI: Body Mass Index; LOS: length of stay; ICU: intensive care unit

3.2. RNase 1 serum levels

We first investigated serum levels of RNase 1 in patients undergoing open or endovascular TAAA repair. RNase 1 concentrations decreased in patients undergoing open TAAA repair from 56.9 ± 44.4 ng/ml before surgery to 40.4 ± 17.5 ng/ml 24 h after surgery (Figure 1). Forty-eight hours after surgery, RNase 1 serum levels increased so that a concentration of 71.1 ± 45.9 ng/ml was reached 72 h after surgery (Figure 1). In patients undergoing endovascular TAAA repair, RNase 1 serum levels increased over time from 46.78 ± 26.7 ng/ml to 77.86 ± 37.2 ng/ml (Figure 1). When comparing the two groups, no significant difference was detected (Figure 1).

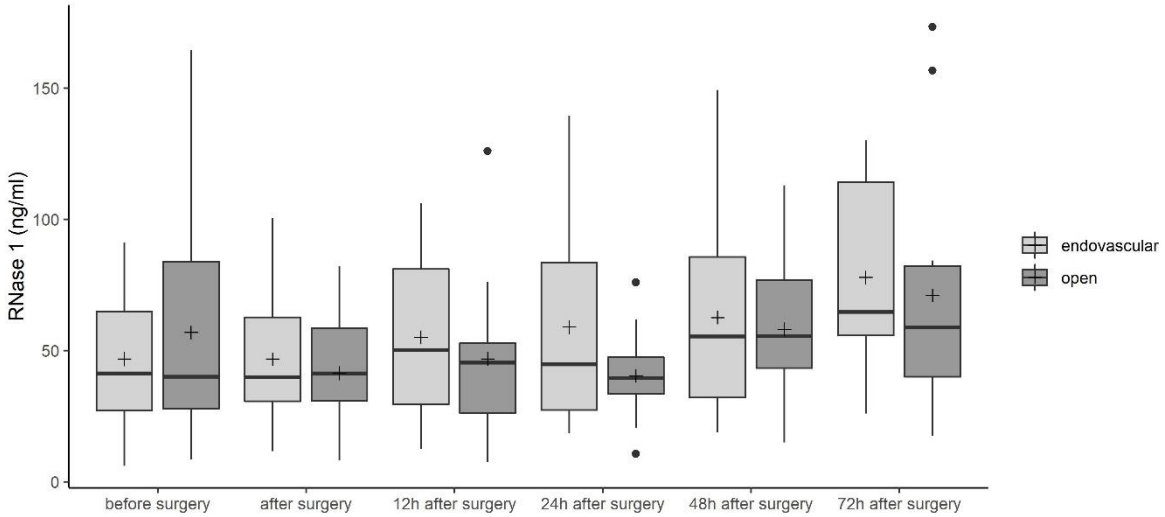

Figure 1. RNase 1 serum levels in patients undergoing open or endovascular TAAA repair. Data are presented as the median with lower and upper quartiles (Q3-Q1). Lines represent the median and pluses the mean. Unpaired t-test (two-tailed) was used for statistical analysis of the two groups. *$p < 0.05$; RNase 1: Ribonuclease 1.

3.3. RNH1 serum levels

As described before, RNH1 is an antagonist of RNase 1 and inhibits its enzymatic activity by direct binding. Therefore, we next investigated serum levels of RNH1 in patients undergoing TAAA repair. In contrast to RNase 1 serum concentrations, we detected increased RNH1 concentrations from 8.3 ± 6.9 ng/ml and 4.2 ± 4.5 ng/ml before open and endovascular TAAA repair to 18.9 ± 5.9 ng/ml and 11.5 ± 10.3 ng/ml, respectively, 12 h after surgery (Figure 2). Afterwards, RNH1 serum concentrations decreased to 13.0 ± 7.3 ng/ml and 5.5 ± 4.3 ng/ml 72 h after open and endovascular TAAA repair, respectively (Figure 2). Before surgery, no significant differences between the two groups were observed. After surgery and 12, 48 and

72 h after admission to the ICU, significantly increased RNH1 serum levels were observed after open TAAA repair compared to endovascular TAAA repair (all $p < 0.05$; Figure 2).

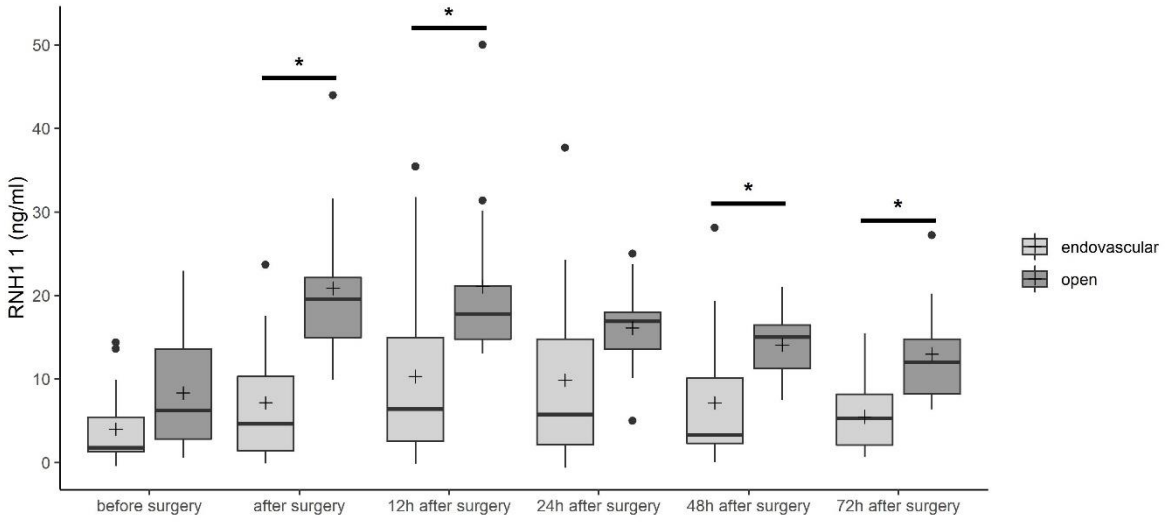

Figure 2. RNH1 serum levels in patients undergoing open or endovascular TAAA repair. Data are presented as the median with lower and upper quartiles (Q3-Q1). Lines represent the median and pluses the mean. Unpaired t-test (two-tailed) was used for statistical analysis of the two groups. *$p < 0.05$; RNH1: RNase inhibitor 1.

3.4. Correlation of RNase 1 and RNH1 with AKI

Analyzing the effect from RNase 1 and RNH1 serum levels on the probability of suffering AKI with a univariable logistic regression model for each point in time, RNase 1 showed a statistically significant effect 12 h after surgery ($p = 0.0327$, OR = 1.035) and 48 h after surgery ($p = 0.0144$, OR = 1.045; Figure 3). Higher RNH1 serum levels conveyed a statistically significant higher probability of experiencing AKI 12 h after surgery ($p = 0.0199$, OR = 1.129), 24 h after surgery ($p = 0.0435$, OR = 1.106), and 48 h after surgery ($p = 0.0194$, OR = 1.178; Figure 3).

Regarding a correlation between RNase 1 and AKI focusing on all patients suffering from post-operative AKI according to the KDIGO classification, a test accuracy of 0.702-0.750 was observed (Figure 3). From 24 h to 48 h after surgery, the sensitivity reached 93.33-100%, and the specificity was 56.25-62.50% (Figure 3). RNH1 showed good test accuracy for post-operative AKI with an AUC between 0.702 and 0.790 for all time points after surgery (Figure 3). Upon admission to the ICU, the test accuracy was 0.781, with a sensitivity of 85.71% and a specificity of 81.25%. Further details can be found in the supplementary materials Table S1 and S2.

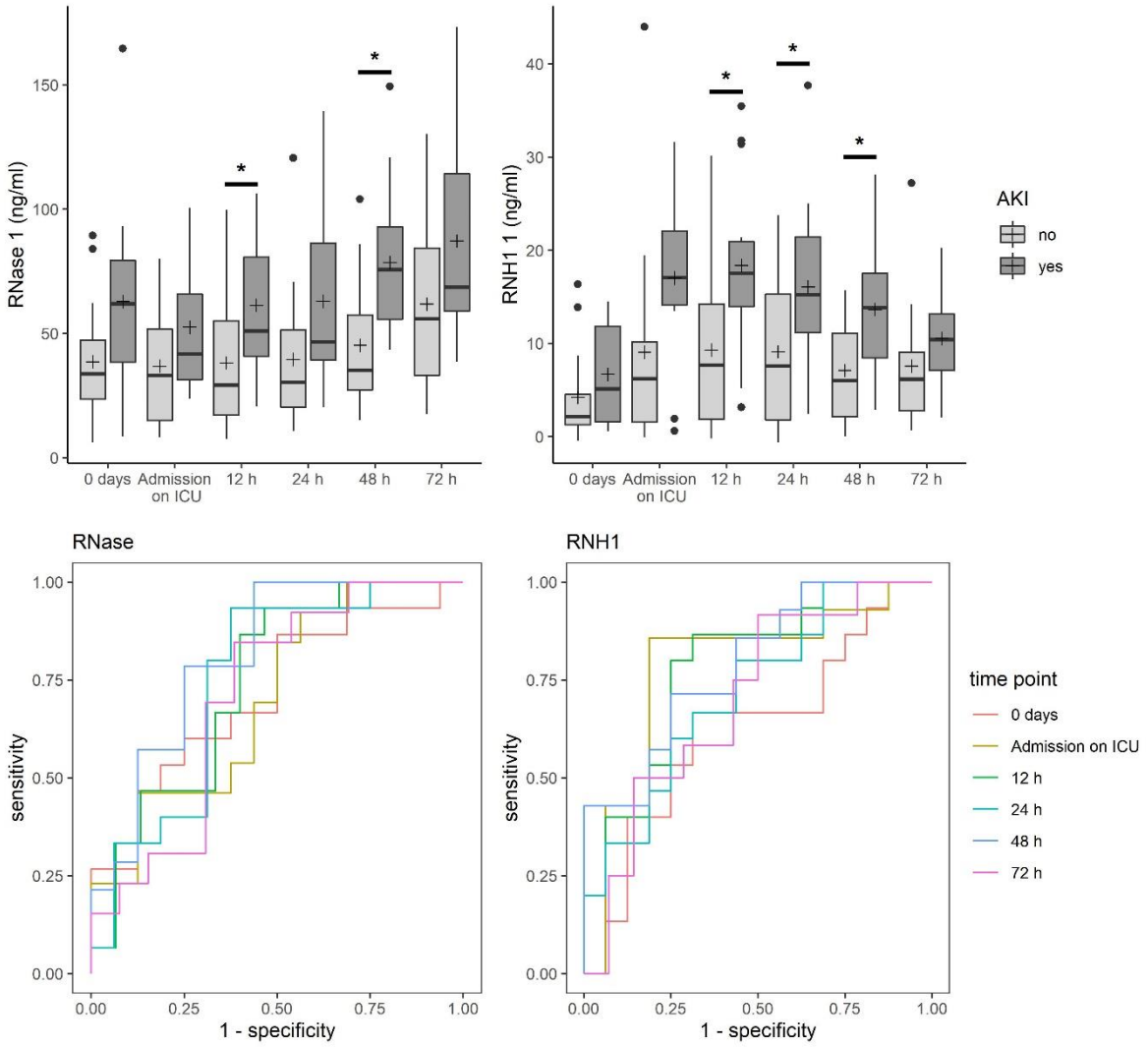

Figure 3. The correlation of RNase 1 and RNH1 with AKI. Data are presented as the median with lower and upper quartiles (Q3-Q1). Lines represent the median and pluses the mean. Unpaired t-test (two-tailed) was used for statistical analysis of the two groups. ROC analysis of the diagnostic accuracy of RNase 1 and RNH1 serum levels for acute kidney injury in patients undergoing endovascular or open TAAA repair. *$p < 0.05$; RNase: Ribonuclease; RNH1: RNase inhibitor 1.

While assessing the predictive abilities of RNase 1 and RNH1 for AKI separated in KDIGO 1 and 3, a favorable test accuracy for RNase 1 measured 48 h after TAAA surgery was observed (AUC: 0.969, sensitivity 100%, specificity 87.5%; Table 2).

Table 2. RNASE to predict AKI (Stadium = 0) vs. AKI (Stadium = 3).

Time of measurement	Optimal Cut-Off (Youden Index)					AUC
	Cut-Off [ng/ml]	Sensitivity [%]	Specificity [%]	LR+	LR-	
0 days	≥ 57.64	75.00 [19.4, 99.4]	81.25 [54.4, 96.0]	4.00	0.31	0.828 [0.595, 0.957]

Admission on ICU	≥ 31.00	100.00 [39.8, 100.0]	50.00 [24.7, 75.3]	2.00	-	0.672 [0.429, 0862]
12 h	≥ 61.42	75.00 [19.4, 99.4]	86.67 [59.5, 98.3]	5.63	0.29	0.817 [0.575, 0.954]
24 h	≥ 32.81	100.00 [39.8, 100.0]	62.50 [35.4, 84.8]	2.67	-	0.797 [0.560, 0.941]
48 h	≥ 67.81	100.00 [39.8, 100.0]	87.50 [61.7, 98.4]	8.00	-	0.969 [0.779, 1.000]
72 h	≥ 62.28	100.00 [39.8, 100.0]	69.23 [38.6, 90.9]	3.25	-	0.904 [0.663, 0.992]

3.4. Correlation of RNase 1 and RNH1 with in-hospital mortality

Analyzing the effect of RNase 1 and RNH1 serum levels on in-hospital mortality with a univariable logistic regression model for each point in time, RNase 1 showed a statistically significant effect 12 h after surgery (p = 0.018, OR = 1.048; Figure 4). For higher RNH1 serum levels, a statistically significant higher probability to die was observed at 0 days (p = 0.0414, OR = 1.174; Figure 4).

The test accuracy of RNase 1 to predict in-hospital mortality increased over time, reaching 0.938 72 h after surgery, with a sensitivity of 100% and a specificity of 91.67% (Figure 4). A moderate predictive accuracy for in-hospital mortality and RNH1 was also observed (Figure 4). All details can be found in Table S3 and S4.

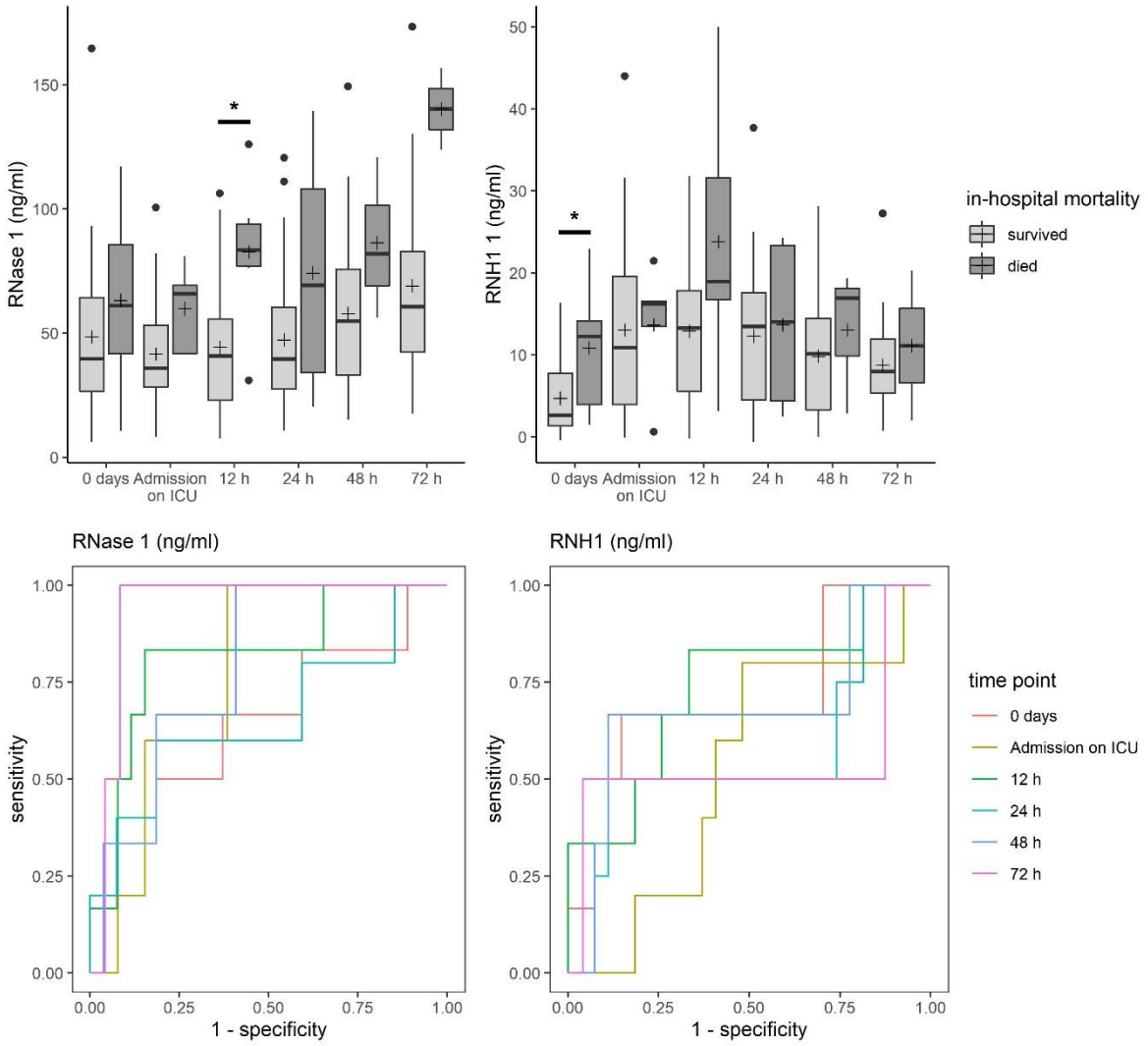

Figure 4. The correlation of RNase 1 and RNH1 with in-hospital mortality. Data are presented as the median with lower and upper quartiles (Q3-Q1). Lines represent the median and pluses the mean. Unpaired t-test (two-tailed) was used for statistical analysis of the two groups. ROC analysis of the diagnostic accuracy of RNase 1 and RNH1 serum levels for in-hospital mortality in patients undergoing endovascular or open TAAA repair. *$p < 0.05$; RNase: Ribonuclease; RNH1: RNase inhibitor 1**.**

3.5. Correlation of RNase 1 and RNH1 with perioperative variables

A significant correlation of RNase 1 and SOFA-Score was observed 48 h and 72 h after surgery ($p = 0.02$ and 0.03, respectively). Regarding the length of stay in the ICU, a significant correlation was observed for RNase 1 levels 48 h after surgery ($p = 0.01$). Significant correlations for several time points of RNH1 measurement and all assessed parameters were observed. Further correlations of RNase and RNH1 with variables are shown in Table 3 and 4.

Table 3. Spearman's correlation coefficient for RNase and perioperative variables. The number of included patients is shown below the coefficient. If the coefficient was statistically significantly different from 0, a *p*-value is stated below the number.

	Before surgery	After surgery	12 h after surgery	24 h after surgery	48 h after surgery	72 h after surgery
SOFA Score	x	0.20292 (n=6)	0.22965 (n=31)	0.25394 (n=29)	0.57451 (n=15) (p = 0.0251)	0.73193 (n=8) (p = 0.0390)
Leucocytes	-0.03862 (n=33)	0.14354 (n=30)	-0.07718 (n=32)	-0.02492 (n=28)	0.37221 (n=22)	0.40294 (n=16)
PCT	-0.86603 (n=3)	0.00000 (n=9)	0.20843 (n=20)	0.24527 (n=24)	0.33636 (n=11)	0.26190 (n=8)
CRP	0.22086 (n=32)	0.10000 (n=9)	0.00351 (n=19)	-0.01342 (n=18)	0.01072 (n=15)	0.20000 (n=15)
IL-6	. (n=1)	-0.08571 (n=6)	0.54545 (n=12)	0.00000 (n=12)	0.54286 (n=6)	. (n=1)
LOS ICU	0.04169 (n=33)	0.11124 (n=31)	0.05597 (n=32)	0.22260 (n=32)	0.46103 (n=30) (p = 0.0103)	0.36988 (n=26)

SOFA: Sequential [Sepsis-related] Organ Failure Assessment; PCT: Procalcitonin; CRP: C-reactive protein; IL: interleukin; LOS: length of stay; ICU: intensive care unit

Table 4. Spearman's correlation coefficient for RNH1 and perioperative variables. The number of included patients is shown below the coefficient. If the coefficient was statistically significantly different from 0, a *p*-value is stated below the number.

	Before surgery	After surgery	12 h after surgery	24 h after surgery	48 h after surgery	72 h after surgery
SOFA Score	x	-0.72471 (n=6)	0.31193 (n=32)	0.41576 (n=29) (p = 0.0249)	0.32676 (n=15)	-0.11119 (n=7)
Leukocytes	-	-0.15045	-	-0.36971	-0.04123	0.27857

	0.03996		0.32879			
	(n=33)	(n=31)	(n=33)	(n=28)	(n=22)	(n=15)
PCT	0.86603	0.69457	0.41986	0.52142	0.60000	0.60714
	(n=3)	(n=9)	(n=20)	(n=24)	(n=11)	(n=7)
		(*p* = 0.0379)		(*p* = 0.0090)		
CRP	-0.05668	-0.13333	-0.43333	0.23220	0.54334	0.43736
	(n=32)	(n=9)	(n=19)	(n=18)	(n=15)	(n=14)
					(*p* = 0.0363)	
IL-6	.	0.54286	0.23776	0.75524	0.08571	.
	(n=1)	(n=6)	(n=12)	(n=12)	(n=6)	(n=1)
				(*p* = 0.0045)		
LOS ICU	0.20422	0.18342	0.20371	0.21947	0.40419	0.44018
	(n=33)	(n=32)	(n=33)	(n=31)	(n=30)	(n=26)
					(*p* = 0.0267)	(*p* = 0.0244)

SOFA: Sequential [Sepsis-related] Organ Failure Assessment; PCT: Procalcitonin; CRP: C-reactive protein; IL: interleukin; LOS: length of stay; ICU: intensive care unit

Discussion:

Thoracoabdominal aortic surgery is associated with several post-operative complications and increased mortality [1]. Multiple organ failure is one of the dreaded post-operative complications after open and endovascular surgical treatment of thoracoabdominal aortic aneurysms (TAAA). AKI is one of the feared post-operative complications. Due to the absence of reliable biomarkers, like serum creatinine, the establishment of new clinically available and reliable biomarkers and therapeutic approaches for the treatment of AKI is essential. In this study, we investigated the role of RNase 1 and RNH1 in open and endovascular TAAA repair, especially their association with post-operative AKI. We showed for the first time that RNase 1 and its antagonist RNH1 play a role in open and endovascular TAAA repair and are associated with post-operative AKI and in-hospital mortality.

As a consequence of TAAA repair, open surgery leads to increased organ damage, while endovascular TAAA repair leads to post-implantation syndrome, both of which result in release of danger associated molecular patterns (DAMPs). Extracellular RNA (eRNA) represents one of these DAMPs. eRNA binds to both TLR 3 and 7, inducing increased

production of pro-inflammatory cytokines, such as tumor necrosis factor alpha, by translocation of nuclear factor kappa B [20,21]. This leads to an increased inflammatory reaction and results in organ dysfunction, such as AKI. Zhou and Yang describe the involvement of eRNA in kidney failure [22]. RNase 1 recognizes pathogenic RNA and degrades it [10]. Therefore, increased release of RNase 1 after TAAA repair is expected. Indeed, we detected increased RNase 1 in the serum of patients after TAAA repair up to 72 h after surgery (Figure 1). An antagonist of RNase 1, RNH1 protects the cytosolic compartments from the toxic effects of RNases, however, this also has the consequence of inhibiting their antimicrobial properties [23]. In previous studies by this group, we also detected increased RNH1 and RNase 1 levels in serum of septic patients compared to healthy subjects [11,12]. In line with these findings, we observed increased RNH1 serum levels after open and endovascular TAAA repair (Figure 2). After open TAAA repair, increased RNH1 serum levels were detected compared to serum levels after endovascular TAAA repair (Figure 2). However, for RNase 1 serum levels, there was no difference between open and endovascular TAAA repair (Figure 1).

Martin et al. reported that elevated RNase 1 serum levels in patients with sepsis indicates dysfunction of two or more organs [11]. In line with Martin et al., we demonstrated that patients with increased RNase 1 serum levels 12 and 48 h after surgery exhibited a higher probability of suffering AKI (Figure 3). Additionally, Martin et al. demonstrated that patients with renal dysfunction experience significantly higher RNase 1 levels compared to those without renal dysfunction [11]. Indeed, we also found that patients with higher RNase 1 serum levels 48 hours after surgery suffered a higher probability of stage 3 than stage 1 AKI (Table 2). Furthermore, we demonstrated that patients with higher RNH1 serum levels 12, 24 and 48 h after surgery also exhibited a higher probability of suffering AKI (Figure 3). Twelve hours after TAAA repair we observed that RNase 1 serum levels correlated with in-hospital mortality (Figure 4). This might be due to the fact that the highest mean RNH1 serum levels were measured 12 h after surgery (15.34 ± 11.29), and thus, RNase 1 is strongly inhibited by RNH1 at this point in time, preventing RNase 1 from degrading eRNA.

We next investigated the correlation of RNase 1 and RNH1 with perioperative variables. We found for the first time that RNase 1 serum levels were significantly correlated with 48 and 72 h time points after surgery, and RNH1 serum levels 24 h after surgery were correlated with SOFA-Score (Table 3 and 4). The length of stay in the ICU was significantly correlated with RNase 1 serum levels 48 h after surgery and with RNH1 serum levels 48 and 72 h after surgery (Table 3 and 4). Based on these findings, RNase 1 and RNH1 serum levels may have the potential to predict post-operative sepsis and thus, the length of hospital stay.

5. Conclusions

In conclusion, our data show for the first time that open TAAA repair results in significantly increased RNH1 serum levels compared to endovascular TAAA repair, after admission to the ICU and 12, 48 and 72 h after surgery. We found that RNase 1 serum levels 12 and 48 h after surgery and RNH1 serum levels 12 , 24 and 48 h after surgery showed a statistically significantly higher probability of suffering AKI. Furthermore, we demonstrated for the first time that RNH1 exhibits good test accuracy for post-operative AKI. In addition, higher RNase 1 serum levels 12 h after surgery and increased RNH1 serum levels on day 0 convey a significantly higher probability for in-hospital mortality. Based on these findings, RNase 1

and RNH1 may be therapeutically important and may represent biomarkers for post-operative AKI and in-hospital mortality.

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Chapter 9

SLPI - a Biomarker of Acute Kidney Injury after Open and Endovascular Thoracoabdominal Aortic Aneurysm (TAAA) Repair

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Contribution to the Field

Thoracoabdominal aortic aneurysm (TAAA) repair is related to a relevant rate of postoperative complications including acute kidney injury (AKI) with the highest incidence, which are closely associated with outcome. Up to now, post-operative AKI detection is mainly based on urine output and serum creatinine levels. Even if serum creatinine is an established biomarker, due to its delayed increase and low sensitivity for the detection of an impaired kidney function a critical assessment is required. Any optimization of the peri-operative and post-operative surveillance could lead to a better understanding of the complex inflammatory processes, which are activated by the required surgical trauma. Biomarkers, such as Secretory leucocyte peptidase inhibitor could enable an earlier detection of severe organ dysfunction, leading to a more appropriate and especially faster diagnosis and therapy. As indicated by our findings, a standardized usage of biomarkers for early detection of organ failure after major surgery such as TAAA repair may improve patients' outcome.

Abstract:
Acute kidney injury (AKI) is a relevant complication following thoracoabdominal aortic aneurysm repair (TAAA). Biomarkers, such as secretory leucocyte peptidase inhibitor (SLPI), may enable a more accurate diagnosis. In this study, we tested if SLPI measured in serum is an appropriate biomarker of AKI after TAAA. In a prospective observational single-center study including 33 patients (51.5 % women, mean age 63.0 ± 16.2 years) undergoing open and endovascular aortic aneurysm repair in 2017, SLPI was measured peri-operatively (until 72 h after surgery). After surgery, the postoperative complications AKI, as defined according to the KDIGO diagnostic criteria, sepsis, death, MACE (major cardiovascular events), pneumonia were assessed. In a subgroup analysis, patients with preexisting kidney disease were excluded. Of 33 patients, 51.5% (n = 17) of patients developed AKI. Twelve hours after admission to the intensive care unit (ICU), SLPI serum levels were significantly increased in patients who developed AKI. Multivariable logistic regression revealed a significant association between SLPI 12 hours after admission to ICU and AKI (P = 0.0181, OR = 1.055, 95% CI = 1.009-1.103). The sensitivity of SLPI for AKI prediction was 76.47% (95% CI=50.1-93.2) and the specificity was 87.5% (95% CI = 61.7-98.4) with an AUC = 0.838 (95% CI = 0.7-0.976) for an optimal cut-off 70.03 ng/ml 12 hours after surgery. In patients without pre-existing impaired renal function, an improved diagnostic quality of SLPI for AKI was observed (Sensitivities of 45.45-91.67%, Specificities of 77.7–100%, AUC = 0.716-0.932). There was no association between perioperative SLPI and the incidence of sepsis, death, MACE (major cardiovascular events), pneumonia. This study suggests that SLPI might be a post-operative biomarker of AKI after TAAA repair, with a superior diagnostic accuracy for patients without preexisting impaired renal function.

Introduction:

Open and endovascular repair of thoracoabdominal aortic aneurysm (TAAA) is related to a high risk of postoperative complications. With an incidence ranging between 13 and 42%, acute kidney injury (AKI) is one of the most common complications and closely associated with increased mortality and cardiovascular morbidity [1-3]. The early detection of impaired kidney function and other organ dysfunctions may enable an immediate start of specific treatment bundles. The diagnosis of AKI is mainly based on patients' urine output and serum creatinine levels. Serum creatinine is an established, yet controversial biomarker due to its delayed increase and low sensitivity for the detection of an impaired kidney function.[4-6] In this context, the necessity of clinically available early and reliable biomarkers of AKI becomes evident.

Secretory leucocyte peptidase inhibitor (SLPI) is a protease inhibitor and regulator of innate and adaptive immunity [7]. It is synthesized predominantly in immune and epithelial cells of mucosal surfaces, such as the pancreas and kidney [8]. Elevated serum SLPI levels have been observed in acute and chronic inflammatory conditions such as acute lung injury [9,10]. In the setting of oxidative stress, SLPI seems to have antioxidant and cytoprotective properties [11,12]. In a murine model of experimental ischemic AKI, Macrophage Migration Inhibitory Factor- 2 (MIF-2) was suggested to exert kidney protection by upregulation of SLPI expression [13].

In human kidney biopsies taken from patients with early post-transplant AKI after kidney transplantation, whole-genome mRNA profiling revealed a significant (15-fold) upregulation of *SLPI* mRNA expression compared to patients not affected by post-transplant AKI. Additionally, patients with post-transplant AKI showed significantly increased SLPI plasma and urine SLPI when compared with patients without AKI [14]. In a recent study, we found SLPI to be a candidate biomarker for the early diagnosis of AKI after cardiac surgery [15]. However, the performance of SLPI as a biomarker of AKI in the setting of TAAA repair has not yet been investigated.

The aim of this study was to evaluate the role of SLPI as a potential biomarker for the prediction of postoperative AKI in patients undergoing complex open and endovascular TAAA repair.

Methods:

Study design

The internal review board of the University Hospital Aachen (EK004/14) approved this study. We performed this study in accordance with the Declaration of Helsinki. Preoperatively, informed consent was obtained from all subjects.

If an elective open or endovascular TAAA repair, defined according to the Crawford classification was planned, Patients were eligible for inclusion was planned. TAAA was [16].

Patients undergoing TAAA repair between January and December 2017 were consecutively screened. After excluding patients, meeting the exclusion criteria emergency procedures, the following exclusion criteria have been applied: Chronic kidney disease with dialysis treatment, age below 18 years, pregnancy, , and immunosuppressive medication. 33 patients were included in this prospective study. Medical history and physiological parameters were taken from medical records and electronic bedside flow charts (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). Serum samples were collected before surgery, after admission to the intensive care unit (ICU), as well as during early follow up on ICU (12, 24, 48, and 72 hours).. AKI was defined according to the KDIGO criteria [17] based on serum creatinine levels and 24-hour urine output detection during the first 72 hours after surgery. Baseline creatinine was defined as the lowest pre-intervention value 24 hours before surgery.

SLPI measurement

Serum samples were collected one day before the TAAA repair, after admission to the ICU as well as 12, 24, 48 and 72 hours afterwards.These samples were centrifuged with 3000 rpm for ten minutes, afterwards supernatants were transferred to cryotubes and stored at -80°C. According to the manufacturer's advice (R&D systems, Minneapolis, MN) serum levels of SLPI were measured by ELISA. The average coefficient of variation (CV) between duplicates was 9.8% (intra-assay CV) and the average inter-assay coefficient was 13.4%.

Surgery

As published before, the protocol for open TAAA repair included aortic clamping, extracorporal circulation with distal aortic perfusion, and visceral perfusion using selective perfusion catheters [18,19] [20]. Renal perfusion was realized by using 4° C tempered Custodiol® (Dr. Franz Köhler Chemie, Austria) to avoid ischemic organ damage [21]. To avoid renal failure, contrast agent was used carefully, leading to a mean application of 65 ± 17 ml per endovascular procedure. Furthermore we applied one fourth of the standard dose for kidney angiography [22].

Endpoints

The assessment of the kinetics of serum SLPI and its applicability as a potential biomarker of AKI after TAAA repair was the motivation for this study. In a subgroup , patients with pre-operativee kidney failure (defined as preoperative serum creatinine > 1.25 mg/dl according to cut-off used in the Cleveland clinic foundation score [23] were excluded, to select those patients with physiological preoperative kidney function and reduce the heterogeneity of the cohort. As secondary endpoints, the association of serum SLPI with the following postoperative adverse events was analyzed: Sepsis, death, MACE (major cardiovascular events), pneumonia. Pneumonia and tracheotomy were defined according to the guidelines of the American Thoracic Society or the Belgian Society of Pneumology, respectively [24,25]. Spinal

cord ischemia was defined post-as operative paraplegia or paraparesis [20]. Major cardiovascular events (MACE) included myocardial infarction, acute heart failure and ventricular tachycardia; all defined according to current guidelines [26-28]. Sepsis was defined according to the guidelines of the German Sepsis Society [29]: Fever above 38 ° C or hypothermia below 36 ° C, tachycardia with a heart rate above 90 beats per minute, tachypnea with a respiratory rate above 20 per minute or a leukocytosis (≥ 12 000/mm³) or leucopenia (≤ 4 000/mm³). For patients and time points when clinical data were available, we additionally correlated serum SLPI with the inflammatory markers CRP, PCT, IL-6 and white blood cell count measured on ICU.

Statistics

The continuous variables are expressed as median with lower and upper quartile (Q1 - Q3) in case of heavily skewed data or as means ± standard deviation (SD) or. Categorical variables are shown as absolute frequencies and percentages. The time course of perioperative serum SLPI is visualized in boxplots. In a linear model with unstructured covariance structure to illustrate the correlation between repeated measurements within each patient,we tested for differences in SLPI between open and endovascular surgery.

The association between the occurrence of an AKI and other clinical outcomes (e.g. pneumonia) was assessed using Fisher's exact test. Firth's bias correction was used in an univariable logistic regression model to identify associations between baseline or operational characteristics and the development of an AKI. Associations between the development of an AKI (dependent variable) and serum SLPI were likewise assessed using a univariable logistic regression model with Firth's bias correction. The time point with the best association (SLPI 12 hours after ICU) was selected as an independent variable for a multivariable logistic regression analysis. The model further included the type of surgery and all patient characteristics from Table 1 that had a *P*-value of at most 0.2 in the univariable logistic regression model as independent variables. For AKI these were SLPI 12 hours after ICU, sex, the presence of a coronary heart disease, hypertension, and the type of surgery.

The diagnostic quality of SLPI for predicting AKI was assessed using receiver operating characteristic curves (ROC curves). Sensitivity (Se), specificity (Sp), positive and negative likelihood ratio (LR+ and LR-), area under the curve (AUC) and the optimal cut-off value according to the Youden index are reported together with the ROC curves. Additional analyses were performed in the subgroup of patients without pre-existing impaired renal function.

The association between SLPI and other outcomes (sepsis, death, MACE, pneumonia) is shown in boxplots in the supplement. Associations were tested using a logistic regression model with the outcome as dependent variable and using Firth's bias correction. The level of significance was set at 5%. No adjustments were made for multiple comparisons due to the exploratory nature of this study. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary NC, USA) and R, version 3.5.1[30].

Table 1. Patient characteristics in the entire collective and by AKI.

Continuous data is reported as mean ± SD, categorical data as absolute and relative frequencies. [a] Compared using a logistic regression model with Firth's bias correction.

		Acute kidney injury (AKI)		
Characteristic	All patients (N = 33)	No (N = 16)	Yes (N = 17)	p-value [a]
Demographics				
Age, years	63.0 ± 16.2	65.4 ± 15.1	60.8 ± 17.3	0.4724
Sex (male)	16 (48.48%)	10 (62.50%)	6 (35.29%)	0.1392
BMI, kg/m^2	25.4 ± 5.0	25.9 ± 5.4	24.9 ± 4.8	0.6156
Smoker	12 (36.36%)	6 (37.50%)	6 (35.29%)	0.7638
Comorbidities				
Chronic kidney disease	5 (15.15%)	3 (18.75%)	2 (11.76%)	0.8403
Coronary heart disease	14 (42.42%)	9 (56.25%)	5 (29.41%)	0.1471
Diabetes mellitus	6 (18.18%)	2 (12.50%)	4 (23.53%)	0.4807
Hypertension	23 (69.7%)	14 (87.50%)	9 (52.94%)	0.0575
COPD	13 (39.39%)	8 (50%)	5 (29.41%)	0.2593
Connective tissue disease (Marfan syndrome)	5 (15.15%)	1 (6.25%)	4 (23.53%)	0.2609
pAVK	4 (12.12%)	2 (12.50%)	2 (11.76%)	0.9503
Maximum aortic diameter, cm	6.6 ± 1.3	6.5 ± 1.4	6.7 ± 1.1	0.6691
Marker at baseline				
Hemoglobin, g/dL	12.9 ± 1.9	12.9 ± 2.3	12.8 ± 1.5	0.9156
Serum creatinine, mg/dL	1.1 ± 0.4	1.2 ± 0.4	1.0 ± 0.3	0.2413
Type of TAAA				
TAAA 1	5 (15.15%)	3 (18.75%)	2 (11.76%)	
TAAA 2	7 (21.21%)	2 (12.50%)	4 (29.41%)	
TAAA 3	7 (21.21%)	1 (6.25%)	6 (35.29%)	0.3272
TAAA 4	10 (30.3%)	7 (43.75%)	3 (17.65%)	
TAAA 5	4 (12.12%)	3 (18.75%)	1 (5.88%)	

Table 2. Operational characteristics in the entire collective and by AKI.

Continuous data is reported as mean ± SD or median (Q1-Q3) in case of heavily skewed data, categorical data as absolute and relative frequencies. [a] Compared using a logistic regression model with Firth's bias correction where skewed characteristics were logarithmically transformed.

		Acute kidney injury (AKI)		
Characteristic	All patients (N = 33)	No (N = 16)	Yes (N = 17)	p-value [a]
Surgery				
Endovascular surgery	19 (57.6%)	12 (75%)	7 (41.18%)	0.0707
Open surgery	14 (42.4%)	4 (25%)	10 (58.82%)	
Operation time, min	374.3 ± 111	329.7 ± 101.3	416.3 ± 105.7	0.0466
ICU ventilation time, min	835 (300 – 1571)	350 (0 – 817.5)	1149 (965 – 2147)	0.1381
Total ventilation time, min	1410 (960 – 2505)	1020 (582.5 – 1410)	1940 (1410 – 4865)	0.0491
Stay on ICU, days	4 (3 – 5)	3 (1.5 – 5)	5 (4 – 9)	0.0595
In-hospital stay, days	26 (11 – 35)	20.5 (10 – 33)	28 (19 – 38)	0.3621
Blood transfusion (blood bags)	8 (4 – 15)	5 (2 – 7)	13 (9 – 27)	0.1290

Results:

The mean patients' age was 63 ± 1.26 years, 51.5% were women. Demographical and baseline information as well as procedural details can be found in tables 1 and 2. Seventeen patients (51.5%) developed postoperative AKI as diagnosed according to the KDIGO classification criteria. From these seventeen patients, ten (58.8 %) were classified as KDIGO 1, two (11.7 %) as KDIGO 2 and five patients (29.4%) as KDIGO 3. All patients with AKI fulfilled the diagnostic criteria of a rise in serum creatinine, but only six patients showed a significantly reduced urine output (KDIGO 1: N = 1, KDIGO 2: N = 1, KDIGO 3: N = 4). All details can be found in table 3.

Patients suffering from AKI had an increased risk of pneumonia (29.41% vs. 6.25%), sepsis (29.41% vs. 6.25%), and in-hospital mortality (29.41% vs. 6.25%).

Table 3. Prevalence of outcomes in the entire collective and by AKI.

		Acute kidney injury (AKI)		
	All patients	No	Yes	p-value [a]
Outcome	(N = 33)	(N = 16)	(N = 17)	
Pneumonia	6 (18.18%)	1 (6.25%)	5 (29.41%)	0.1748
Tracheotomy	4 (12.12%)	1 (6.25%)	3 (17.65%)	0.6012
Spinal cord ischemia	3 (9.09%)	2 (12.50%)	1 (5.88%)	0.6012
Major cardiovascular events		3 (18.75%)	7 (41.18%)	0.2587
(MACE)	10 (30.30%)			
Sepsis	6 (18.18%)	1 (6.25%)	5 (29.41%)	0.1748
In-hospital mortality	6 (18.18%)	1 (6.25%)	5 (29.41%)	0.1748

Data is reported as absolute and relative frequencies. [a] The association between AKI and other outcomes was assessed using Fisher's exact test.

Association of serum SLPI with AKI and postoperative adverse events

SLPI serum levels showed a biphasic course with a significant decline from the day before surgery to admission to ICU after surgery (57 vs. 32 ng/ml, $P = 0.0002$) and a significant increase during the first 12 hours (Table 4). Serum SLPI remained high until 48 hours and reached baseline values at 72 hours after admission to ICU. No significant differences in serum SLPI were observed between patients undergoing open or endovascular TAAA repair (linear mixed model, $P = 0.7691$, figure 1).

Twelve hours after admission to ICU, patients who developed AKI displayed significantly higher serum SLPI (AKI: $P = 0.0058$) (Table 4, figure 2). In the subgroup of patients without pre-existing renal function impairment (preoperative creatinine $\leq$1.25 mg/dl), significantly increased serum SLPI was observed 12 and 24 hours post-interventionally in patients with AKI (Figure 3). Besides, serum SLPI 12 hours after surgery was negatively correlated with urine output during the first 24 hours after surgery (Spearman coefficient = -0.48 and 95% CI = -0.71 - -0.16). Serum SLPI did not differ between patients affected by sepsis, MACE, death, or pneumonia compared to patients not affected by these adverse events (Figure S1-4). Serum SLPI was significantly correlated with procalcitonin 24 and 72 hours after surgery, but did not show a significant correlation with CRP, IL-6 and white blood cells at any time point analyzed (PCT 24 h: $P = 0.018$, $R^2 = 0.288$; PCT 72 h: $P = 0.025$, $R^2 = 0.226$, figure S5).

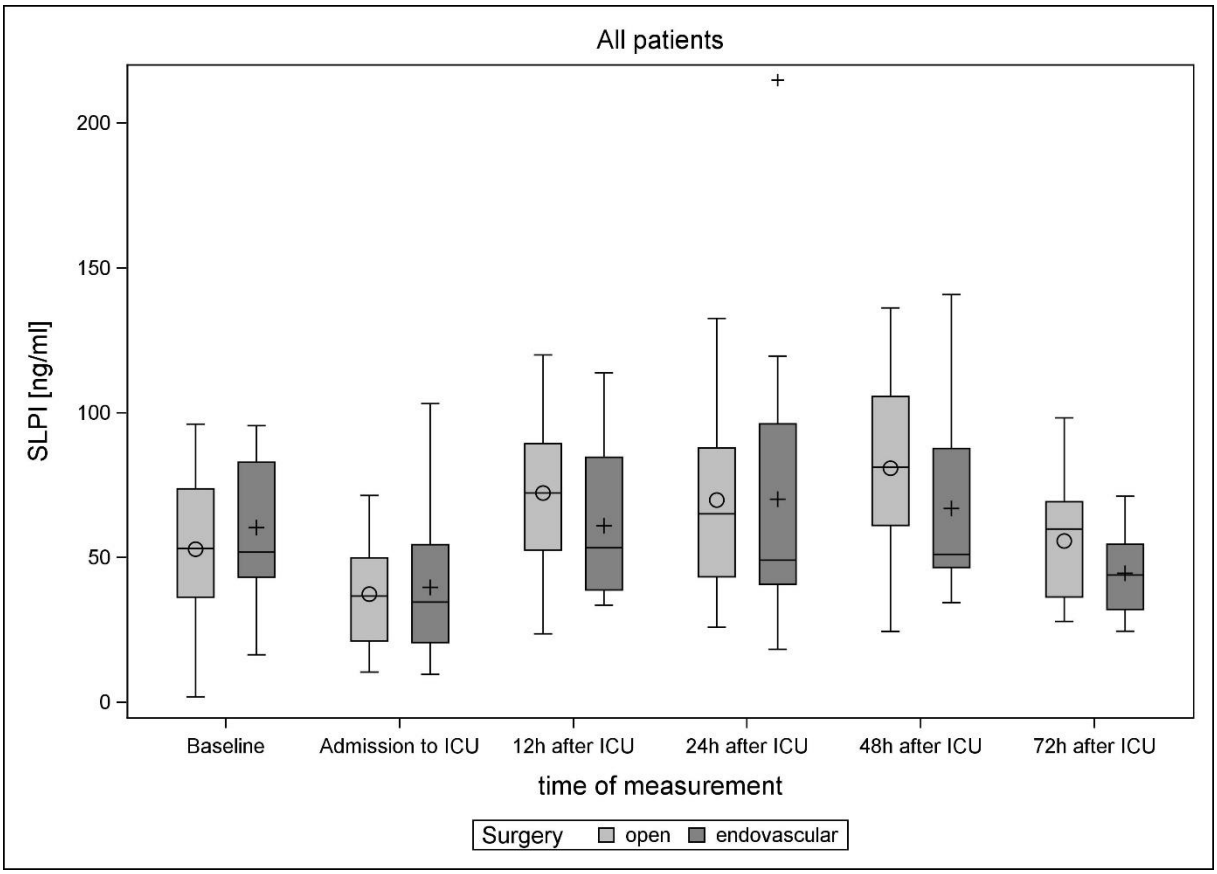

Figure 1: Boxplots illustrating SLPI levels before and after surgery in patients undergoing endovascular and open TAAA repair. There was no statistically significant difference in serum SLPI levels between patients undergoing open or endovascular TAAA repair (linear mixed model, P = 0.7691).

Table 4. SLPI in ng/ml measured at different times in the entire collective and in a subgroup by AKI.

All patients				
		Acute kidney injury (AKI)		
Time	All patients (N=33)	No (N = 16)	Yes (N = 17)	p-value [a]
Baseline	51.85 (43.05 - 75.12)	61.11 (43.36 - 80.59)	50.45 (38.89 - 73.66)	0.4342
Admission to ICU	35.13 (20.63 - 53.36)	33.28 (21.12 - 35.80)	48.03 (20.63 - 56.43)	0.3807
12h after ICU	64.00 (42.51 - 84.59)	45.46 (35.91 - 61.04)	84.21 (70.03 - 101.93)	0.0058
24h after ICU	58.15 (40.77 - 96.12)	44.17 (36.54 - 61.19)	71.47 (51.90 - 98.59)	0.3735
48h after ICU	62.90 (46.96 - 93.05)	51.01 (43.54 - 64.74)	87.64 (61.05 - 100.12)	0.2077
72h after ICU	50.40 (32.03 - 67.25)	40.60 (32.03 - 54.57)	54.21 (33.71 - 69.24)	0.2032
Patients with serum creatinine at baseline ≤ 1.25 mg/dL				
		Acute kidney injury (AKI)		
Time	All patients (N=22)	No (N = 9)	Yes (N = 13)	p-value [a]
Baseline	51.33 (36.29 - 74.36)	45.08 (43.52 - 74.36)	52.21 (36.29 - 73.66)	0.8365
Admission to ICU	34.86 (20.58 - 54.40)	26.86 (20.58 - 40.50)	43.67 (19.97 - 55.42)	0.9141
12h after ICU	52.93 (37.13 - 84.21)	36.23 (33.47 - 45.64)	75.80 (57.02 - 89.25)	0.0240
24h after ICU	49.04 (36.66 - 87.77)	36.66 (32.89 - 40.77)	78.51 (51.64 - 98.59)	0.0339
48h after ICU	67.99 (45.96 - 96.58)	46.96 (37.56 - 51.01)	90.84 (71.24 - 105.57)	0.0660
72h after ICU	36.33 (30.87 - 68.81)	33.90 (29.38 - 39.32)	55.00 (31.48 - 70.63)	0.1288

Data is reported as median (Q1-Q3). [a] Compared using a univariable logistic regression model with Firth's bias correction.

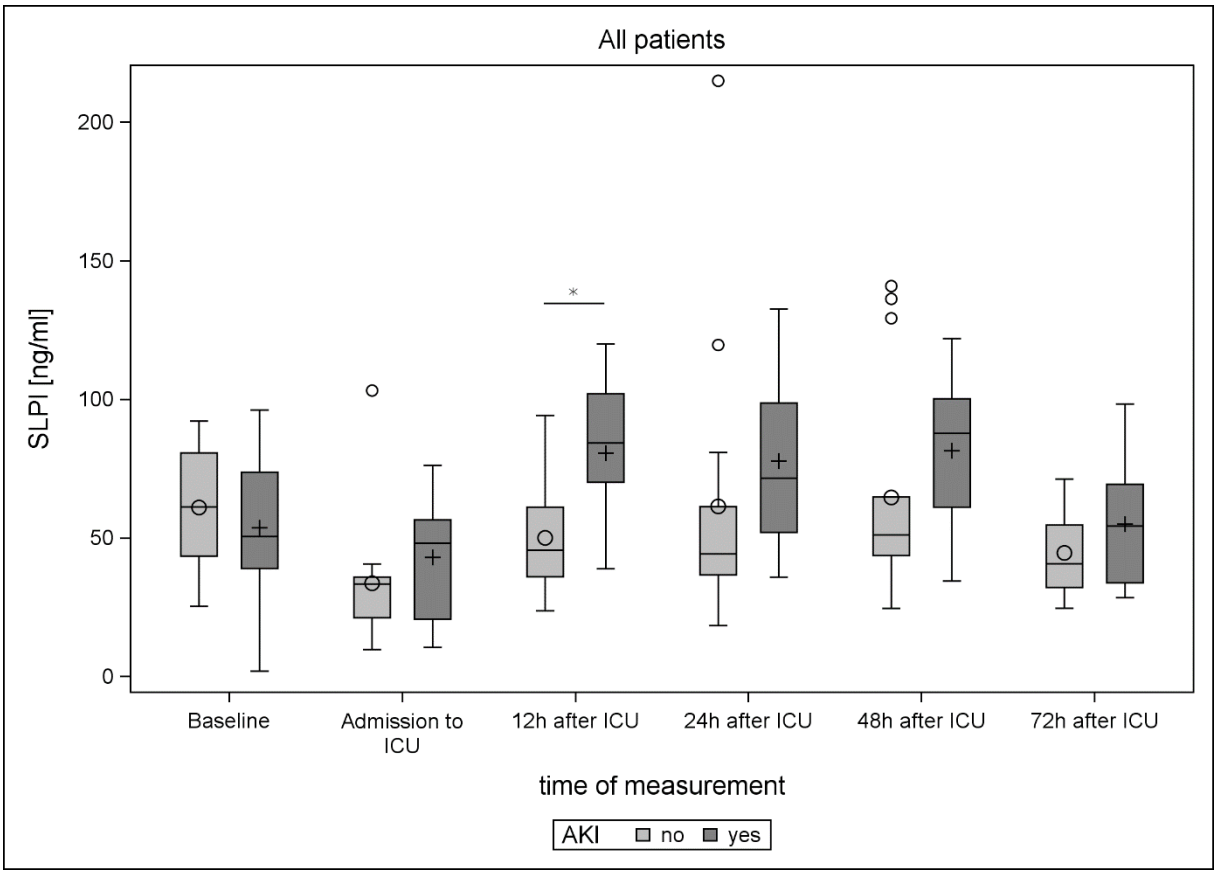

*Figure 2: Boxplots illustrating SLPI levels before and after surgery in AKI versus non-AKI patients. Significant differences (P-values < 0.05 in the corresponding analysis from Table 4) are indicated by *.*

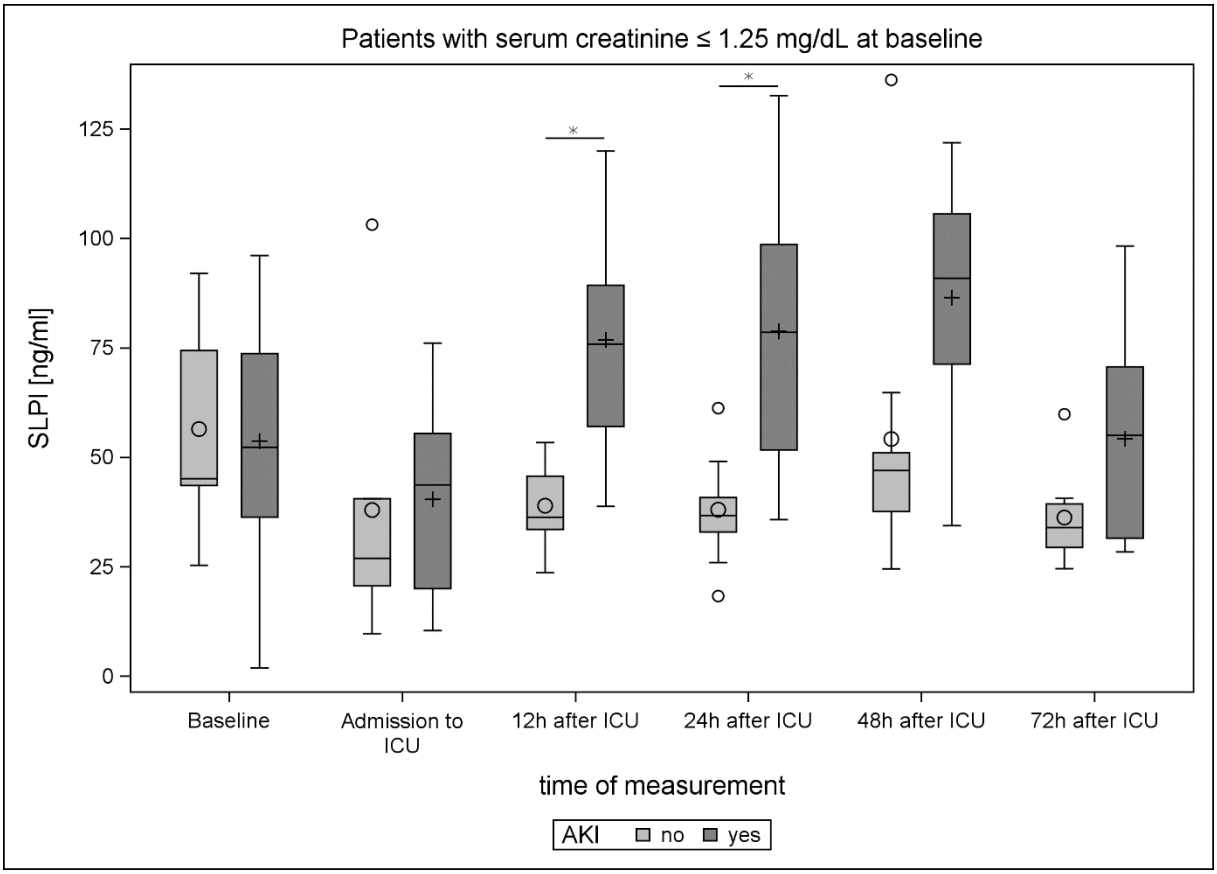

*Figure 3: Boxplots of the subgroup of patients without pre-existing renal functional impairment illustrating the SLPI-levels before and after surgery in AKI versus non-AKI patients. Significant differences (P-values < 0.05 in the corresponding analysis from Table 4) are indicated by *.*

Multivariable logistic regression model

Next, we applied a multivariable logistic regression analysis to characterize the prognostic value of serum SLPI for AKI (Table 5). In this model, SLPI 12 hours after admission to ICU was significantly associated with the occurrence of an AKI (OR = 1.055, 95% CI = 1.009-1.103, *P* = 0.0181). None of the other independent variables showed a statistically significant association with AKI.

Table 5. Multivariable logistic regression model for AKI using Firth's bias correction.

Independent variable	Odds ratio [95% Confidence interval]	p-value
Serum creatinine, mg/dL	0.002 [<0.001, 0.574]	0.0320
Blood transfusion (blood bags)	3.425 [0.802, 14.633]	0.0966
SLPI 12h after ICU, ng/ml	1.116 [1.022, 1.218]	0.0145

The independent variables were selected through a stepwise variable selection technique.

Diagnostic accuracy of SLPI as a predictor of AKI

The analysis by Receiver Operation Characteristics (ROC) curves revealed an adequate predictive accuracy of SLPI to detect AKI 12 and 24 hours after admission to ICU (for the optimal cut-off 70.03 ng/ml at 12 hours: Sensitivity 76.47 %, 95% CI = 50.1-93.2, Specificity 87.5 %, 95% CI = 61.7-98.4, AUC = 0.838, 95% CI = 0.7-0.976; for the optimal cut-off of 56.33 ng/ml at 24 hours: Sensitivity 75 %, 95% CI = 47.6-92.7%, Specificity 71.4 %, 95% CI = 41.9-91.6% AUC = 0.723, 95% CI = 0.523-0.923, Table 6, figure 4).

Table 6. Diagnostic ability of SLPI to predict AKI.

Time of measurement	Optimal Cut-Off (Youden index)					AUC
	Cut-Off, ng/ml	Sensitivity [%]	Specificity [%]	LR+	LR-	
Baseline	≥ 95.52	11.76 [1.4, 36.4]	100 [79.4, 100]	-	0.88	0.438 [0.234, 0.641]
Admission to ICU	≥ 46.38	57.14 [28.9, 82.3]	91.67 [61.5, 99.8]	6.86	0.47	0.649 [0.414, 0.883]
12h after ICU	≥ 70.03	76.47 [50.1, 93.2]	87.50 [61.7, 98.4]	6.12	0.27	0.838 [0.7, 0.976]
24h after ICU	≥ 56.33	75.00 [47.6, 92.7]	71.43 [41.9, 91.6]	2.63	0.35	0.723 [0.523, 0.923]
48h after ICU	≥ 61.05	80.00 [51.9, 95.7]	73.33 [44.9, 92.2]	3.00	0.27	0.693 [0.477, 0.909]
72h after ICU	≥ 67.25	42.86 [17.7, 71.1]	92.31 [64.0, 99.8]	5.57	0.62	0.648 [0.432, 0.865]

ROC analysis was performed to evaluate the diagnostic ability of perioperative SLPI levels during the first 72h on ICU with regard to AKI. If an elevated SLPI value indicates that the patient is likely to develop an AKI, the ROC curve should be farther from the bisecting line (Sensitivity=1-Specificity). Sensitivity, specificity and likelihood ratios (LR+/-), are reported for the Youden optimal cut-off. 95%-confidence intervals are shown in parentheses.

Diagnostic accuracy of SLPI in a subgroup without preoperative impaired renal function

The diagnostic performance of SLPI to predict AKI was improved in the subgroup of patients without pre-existing renal functional impairment (e.g. for 12 hours after admission to ICU: AUC=0.932, 95% CI=0.83-1) (Table 7, figure 4).

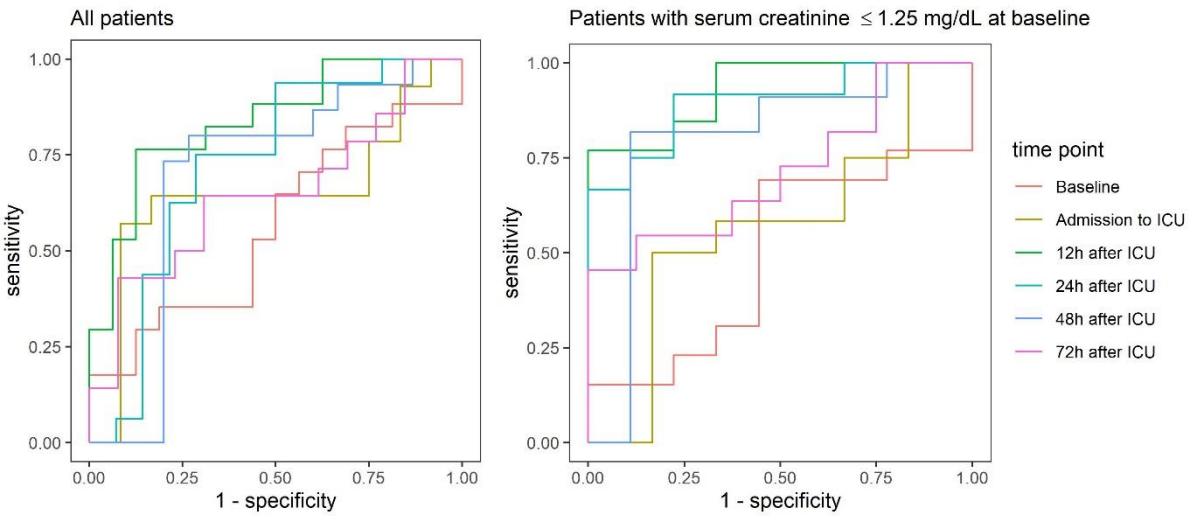

Figure 4: ROC analysis of the diagnostic accuracy of SLPI-levels for acute kidney injury in all patients and in the subgroup of patients without pre-existing renal functional impairment.

Table 7. Diagnostic ability of SLPI to predict AKI in the subgroup of patients with serum creatinine at baseline ≤ 1.25 mg/dL.

Time of measurement	Optimal Cut-Off (Youden index)					AUC
	Cut-Off, ng/ml	Sensitivity [%]	Specificity [%]	LR+	LR-	
Baseline	≥ 49.84	69.23 [38.6, 90.9]	55.56 [21.2, 86.3]	1.56	0.55	0.496 [0.235, 0.756]
Admission to ICU	≥ 49.67	50.00 [21.1, 78.9]	83.33 [35.9, 99.6]	3.00	0.60	0.569 [0.253, 0.886]
12h after ICU	≥ 57.02	76.92 [46.2, 95.0]	100 [66.4, 100]	-	0.23	0.932 [0.832, 1]
24h after ICU	≥ 43.20	91.67 [61.5, 99.8]	77.78 [40.0, 97.2]	4.13	0.11	0.898 [0.763, 1]
48h after ICU	≥ 71.24	81.82 [48.2, 97.7]	88.89 [51.8, 99.7]	7.36	0.20	0.798 [0.560, 1]
72h after ICU	≥ 68.81	45.45 [16.7, 76.6]	100 [63.1, 100]	-	0.55	0.716 [0.477, 0.955]

ROC analysis was performed to evaluate the diagnostic ability of perioperative SLPI levels during the first 72h on ICU with regard to AKI. If an elevated SLPI value indicates that the patient is likely to develop an AKI, the ROC curve should be farther from the bisecting line (Sensitivity=1-Specificity). Sensitivity, specificity and likelihood ratios (LR+/-), are reported for the Youden optimal cut-off. 95%-confidence intervals are shown in parentheses.

Discussion:

Mortality and morbidity after open and endovascular TAAA Repair remain high [31 32]. In our observational study including those patients AKI was the most frequent complication after surgery and showed a crucial association with additional severe complications.

Previous studies demonstrated that serum creatinine as an indirect marker of impaired renal function is inappropriate to detect early stages of AKI [5,6] As treatment options of AKI are limited, the early identification of AKI by biomarkers and the immediate initiation of treatment are urgently needed to decrease the incidence and clinical consequences of AKI. The KDIGO clinical practice guideline recommends different preventive measures for the treatment of AKI. Next to the eradication of potentially nephrotoxic agents, an appropriate fluid management is important to prevent AKI in critically ill patients [33]. Besides, an early initiation of renal replacement therapy was suggested to improve the long-term survival of patients who suffered from AKI [34].

To date, only a few biomarkers of postoperative complications have been investigated in the setting of TAAA. Recently, the diagnostic relevance of urinary neutrophile gelatinase associated lipocalin (NGAL) for postoperative AKI requiring dialysis was evaluated [35]. Up to now the quantification of NGAL has failed to reliably predict AKI [35]. One potential reason for why the postoperative detection of NGAL in the urine has not yet been put into clinical practice might be the circumstance, that urine samples are not routinely drawn for clinical chemistry analysis. Thus, it might be beneficial to identify appropriate kidney injury markers in the serum, which would be more feasible to be established as a routine diagnostic biomarker for AKI.

SLPI (12 kDa) is a serine protease inhibitor and is expressed by macrophages, neutrophils, and many epithelial cells including the lung and kidney [36].

By inhibiting neutrophil elastase, SLPI protects proteins from digestion [37]. Besides, SLPI was shown to inhibit the proinflammatory transcription factor NF*k*B and excessive inflammatory responses [38]. Apart from its anti-inflammatory functions, SLPI may control the growth of bacteria and fungi in a charge-dependent manner similar to other cationic peptides, such as defensins by disrupting microbial membranes [39,40] By its immunomodulatory, anti-proteolytic, and anti-microbial action, SLPI functions as a regulator of innate and adaptive host defense [8,41].

In this prospective, observational study with 33 patients undergoing open or endovascular TAAA repair, we found SLPI to be a candidate biomarker of postoperative AKI with the best predictive accuracy during the first 12 to 24 hours deeming SLPI as an early biomarker. While serum SLPI was significantly elevated in the postoperative time course on the ICU, serum SLPI levels were significantly reduced directly after surgery at the time point of admission to ICU. The half-life of serum SLPI was shown to range between 10 and 120 minutes [42]. Potentially, dilutions effects by perioperative volume management along with accelerated degradation of SLPI and a reduced *de novo* synthesis during the operative procedure could contribute to the decline in serum SLPI. However, as functional data on the regulation of SLPI expression and degradation in the setting of surgical interventions are missing, to date we can only speculate on potential reasons for this observation.

Despite the different invasiveness and divergent pathophysiological mechanisms leading to AKI, there was no relevantdifference in serum SLPI levels in the endovascular and open repair group.. Twelve hours after complex aortic intervention, patients with AKI depicted significantly increased serum SLPI and SLPI was negatively correlated with urine output. Serum SLPI performed well to predict AKI, with a promising diagnostic accuracy of

12 and 24 hours after admission to ICU. A multivariable analysis confirmed the additional prognostic value of postoperative serum SLPI to predict AKI.

Pre-operatively increased serum creatinine > 1.25 mg/dl is one of the parameters used for perioperative risk stratification of AKI after major surgery [43]. As awareness regarding the occurrence of AKI might be not appropriate in those patients without pre-existing kidney function impairment, there is a special interest to elucidate the risk of AKI in patients with non-compromised preoperative renal function[44]. Hence, patients suffering from pre-existing renal dysfunction were excluded in an additional analysis. Interestingly, after exclusion of these patients the test accuracy significantly improved for all postoperative time points analyzed. The reason why the prognostic performance of SLPI is better in patients without chronic kidney dysfunction remains elusive. One potential explanation might be the fact, that SLPI is a protein that under physiologic conditions is efficiently degraded in tubular cells whereas in uremic patients increased plasma levels of SLPI are found which might impair the performance of SLPI as an acute biomarker of AKI [45,46].

The results obtained from this observational study remain correlative and cannot explain causality. Therefore, the pathophysiological function of elevated serum SLPI needs to be discussed and investigated in different settings of cardiovascular surgery. Studies investigating the effect of SLPI during organ damage, overall establish a tissue protective role of SLPI by modulating inflammation. In an animal model, myocardial contractility was impaired in *Slpi*$^{-/-}$ hearts and fully restored when SLPI was added to the preservation solution [47]. In the context of acute and chronic lung injury, animal models revealed a protective role of SLPI by limiting neutrophil elastase induced inflammation and anti-inflammatory, and antimicrobial activity [48]. Similarly, a dysregulated inflammation may be involved in the pathogenesis of AKI after TAAA repair. Hence, the extensive release of SLPI during aortic surgery may be part of the inflammatory response and a compensatory mechanism to balance the inflammatory reaction [47]. This hypothesis is supported by our observation of a significant correlation between serum SLPI and procalcitonin, an early inflammatory marker of the immune response, 24 and 72 hours after surgery.

Although, SLPI was suggested to exert kidney protection via promoting tubular cell regeneration, data on the functional role of SLPI in AKI and in critically ill patients are scarce [13]. Thus, experimental studies elucidating the pathophysiological effects of SLPI on oxidative stress and kidney injury are needed. Of note, the assumed protective role of SLPI during organ dysfunction could potentially be exploited therapeutically by mimicking SLPI´s organ protective functions.

Regarding the potential limitations of this study, the following aspects need to be mentioned:. As only few patients suffering from TAAA are treated by open or endovascular means annually world-wide, only few patients could be included in this study. Furthermore it would have been favorable to include only patients treated by one treatment modality As for most observational studies investigating the diagnostic accuracy of biomarkers of AKI, another limitation is that kidney biopsies are not routinely available for the diagnosis of AKI based on histopathological tubular injury (“gold standard”), but that the diagnosis is commonly based on the two parameters serum creatinine and urine output, lacking sensitivity and specificity for the detection of kidney tubular injury. In the future, this general restriction will potentially be resolved by the identification of damage associated AKI biomarkers as the new gold standards of AKI. Even if the results of our study are promising and the test quality is good, the hypothesis-generating manner of this study needs to be emphasized: The results should be validated by follow-up clinical studies to verify the clinical significance of SLPI as

a promising new biomarker of acute kidney failure and other severe complications after major surgical interventions.

Conclusion:
The presented results highlight SLPI as a promising, new biomarker for the detection of postoperative acute kidney after open and endovascular TAAA repair within 72 hours, which may enable the early initiation of organ-protective therapy and reduce the frequence and consequences of AKI and other postoperative complications.

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Chapter 10

Increase of urinary TIMP-2 and IGFBP7 as potential predictor of Acute Kidney Injury requiring Renal Replacement Therapy and Patients' Outcome following complex endovascular and open thoracic abdominal aortic aneurysm surgery: a prospective observational study

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Abstract:

Background:

Acute kidney injury (AKI) as complication after open and endovascular repair of thoracoabdominal aortic aneurysm (TAAA) is one major predictor of mortality and postoperative complications. We evaluated tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) as combined early biomarker for AKI detection and predictor of patients' outcome.

Methods:

Between 2014 and 2015, 52 patients have been enrolled in this observational study, of whom 29 (55.8%) underwent elective open repair and 23 (44.2%) endovascular repair. TIMP2*IGFBP7 were measured until 48 hours after admission on intensive-care unit (ICU) and were analyzed regarding their predictive ability for AKI (defined according to the KDIGO criteria) requiring temporary renal replacement therapy (RRT) and 90-day mortality using ROC curves.

Results:

Mean patient age was 64.5 years (Min: 43, Max: 85), endovascular treated patients were older ($p < 0.0001$). 40.4 % ($n = 21$) developed AKI, and 21.2% ($n = 11$) required renal replacement therapy. In-hospital and total mortality rates were 7.7% ($n = 4$) and 9.6% ($n = 5$), respectively. At no time a significant difference in TIMP2*IGFB7 levels between patients undergoing open or endovascular surgery was observed. The predictive quality of the TIMP2*IGFBP7 value on ICU admission was sound regarding AKI requiring temporary renal replacement therapy (sensitivity: 55.56% [38.1-72.1%], specificity: 90.91% [58.7-99.8%] with an area under the curve [AUC]: 0.694 [0.543-0.820]).

Mean follow-up was 13.2 months (Min: 2, Max: 20), regarding the 90-day mortality, the predictive property of the TIMP2*IGFBP7 value was not sufficient (sensitivity: 80% [28.4-99.5%], specificity: 52.38% [36.4-68%], and AUC: 0.607 [0.454–0.746]).

Conclusion:

TIMP2*IGFBP7 level measured 6-12 hrs postoperatively may be useful as an early detectable biomarker for AKI requiring temporary renal replacement therapy. It seems not suited to predict patients' outcome following complex thoracoabdominal aortic surgery, regardless if performed by open or endovascular repair.

Introduction:

A relevant risk of acute kidney injury after open and endovascular repair of thoracoabdominal aortic aneurysms (TAAAs) has been described in literature, which is furthermore related to increased mortality rates (1-5).

Novel biomarkers of cellular injury in early AKI may allow initiation of renal protection strategies and may improve clinical outcome following thoracoabdominal surgery(6). Tissue inhibitor of metalloproteinase-2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7) were validated in recent studies as predictor for moderate and severe AKI in critically ill patients (7). TIMP2 and IGFBP7 are indicator of cellular stress in the early phase of tubular damage due to inflammation, ischemia or further triggers (8, 9). Furthermore, these biomarkers operate as paracrine signaling molecules in case of cellular damage (10, 11). In the context of early AKI detection, TIMP2*IGFBP7 levels may have favorable predictive abilities, which are comparable to neutrophil gelatinase-associated lipocalin or kidney injury molecule 1 (12). In patients undergoing cardiac or major, non-cardiac surgery, TIMP2*IGFBP7 value may be a predictor of AKI (7, 13). The potential role of TIMP2*IGFBP7 as marker for the early detection of AKI and patients' outcome following complex thoracoabdominal aortic repair was the motivation for this study.

Methods:

Ethics approval and consent to participate: The local ethics committee of the University Hospital RWTH Aachen approved this study (EK004/14). This study was performed in accordance with the Declaration of Helsinki in its actual form. Written informed consent was obtained preoperatively from all subjects.

Study design

The cohort of patients has been used for another study of this research group, hence some overlaps regarding the methodology, the applied statistics and patients' data in this manuscript are inevitable(14). The datasets supporting the conclusions of this article are available upon request.

Patients suffering from TAAA larger than 6 cm and receiving elective surgical treatment. were eligible for inclusion. Depending on patients' age and comorbidities, open or endovascular treatment was conducted. Exclusion criteria were pregnancy, chronic kidney disease requiring dialysis treatment, age < 18 years, and physical or mental disability. No emergency procedures were included either. All patients who survived the first 30 days were retrospectively contacted between December 2015 and January 2016. The study is registered at clinicaltrials.gov (NCT03093857).

Clinical and laboratory data collection

Prospective data collection was performed using paper case report forms. Data on demographics, medical history, and admission diagnosis as well as daily physiological variables, surgical interventions, need for dialysis, and any kind of adverse event were extracted from electronic bedside flow charts (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). Kidney Disease Improving Global Outcomes (KDIGO) criteria were used to define AKI(15). Baseline creatinine was the lowest pre-intervention value. AKI was defined by a reduction in kidney function with an increase in serum creatinine (>26.4 µmol/L) or percentage increase of serum creatinine above

50%, as recommended in current guidelines. During the surgical treatment, according to standardized and consistent operations, blood and urine samples were collected from patients at five predefined time intervals: pre-interventionally, on admission to the intensive care unit (ICU), and at 12 h, 24 h, and 48 h after ICU admission. In this pilot study, we focused on AKI requiring temporary renal replacement therapy.

Endpoints

The correlation of TIMP2*IGFB7 levels between patients undergoing open or endovascular surgery and AKI requiring renal replacement therapy defined according to KDIGO as well as the 90-day mortality were the endpoints of this study.

Biomarkers

Urine samples were incubated (3G and 4°C) for 10 min. The samples were stored at -20°C for less than 2 weeks and afterwards at -80°C until further processing by enzyme-linked immunosorbent assay (ELISA), (manufacturer's instructions BOSTER, Pleasanton, US). Tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) levels have been assessed according to manufacturer's protocol. All values for [TIMP-2]*[IGFBP7] product are reported in units of $(ng/mL)^2$ /1000. Laboratory investigations were blinded to the clinical status of the patients throughout the study. Following the recommendations of the manufacturer, normal and pathological reference ranges were established based on the control sample in our laboratory.

Surgical protocol

The surgical protocol for open TAAA repair has prior been published in detail, including cerebrospinal fluid drainage, perioperative monitoring of motor evoked potentials (MEP), sequential aortic clamping where possible, extracorporeal circulation with distal aortic

perfusion, selective visceral perfusion and mild hypothermia 32°C to 33°C. (16-18) To protect the kidneys against ischemic damage Custodiol® (Dr. Franz Köhler Chemie, Austria) at 4°C was used for renal perfusion (19).

The detailed procedure of fenestrated or branched endovascular repair has been described earlier (20). Iodinated contrast solution was used in a one-fourth standard dosage to reduce the nephrotoxic effects (21).

Statistical analyses

Continuous variables are expressed as mean values ± standard deviation (SD) or as median with lower and upper quartile (Q1 - Q3) in case of heavily skewed data. Categorical variables are given as absolute frequencies and percentages. Continuous baseline characteristics between the two surgical procedures were compared using either an unpaired t-test or Welch's t-test. Categorical characteristics were tested using a Chi-square test or Fisher's exact test. Procedural characteristics and outcomes between the two surgical methods were compared using (generalized) linear models and logistic regression models. The temporal course of TIMP2*IGFBP7 levels are presented as boxplots, separated by surgical procedure. For comparison of the two surgical procedures, logarithmized values were calculated and a linear mixed model with an unstructured covariance structure was used to account for the repeated measurements per patient.

The correlation between logarithmized TIMP2*IGFBP7 levels and logarithmized serum creatinine levels was assessed using Pearson's correlation coefficient for single time points and using a linear mixed model to estimate an overall correlation in repeated measurements. Spearman correlation coefficients were computed to evaluate the monotone correlation between the maximum TIMP2*IGFBP7 levels measured until 48h after admission on ICU and other procedural characteristics or intensive markers.

The ability of TIMP2*IGFBP7 levels for predicting AKI requiring temporary RRT or 90-day mortality (survival) was assessed using receiver operating characteristic curves (ROC curves). Sensitivity (Se), specificity (Sp), positive and negative likelihood ratio (LR+ and LR-), area under the curve (AUC) and the Youden-optimal cut-off are pictured together with the ROC curves. For the prediction of AKI requiring temporary renal replacement therapy, an additional cross-validation was made to illustrate how the predictive quality would generalize to another comparable data set.

The level of significance was set at 5%. No adjustments were made for multiple comparisons. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary NC, USA) and MedCalc for Windows, version 15.2 (MedCalc Software, Ostend, Belgium).

Results:

Patient demographics

52 patients (mean age was 64.5 ± 10.4 years [Min: 43, Max: 85 years]) treated for TAAA have been assessed between 2014 and 2015. Patients treated by endovascular means were statistically significantly older (p <0.0001). All details can be found in table 1.

Table 1. Patient characteristics in the open and endovascular surgery group.

	All patients	**Open surgery**	**Endovascular surgery**	***p*-value** [a]
	(N = 52)	(N = 29; 55.8%)	(N = 23; 44.2%)	
Patients characteristics and treatment				
TAAA Repair				
Type II Repair	16	10 (34.5%)	6 (26.1%)	
Type III Repair	11	6 (20.7%)	5 (21.7%)	0.8007
Type IV Repair	25	13 (44.8%)	12 (52.2%)	
Age, years	64.5 ± 10.4	59.8 ± 10.7	70.5 ± 6.2	<0.0001
Male gender	39 (75.0%)	22 (75.9%)	17 (73.9%)	0.8719

BMI, kg/m²	27.1 ± 3.9	26.4 ± 4.0	28.0 ± 3.8	0.1530
Smoker	22 (42.3%)	10 (34.5%)	12 (52.2%)	0.1997
Diabetes	6 (11.4%)	2 (6.9%)	4 (17.4%)	0.3870
Hypertension	47 (90.4%)	27 (93.1%)	20 (87.0%)	0.6443
Chronic kidney disease	7 (13.5%)	2 (6.9%)	5 (21.7%)	0.2192
Aortic aneurysm diameter, mm	63.9 ± 5.0	66.1 ± 4.6	61.1 ± 4.1	0.0002
Degenerative aneurysm	46 (88.5%)	23 (79.3%)	23 (100%)	0.0283
Coronary artery disease	21(40.4%)	13 (44.8%)	8 (34.8%)	0.4634
PAD	10 (19.2 %)	6 (20.7%)	4 (17.4%)	1
COPD	10 (19.2%)	3 (10.3%)	7 (30.4%)	0.0866
Risk scores				
APACHE II	17.8 ± 6.7	17.7 ± 6.5	18.1 ± 7.1	0.8516
SAPS	35.7 ± 13.7	36.3 ± 15.4	35.0 ± 11.4	0.7334
SOFA	7.4 ± 4.3	7.4 ± 4.1	7.4 ± 4.6	0.9730

Table 1: Continuous data is reported as mean ±SD, categorical data as absolute and relative frequencies. [a] Compared using an unpaired t-test for continuous outcomes and a Chi-square or Fisher's exact test for categorical outcomes. PAD: Peripheral Artery Disease; COPD: Chronic Obstructive Pulmonary Disease

Mortality

In-hospital mortality rate was 7.7 % (*n* = 4), and total mortality rate during the follow-up (mean follow-up, 13.2 months ±5.3, [2–20 months]) was 9.6% (*n* = 5). Two patients deceased because of pneumonic sepsis, one each of cerebral bleeding and small intestine ischemia. 19 weeks after type IV repair one patient suffered from fatal thoracic aortic rupture. All details can be found in table 2.

Table 2. Procedural characteristics and outcomes in the open and endovascular surgery group

	All patients	Open surgery	Endovascular surgery	*p*-value [a]
	(N = 52)	(N = 29; 55.8%)	(N = 23; 44.2%)	
Procedural characteristics				
Operation time, min	401.3 ± 98.9 [b]	403.4 ± 96.5 [b]	398.7 ± 103.9	0.8680
Total ventilation time, min	980 (570 - 1980) [c]	1212.5 (630 - 2472.5) [b]	885 (485 - 1590) [b]	0.2527
In-hospital stay, days	21 (11 – 32)[b]	26 (18 – 37)	13.5 (9 – 23)[b]	0.0266
ICU stay, days	3 (1 – 7) [b]	5 (1.5 – 7) [b]	2 (1 – 5)	0.3906
Complications				
Pneumonia	11 (21.2%)	6 (20.7%)	5 (21.7%)	0.9144
Tracheotomy	10 (19.2%)	7 (24.1 %)	3 (13%)	0.3594
Stroke	1 (1.9%)	0	1 (4.3%)	0.4169
Paraplegia	2 (3.8%)	1 (3.4%)	1 (4.3%)	0.8453
MI	0	0	0	-
Sepsis	3 (5.8%)	2 (6.9%)	1 (4.3%))	0.7765
Major Revision	3 (5.8%)	2 (6.9%)	1 (4.3%)	0.7765
AKI	21 (40.4%)	9 (31.0%)	12 (52.2%)	0.1399
AKI requiring temporary RRT	11 (21.2%)	5 (17.2%)	6 (26.1%)	0.4531
AKI requiring permanent RRT	3 (5.8%)	3 (10.3%)	0	0.2450
90-Day Mortality	5 (9.6%)	4 (13.8%)	1 (4.4%)	0.3333
Hereof pneumonia	2	2	0	-
Hereof hemorraghic	1	1	0	-
Hereof small intestine ischemia	1	0	1	-
Hereof cerebral bleeding	1	1	0	-

Table 2: Continuous data is reported as mean ± SD or as median (Q1-Q3) in case of heavily skewed data, categorical data as absolute and relative frequencies. [a] Compared by means of a multivariable (generalized) linear model for continuous outcomes or a multivariable logistic regression model for categorical outcomes, [b] one observation missing, [c] two observations missing. ICU: Intensive care unit; AKI: Acute kidney injury; RRT: renal replacement therapy, MI: Myocardial Infarction

Perioperative course of TIMP2*IGFBP7 product after TAAA-Repair by treatment modality (open or endovascular)

With regard to the perioperative course of TIMP2*IGFBP7 values, an increase of the urine biomarker-levels could be observed, which reached its maximum 24h after ICU admission (supplemental table 1). Regarding the two surgical methods, no statistically significant

difference for the course of TIMP2*IGFBP7 values could be observed between open and endovascular treatment (Figure 1, p-value = 0.9825).

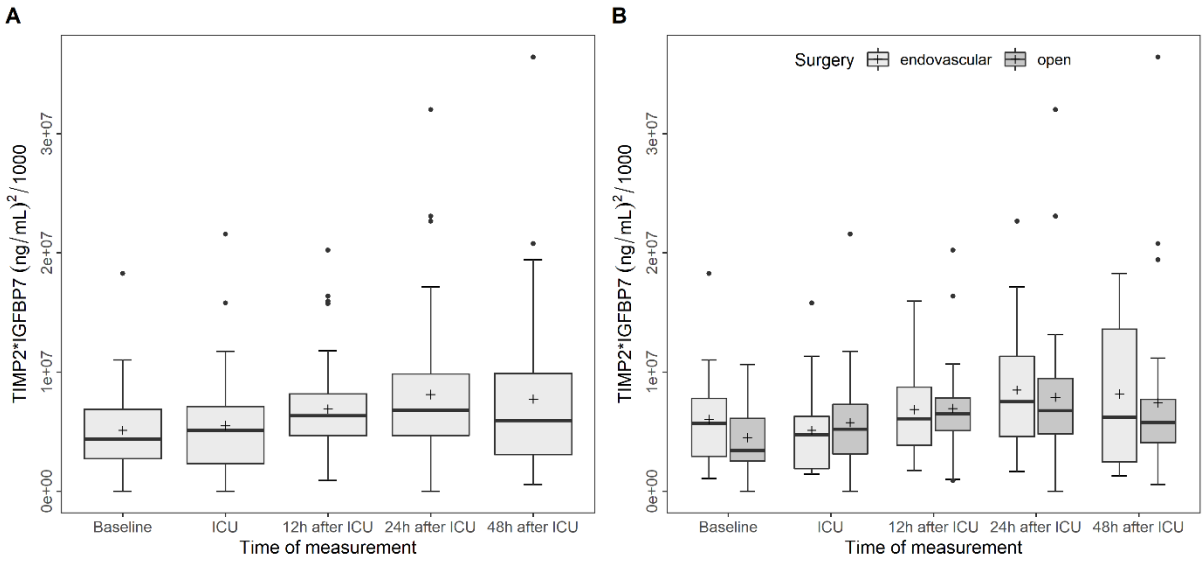


*Figure 1: A) Boxplots showing TIMP2*IGFBP7 levels by time of measurement. B) Boxplots showing TIMP2*IGFBP7-levels by time of measurement and type of surgery. There was no statistically significant difference between the TIMP2*IGFBP7 levels of the two surgical groups, p-value = 0.9825 (Linear mixed model applied to the logarithmized TIMP2*IGFBP7 levels with an unstructured covariance structure to account for repeated measurements).*

Association of perioperative TIMP2*IGFBP7-levels and serum creatinine-levels

TIMP2*IGFBP7 levels have been compared with increased serum creatinine levels indicating AKI according to the KDIGO-criteria. There appears to be a low to moderate correlation between the early measurements of TIMP2*IGFBP7 product and the serum creatinine levels after 24h on ICU (0.3107 [0.0114, 0.5589]) and after 48h (0.3518 [0.0650, 0.5849]). The increasing correlation of TIMP2*IGFBP7 product-levels and serum creatinine over time was mainly based on increasing serum creatinine levels, which suggests an earlier correlation of AKI and increased TIMP2*IGFBP7 values compared to serum creatinine levels (supplemental table 2).

Ability of TIMP2*IGFBP7-levels in predicting AKI requiring RRT

40.4 % (*n* = 21) of patients developed AKI, of whom 21.2% (*n* = 11) required RRT, 5.8%, (*n* = 3) of these needed permanent RRT after discharge. Figure 2 displays levels of TIMP2*IGFBP7 for each time point separated by AKI requiring temporary RRT or normal kidney function (Figure 2).

Overall, the ability of TIPM2*IGFBP7 product-levels for predicting AKI requiring temporary RRT was good, with a sensitivity and a specificity between 38.89-63.89% and 81.82%-100%, respectively and depending on the time of measurement (Figure 3). At admission on ICU, sensitivity was 55.56% [38.1-72.1%], specificity was 90.91 [58.7-99.8%] and AUC was 0.694 [0.543-0.820]. 48 hours after admission on ICU sensitivity was 61.11% [43.5-76.9%], specificity was 90.91% [58.7-99.8%] and the AUC was 0.745 [0.597-0.861*]*. (Figure 3).

As expected, the predictive quality of TIMP2*IGFBP7 values for the cross-validated scenario deteriorates slightly compared to the present derivation data set (supplemental figure 1). Nevertheless, AUC-values above 0.5 at all time points confirm the hypothesis that elevated TIMP2*IGFBP7 values indicate a higher risk of AKI requiring temporary RRT.

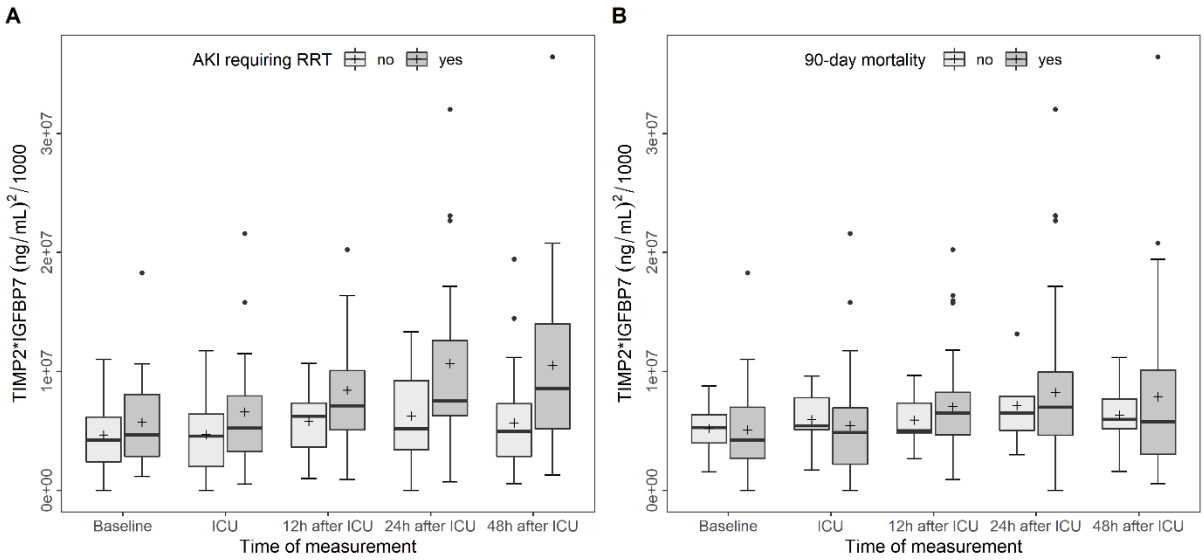
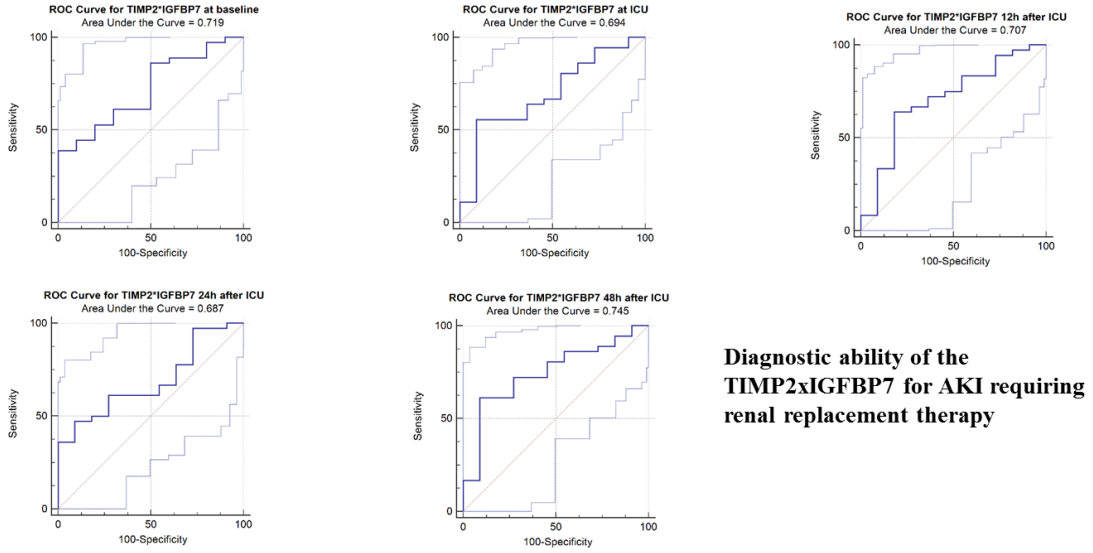


*Figure 2: A) Boxplots showing the correlation of TIMP2*IGFBP7 levels and AKI requiring temporary RRT (as categorized by serum creatinine levels according to the KDIGO-classification). B) Boxplots showing the correlation of TIMP2*IGFBP7 levels and 90-day mortality.*



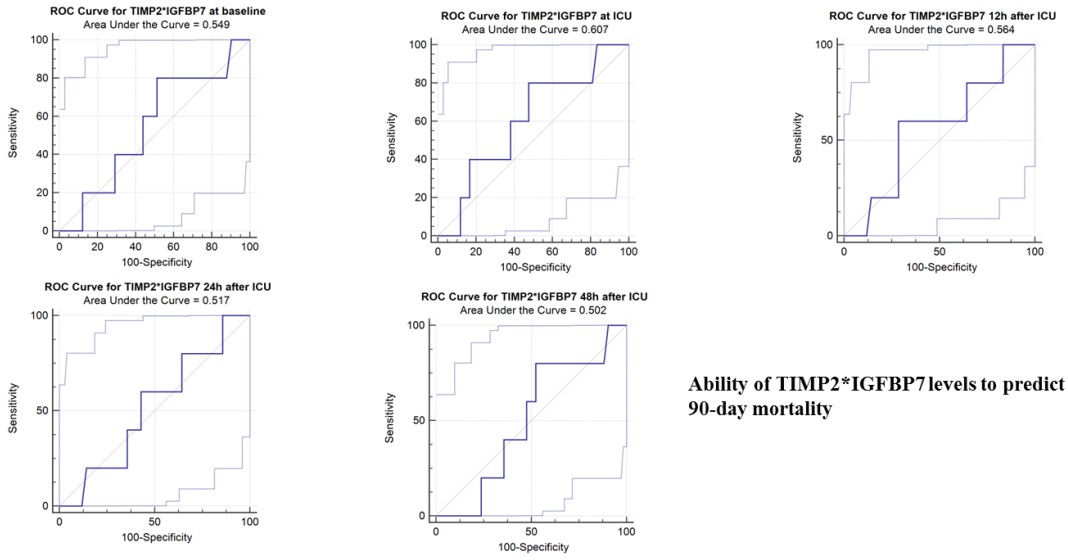
Diagnostic ability of the TIMP2xIGFBP7 for AKI requiring renal replacement therapy

Time of measurement	Optimal Cut-Off (Youden index)					AUC
	Cut-Off	Sensitivity [%]	Specificity [%]	LR +	LR-	
Baseline	≤2.9025E6	38.89 [23.1 - 56.5]	100.00 [69.2 - 100.0]	-	0.61	0.719 [0.568 - 0.842]
Admission on ICU	≤4.5525E6	55.56 [38.1 - 72.1]	90.91 [58.7 - 99.8]	6.11	0.49	0.694 [0.543 - 0.820]
12h after ICU	≤6.5972E6	63.89 [46.2 - 79.2]	81.82 [48.2 - 97.7]	3.51	0.44	0.707 [0.556 - 0.831]
24h after ICU	≤5.1692E6	47.22 [30.4 - 64.5]	90.91 [58.7 - 99.8]	5.19	0.58	0.687 [0.535 - 0.814]
48h after ICU	≤5.6204E6	61.11 [43.5 - 76.9]	90.91 [58.7 - 99.8]	6.72	0.43	0.745 [0.597 - 0.861]

*Figure 3: Ability of TIMP2*IGFBP7 levels to predict AKI requiring temporary RRT. A ROC analysis was performed to evaluate the predictive ability of perioperative TIMP2*IGFBP7 levels during the first 48h on ICU with regard to AKI requiring temporary RRT. Sensitivity, specificity and likelihood ratios (LR+/-), are reported for the Youden optimal cut-off. 95%-confidence intervals are shown in parentheses.*

Ability of TIMP2*IGFBP7-levels to predict 90-day mortality

The 90-day mortality rate was 9.6 % (n =5) with no statistical significant difference between the open and endovascular group. A survey of the post-operative measured TIMP2*IGFBP7 for all time points revealed no correlation between elevated TIMP2*IGFBP7 levels and an increased mortality-rate within 90 days. At admission on ICU, we found a sensitivity of 80% [28.4-99.5%], specificity of 52.38 % [36.4-68%], and AUC of 0.607 [0.454–0.746]. 48 h after admission to ICU, a sensitivity of 80.0% [28.4–99.5%], a specificity of 47.62 % [32–63.6 %], and an AUC of 0.502 [0.353–0.652] was observable (Figure 4).



Time of measurement	Optimal Cut-Off (Youden index)					AUC
	Cut-Off	Sensitivity [%]	Specificity [%]	LR+	LR-	
Baseline	> 3.4099E6	80.00 [28.4 - 99.5]	48.78 [32.9 - 64.9]	1.56	0.41	0.549 [0.395 - 0.696]
0h after ICU	> 4.9583E6	80.00 [28.4 - 99.5]	52.38 [36.4 - 68.0]	1.68	0.38	0.607 [0.454 - 0.746]
12h after ICU	≤ 5.0012E6	60.00 [14.7 - 94.7]	71.43 [55.4 - 84.3]	2.10	0.56	0.564 [0.412 - 0.708]
24h after ICU	≤ 6.4819E6	60.00 [14.7 - 94.7]	57.14 [41.0 - 72.3]	1.40	0.70	0.517 [0.366 - 0.665]
48h after ICU	> 5.0562E6	80.00 [28.4 - 99.5]	47.62 [32.0 - 63.6]	1.53	0.42	0.502 [0.353 - 0.652]

*Figure 4: Ability of TIMP2*IGFBP7 levels to predict 90-day mortality. A ROC analysis was performed to evaluate the predictive ability of perioperative TIMP2*ILGBP7 levels during the first 48h on ICU with regard to 90-day survival. Sensitivity, specificity and likelihood ratios (LR+/-), are reported for the Youden optimal cut-off. 95%-confidence intervals are shown in parentheses.*

Correlation of TIMP2*IGFBP7 with further clinical parameters

With regard to further potentially relevant correlations, the maximum TIMP2*IGFBP7-levels were related to several postinterventional parameters. No relevant correlation could be observed.

Discussion:

The present study assessed the use of the TIMP2*IGFBP7 product as a biomarker of AKI after complex open and endovascular TAAA surgery. According to previous findings, which described TIMP2*IGFBP7 product as favorable biomarker in different surgical settings, TIMP2*IGFBP7 was demonstrated as valuable approach to detect early post-operative AKI

after complex aortic surgery (7, 13). AKI is a relevant and underestimated threat after open and endovascular surgical repair of TAAA (1, 22). Hence, an early marker to index severe AKI seems to be favorable. Despite numerous randomized control trials emphasizing a favorable outcome after early postoperative renal replacement therapy in case of AKI after cardiac surgery, no such studies have been conducted in fields of aortic surgery (23). In this trial we investigated and compared outcome and data of patients' undergoing endovascular and open TAAA repair. Regarding potential differences of TIMP2*IGFBP7 levels, no significant difference could be observed for any perioperative time point in this study. Accordingly, with respect to the assessed risk scores (SOFA, APACHE II and SAPS), no significant difference between the open and endovascular group could be observed.

While comparing TIMP2*IGFBP7-levels within 48 hours perioperatively with increased serum creatinine levels, only a moderate correlation could be observed. This finding does not correspond to the existing literature, which revealed a correlation between increasing serum creatinine levels and TIMP2*IGFBP7-levels (7). Yet, as literature regarding post-interventional AKI following open or endovascular TAAA is scarce, only few is known about the corresponding relevance of postoperative serum creatinine levels in the TAAA surgery setting. As the increased release of creatinine after major surgery is multifactorial and as the postoperative predictive ability of TIMP2*IGFBP7 for AKI is good, the relevance of this limitation is moderate.

The predictive quality of TIMP2*IGFBP7 regarding postoperative renal failure was good. Especially the favorable specificity-level for each assess point has to be mentioned. According to the applied statistics, the optimum cut-off value of the Youden index is associated with a high level of specificity and a moderate to low level of sensitivity. Hence, the presented results may underline the value of postoperative TIMP2*IGFBP7-testing as a rule-in marker. These findings correspond with the previously described usage of the TIMP2*IGFBP7 product as an early indicator for postoperative AKI requiring RRT. As

reported before, an established and commercially available rapid testing system enables bed-site testing of patients at risk directly after admission on ICU (24).

Only few biomarker studies deal with post-operative organ dysfunction and patients' outcome after open or endovascular TAAA surgery, although the morbidity and mortality rates surpass those reported in cardiac surgery studies (25). Based on the high volume turnover in this group of surgical interventions and the major release of inflammatory biomarkers, a critical evaluation is required (26, 27). In this context, already pre-operatively elevated levels of TIMP2*IGFBP7 may indicate cellular stress, which could influence postoperative outcomes(28). Besides, elevated TIMP2- Matrix Metalloproteinase levels may be related to aortic aneurysm development as well as adverse outcome in animal models of aortic aneurysm. This indicates a pre-operative predictive value of elevated biomarker levels (29, 30). Further attempts to improve the understanding of postoperative organ dysfunction after TAAA repair seems crucial, as the mortality rates could not be improved by adjustments in open TAAA surgery within the last decades (1, 31). Furthermore, even if applicable in more frail and older patients, endovascular therapy may be related to similar mortality and morbidity rates especially with regard to renal failure (32, 33).

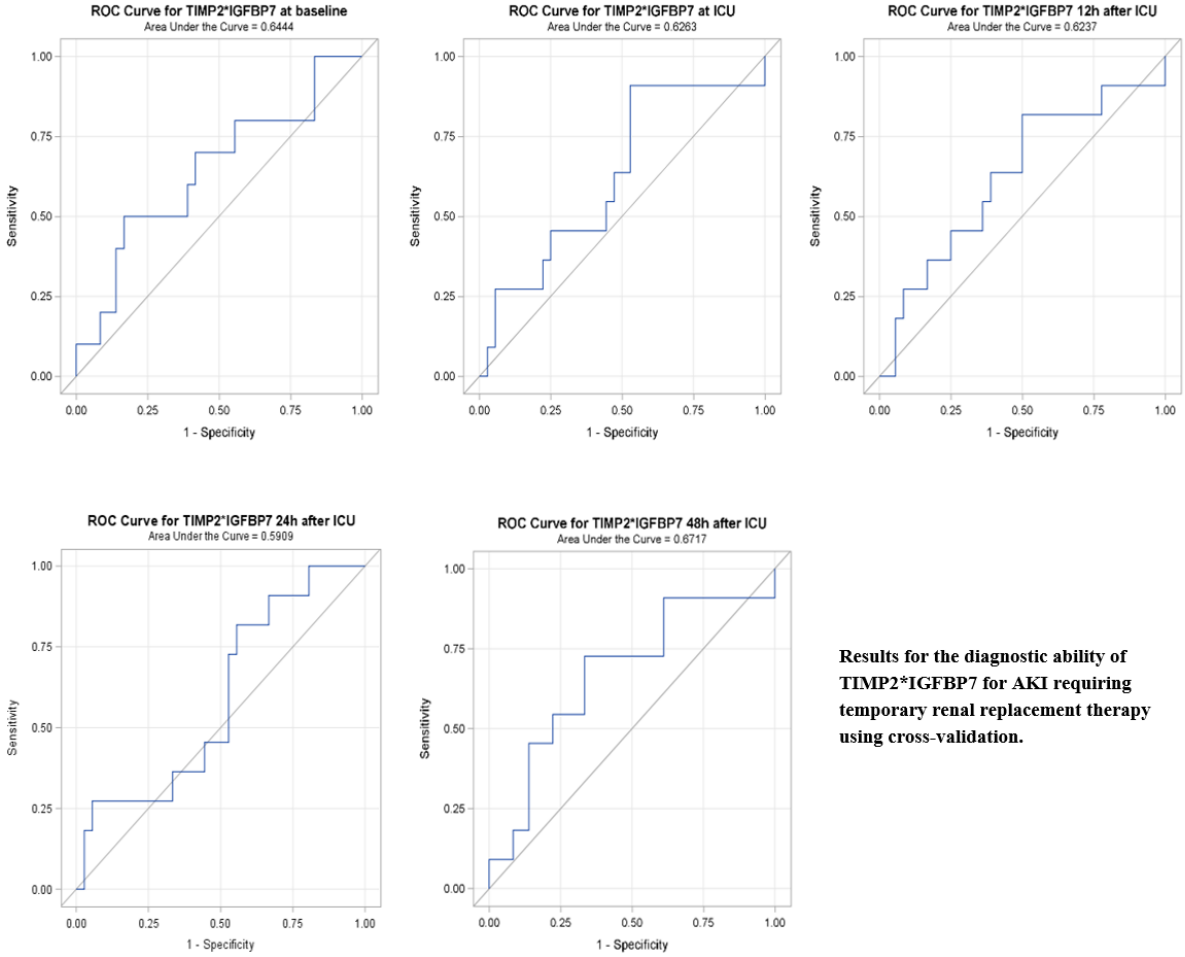
In their study, Gocze et al., TIMP2*IGFBP7-testing observed no correlation with patients' survival e.g. postoperative mortality rates (13). Even if an association of AKI and mortality rates have been described for patients undergoing complex TAAA repair, the study design as well as the number of patients included may be an explanation for the missing correlation (2, 3). However, the specificity of TIMP2*IGFBP7 product as a biomarker for early AKI detection is not compulsory disadvantageous. As this study was performed as a pilot study, we focused on AKI requiring RRT as dichotomous endpoint without separate assessment of all categories of AKI according to the KDIGO classification (34). Yet, as 11 of 14 patients (78%) who develop a postoperative AKI required RRT, our endpoint seems to be clinically relevant.

While assessing the potential value of this study, certain limitations must be taken into account. The non-randomized, observational single-center character of this study is a mayor limitation for each finding. Yet, literature focusing on postoperative biomarkers for organ dysfunction after open or endovascular TAAA repair is scarce. With regard to patients' comorbidities and the chosen treatment option, a more homogenous cohort of patients would be favorable. Yet, as the influence of the open or endovascular approach seems to be non-significant in this study, the presented findings emphasize the relevance of urinary TIMP2*IGFBP7-levels as a postoperative biomarker of AKI after thoracic abdominal aortic surgery. This study was performed in a hypothesis-generating manner, yet our findings are similar to results reported in the literature.

Conclusion:

6-12h postoperatively measured TIMP2*IGFBP7 levels may be useful as early detectable biomarker for AKI requiring temporary renal replacement therapy for patients undergoing open or endovascular aortic surgery. With regard to patients' outcome, no relevant correlation could be assessed after complex thoracoabdominal aortic surgery with TIMP2*IGFBP7 levels.

Supplemental Data:



Results for the diagnostic ability of TIMP2*IGFBP7 for AKI requiring temporary renal replacement therapy using cross-validation.

*Supplemental Figure 1: Ability of TIMP2*IGFBP7 levels to predict AKI requiring temporary RRT using cross-validation. A cross-validated ROC analysis was performed to evaluate how the predictive ability of perioperative TIMP2*IGFBP7 levels during the first 48h on ICU for AKI requiring RRT would generalize to other data sets. Sensitivity, specificity and likelihood ratios (LR+/-), are reported for the Youden optimal cut-off. 95%-confidence intervals are shown in parentheses.*

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Chapter 11

Perspective: Multicentric, prospective assessment of bedside biomarkers of acute kidney injury following open TAAA repair: the Nephrocheck® trial

Presentation of 24 patients assessed in the Department of Vascular Surgery, University Hospital RWTH Aachen

The mindset regarding usage of novel biomarkers for acute kidney injury (AKI) has changed. According to a recently published consensus paper, novel biomarkers are recommended for AKI prediction in critical care patients and patients after major cardiac surgery (1, 2).
In accordance with these developments and based on the findings of our previous studies, we started a prospective, multicentric study in 2019 involving three high-volume centres for aortic surgery, namely the Department of Vascular Surgery of the University of Muenster and Aachen, as well as the Department of Vascular Surgery of the Charité. In this study, our focus is on AKI following open thoracoabdominal aortic repair. Since the study began in December 2019, more than 45 patients have been included. The COVID-19 pandemic is a relevant confounder of patient inclusion, and we have been able to assess only patients treated in the University Hospital of Aachen. We hope to complete patient inclusion in 2022.

Methods:

Study design

This multicentric prospective, one-arm observational study is taking place at three German centres for aortic surgery (Muenster and Aachen, Charité Berlin) and is planned to include 60 patients according to an *a priori*–designed study protocol based on a power calculation.
The study was registered at Clinicaltrials.gov (number: NCT04087161), and all participants have given informed consent. The internal review board of the University Hospital Aachen (EK010/19) approved this study. We performed this work in accordance with the Declaration of Helsinki. All patients undergoing elective open or endovascular TAAA repair, defined according to the Crawford classification, were included (3).
Patients undergoing TAAA repair from December 2019 until December 2021 were consecutively screened. The following exclusion criteria were applied: chronic kidney disease

with dialysis treatment, age <18 years, pregnancy, and use of immunosuppressive medication. Medical history and physiological parameters were taken from medical records and electronic bedside flow charts (IntelliSpace Critical Care and Anesthesia; Philips Healthcare, Andover, Massachusetts, USA). Serum samples were collected before surgery, after admission to the intensive care unit (ICU), and during early follow-up in the ICU (12, 24, 48, and 72 hours). AKI was defined according to KDIGO (Kidney Disease Improving Global Outcomes) criteria (4) based on serum creatinine levels and 24-hour urine output detection during the first 72 hours after surgery. Baseline creatinine was defined as the lowest pre-intervention value 24 hours before surgery.

Endpoints

The primary endpoint was AKI within the first 30 days after surgery, classified according to KDIGO. Accordingly, the results of [TIMP-2*IGFBP7] bedside tests were analysed and compared.

The secondary endpoints were further complications such pneumonia, sepsis, multi-organ failure, death within 30 days after surgery, and 12-month mortality rate. The following postoperative adverse events were analysed: sepsis, death, MACE (major cardiovascular events), and pneumonia. Pneumonia and tracheotomy were defined according to the guidelines of the American Thoracic Society or the Belgian Society of Pneumology, respectively (5, 6). Spinal cord ischaemia was defined as post-operative paraplegia or paraparesis (7). MACE included myocardial infarction, acute heart failure, and ventricular tachycardia, all defined according to current guidelines (8-10). Sepsis was defined according to the guidelines of the German Sepsis Society (11), as follows: fever (body temperature >38 °C) or hypothermia (<36 °C), tachycardia (heart rate >90 beats per minute), tachypnoea (respiratory rate >20/minute), or leucocytosis (≥12,000/mm³) or leucopenia (≤4000/mm³). For patients and time points with available clinical data, we additionally correlated serum SLPI with the inflammatory markers CRP, PCT, IL-6, and white blood cell count, measured in the ICU.

Power calculation

To reach an area under the curve (AUC) of 80% for the prediction of AKI significantly different from 50% (H0: AUC = 0.5; H1: AUC = 0.8), a total of 60 patients would need to be included, with significance set at 5% (~75%/25% = N-/N+; N+ = 15; N- = 45) and a power of 96%. linically relevant sensitivity and specificity of 80% for AKI detection, respectively,

would lead to a 90% confidence interval (exact Clopper–Pearson) of 56%–94% (confidence level of 0.38).

[TIMP-2*IGFBP7] measurement

[TIMP-2*IGFBP7] was measured in urinary samples after admission to the ICU as well as at 12, 24, 48, and 72 hours in the ICU, according to manufacturer's instructions. Urine samples were analysed within one hour using Nephrocheck® (Astute Medical, San Diego, CA, USA), based on a florescence-labelled immunoassay. Quality controls were provided by the manufacturer. After measuring [TIMP-2] and [IGFBP7] concentrations, the signals were calculated into a single metric number. Values ≥0.3 indicate elevated risk for AKI.

Surgery

As previously published, the protocol for open TAAA repair includes aortic clamping, extracorporeal circulation with distal aortic perfusion, and visceral perfusion using selective perfusion catheters (12, 13) (7). Renal perfusion was achieved with 4 °C tempered Custodiol® (Dr. Franz Köhler Chemie, Austria) to avoid ischaemic organ damage (14).

Statistics

Continuous variables are expressed as medians with lower and upper quartiles (Q1, Q3) in case of heavily skewed data or as means ± standard deviations (SDs). Categorical variables are shown as absolute frequencies and percentages. The time course of perioperative [TIMP-2*IGFBP7] is visualized in boxplots. The association between the occurrence of AKI and other clinical outcomes (e.g., pneumonia) was assessed using Fisher's exact test. Firth's bias correction was applied in an univariable logistic regression model to identify associations between baseline or operational characteristics and the development of AKI. Associations between the development of AKI (dependent variable) and [TIMP-2*IGFBP7] were likewise assessed using a univariable logistic regression model with Firth's bias correction.

The diagnostic quality of [TIMP-2*IGFBP7] for predicting AKI was assessed using receiver operating characteristic (ROC) curves. Sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR-, respectively), AUC, and the optimal cut-off value according to the Youden index are reported together with the ROC curves. Additional analyses were performed in the subgroup of patients without pre-existing impaired renal function.

The association between [TIMP-2*IGFBP7] and other outcomes (sepsis, death, MACE, pneumonia) is shown in boxplots in the supplement. Associations were tested using a logistic

regression model with the outcome as the dependent variable and applying Firth's bias correction. The level of significance was set at 5%. No adjustments were made for multiple comparisons due to the exploratory nature of this study. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA) and R, version 3.5.1 (15).

Preliminary results (single-centre assessment of n = 24 treated by open TAAA repair in Aachen)

Between October 2019 and June 2021, 24 patients underwent open TAAA repair, of whom seven (29.2%) were female. The mean age was 52 (16–68) years.

AKI as defined according to KDIGO was observed in 15 patients (62.5%), with a median time of occurrence on the first day after surgery.

The median Nephrocheck™ measurements of patients experiencing AKI were significantly higher at 12 hours than in patients without AKI(t [14.776] = -2.485, p = .025), 24 hours (t [17.419] = -2.152, p = .046), and 48 hours (t [12.032] = -2.565, p = .025) after surgery.

Regarding diagnostic quality, test accuracy was good. Using the Youden index, we identified the optimal cut-off for AKI prediction at 12 hours after surgery as 0.37, for a sensitivity of 84.6%, specificity of 77.8%, LR+ of 3.81, LR- of 0.2, and AUC of 0.83 (95% CI 0.65–1).

At 24 and 48 hours after surgery, the diagnostic accuracy was of adequatequality for AKI prediction (cut-off 0.49; at 24 hours: sensitivity 83.3%, specificity 77.8%, LR+ 3.75, LR- 0.2, and AUC 0.843 [95% CI 0.661–1]; at 48 hours: sensitivity 84.6%, specificity 77.8, LR+ 3.8, LR- 0.2, and AUC 0.91 [95% CI 0.768–1]).

Discussion

Although the findings must be considered against the limitation of the preliminary nature of these analyses, these prospective data from one centre suggest promise for the applicability of Nephrocheck™ in enabling timely and appropriate assessment of AKI in complex open aortic surgery. The inclusion of novel biomarkers that are immediately available after surgery may lead to better treatment and improved outcomes for patients experiencing complex aortic pathologies such as thoracoabdominal aortic aneurysm.

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Chapter 12

Discussion

Historical introduction to the repair of thoracoabdominal aortic aneurysm

A short historical summary of the important milestones in aortic aneurysm surgery could be useful in assessing the unresolved issues of medical therapy for complex aortic aneurysm in the 21st century.

The first scientific reports of surgical treatment for aortic aneurysm date to the 18th century, when John Hunter performed ligation of the vessel proximal to the aneurysm and Matas described endoaneurysmorrhaphy. In the last century, Albert Einstein was treated by Dr. Rudolph Nissen in Brooklyn in 1948, who wrapped Einstein's infrarenal aortic aneurysm with cellophane. Einstein survived the surgery for 7 years and died from rupture of the aneurysm in 1955 (1). In the 1950s, surgeons such as DeBakey and Crawford developed different approaches to repairing more complex aneurysms, such as thoracoabdominal aortic aneurysm (TAAA) (2).

During the 1950s and 1960s, the Dacron prosthesis, first implanted by DeBakey, was favoured as graft material instead of a homograft. In this era, giants of aortic surgery in Houston, Texas, already had described the negative and life-threatening effects of postoperative organ failure, such as acute renal failure and spinal cord ischaemia (SCI) (3).

Facing these fundamental issues, in the 1970s, Crawford popularised techniques for TAAA repair that followed anatomic approaches to aortic replacement, drawing on the earlier work of Carrel and Guthrie. He re-implanted patches of intercostal arteries to increase perfusion to the spinal cord, reattached the visceral arteries as an island in the graft, and avoided full resection of the aneurysm, instead wrapping the remaining wall around the replacement graft. Crawford's techniques leading toearly survival after open TAAA repair of 92% (4).

In the last decades of the 20th century, the organ-protective approach during open TAAA surgery was further developed and improved by surgeons, such as Safi, Coselli, and Jacobs, by establishing distal aortic perfusion using a heart-lung machine, cerebrospinal fluid drainage, moderate hypothermia, and sequential clamping to decrease the incidence of neurological deficit from SCI and further severe organ damage (5). In the 1990s, Jacobs et al. captured the concept of neuro-protective measures during open TAAA surgery and supported

the intra-operative and postoperative application of neuro-monitoring based on large animal studies and improved clinical outcomes (6). During that same period, based on increasingly better results with endovascular aortic surgery, the first surgeon-modified and fenestrated aortic endografts were implanted by vascular surgical colleagues, including Roy Greenberg (7). Patients who had been deemed poor candidates for open TAAA repair had a chance to undergo curative treatment for their underlying aortic disease. In addition to reports of notable re-intervention rates and aortic-related mortality during follow-up, however, organ failure, such as acute kidney injury (AKI) and SCI, remained common. By improving endovascular techniques, Oderich et al. further enhanced patient outcomes after endovascular TAAA repair. Although a recently published meta-analysis pointed out that a notable peri-operative mortality and morbidity rate persists, the short- and long-term results of endovascular TAAA are more than promising and are calling into question the indication for open TAAA repair (8, 9). However, prospective studies had not yet confirmed these promising findings regarding peri-operative outcomes with endovascular TAAA repair (10). Accordingly, in the current ESVS guidelines published in 2017, open TAAA repair was considered the gold standard treatment for patients suffering from extended aortic aneurysms, as long as the patients were in appropriate physical condition (11).

Regardless of whether TAAA surgery is open or endovascular, it involves considerable morbidity and mortality (12). Although large prospective studies have proven the beneficial application of peri-operative biomarkers for patients undergoing major surgery, peri-operative risk assessment using biomarkers has yet to be established in the aortic surgery fields (13). Focussing on AKI, several studies have underlined the benefit of early AKI detection using multiple biomarkers combined in a panel (14). This short historical overview illuminates the boundary conditions for this doctoral thesis, leading to the following findings.

Key findings in perspective

1. Even with current state-of-the-art organ-protective measures, open TAAA repair remains associated with a considerable rate of acute organ damage. The rate of SCI seems to be favourably low, but the rate of AKI requiring renal replacement therapy remains high.

2. The biomarkers adrenomedullin and NGAL were confirmed as being predictive of mortality and peri-operative adverse events after open and endovascular TAAA repair in both retrospective and prospective settings.
3. Biomarkers for assessing AKI, in particular SLPI, TIMP-2, and IGFBP7, and ribonuclease (RNase) 1 and ribonuclease inhibitor 1 (RNH1), emerged as reliable sources of information, delivering fast, timely, and appropriate findings on post-operative kidney function after open TAAA repair.
4. A bedside testing kit for assessing postoperative kidney failure (Nephrocheck™) after open TAAA repair could detect AKI in a more timely way than with the clinically established biomarker serum creatinine. Using this point-of-care assessment, early measures such as renal replacement therapy could be introduced in a more timely way, potentially improving patient outcomes.

Open TAAA repair is related to a notable rate of acute organ damage (Chapters 2 and 3)

The first study retrospectively analysed outcomes with TAAA repair following elephant trunk or frozen elephant trunk implantation, and included 32 patients with different acute or chronic aortic pathologies. A high in-hospital mortality rate was observed (18.75%), and SCI occurred in two cases and temporary AKI in 42% (n = 13).

The estimated 1-year survival rate was 78.1% (95% CI 63.9%–95.6%) with a median follow-up of 1.29 years (IQR 0.26–3.88). We identified no procedure-related re-interventions and recorded one case of aortic-related mortality, namely sepsis because of graft infection. Thus, open TAAA repair following aortic arch repair including ET or FET because of acute or chronic aortic pathologies was associated with a relevant peri-operative morbidity and mortality rate. Yet, aortic-related mortality and procedure-related re-intervention rates during follow-up were low.

The second retrospective study evaluated 255 patients treated under emergency and elective conditions by open TAAA repair. The TAAA distribution was 25% type I, 26% type II, 23% type III, 18% type IV, and 7% type V. A total of 51 (20%) patients had an emergency procedure. Among all patients, 51% had a history of aortic surgery, 58% had experienced post-dissection TAAA, and 26% had a connective-tissue disease. In-hospital mortality rate among electively treated patients was 16% (n = 33) versus 35% (n = 18) in the emergency subgroup; the total mortality rate was 20% (n = 51). The adjusted odds ratio for in-hospital mortality following emergency repair compared with elective repair was 2.52 (95% CI 1.15–5.48). Temporary renal replacement therapy because of AKI was required in 29% (n = 74) of all patients, sepsis from some other cause was observed in 37% (n = 94), and SCI occurred in 7% (3% paraplegia).

The mean follow-up time was 3.0 years (median 1.5, 0–12.8 years). The aortic-related re-intervention rate was 2.8%, and the total mortality rate during follow-up was 22.5% (n = 46); 5.3% (n = 11) of all patients died because of aortic-related events.

Open TAAA repair was related to a relevant morbidity and mortality rate, yet the incidence of SCI was favourably low when a dedicated neuromonitoring protocol was applied. Aortic-related re-intervention and aortic-related mortality rate during follow-up were low.

Complications after open TAAA repair are relevant and common for post-dissectional or degenerative aneurysm under elective or emergency conditions.

Based on the retrospective character of these observational studies, certain limitations need to be considered in assessing the findings. Their retrospective nature and the sample size of the studies described in Chapter 2 carry a risk for selection bias, despite reporting being done according to the STROBE criteria and involving at least three researchers and one independent medical statistician during the data assessment. The decision to perform open repair in each included case was made by a panel of specialists from multiple medical disciplines, which as a non-randomised decision-making process could have been prone to bias, as well.

Nevertheless, these two retrospective studies summarise the results from specialised aortic centres focussed on open TAAA repair. Several aspects must be discussed when assessing patient outcomes after surgery. In the smaller series (Chapter 2), with a focus on open TAAA repair following previous ascending aortic and arch repair with elephant trunk or frozen elephant trunk, we identified no impact of emergency procedures on patient outcomes. In general, a relevant mortality rate was observed in this series, underlining the major impact of this surgical therapy in cases for which no endovascular options then existed. In the retrospective study assessing outcome after elective and emergency TAAA repair (Chapter 3), emergency procedures were related to significantly increased mortality and complication rates, which had a major impact on the total mortality rate in this cohort. In that study, even during follow-up after surgical repair under emergency conditions, the survival rate decreased compared with electively treated TAAA.

The frequency of AKI following open TAAA repair in both studies is important, particularly when renal replacement therapy is required. Under emergency conditions, no increased AKI rate was observed. Even when a specific intraoperative protocol to protect the kidneys against injury was applied, i.e., distal aortic perfusion and selective kidney perfusion using cardioplegia solution, an impact of ischaemia-reperfusion damage was observed. A dysregulated inflammatory reaction is likely involved in the pathogenesis of AKI and multi-organ failure after open TAAA repair (15). Further major findings of these studies are the frequency of pulmonary complications, the most common complication in both cohorts. Surgical access and temporary one-lung ventilation are well-known risk factors for postoperative pneumonia and acute respiratory failure, as confirmed in both studies (16). In

this context, the impact of increased organ damage, leading to the release of damage-associated molecular pattern (DAMP), may be a further cause of postoperative pneumonia (17-19).

SCI is a dreaded complication following open and endovascular TAAA repair and is related to increased peri-operative and total mortality rates after complex aortic surgery (20). In both studies, a comparatively moderate rate of SCI was observed. Specific strategies to prevent SCI, established over the last two decades by the leading surgeon, are likely behind this finding. Distal aortic perfusion seems to be important for reducing the inflammation-reperfusion reaction following aortic cross-clamping in open thoraco-abdominal aortic surgery and to enable improved spinal cord supply in the collateral network of the spinal cord (21). Cerebrospinal fluid drainage should be considered as a useful and established tool for observing and treating increased spinal cord pressure, particularly if combined with MEPs, which enables intra-operative assessment of spinal cord perfusion (21, 22). In the case of open thoracoabdominal aortic repair, the combination of distal aortic perfusion with implantation of selective intercostal artery bypass is a possible option for re-establishing perfusion of the spinal cord if MEPs are decreasing and the spinal cord is at risk of ischaemic injury (23). Accordingly, intercostal artery bypass was applied in almost every case with pathological findings during the intraoperative MEP assessment.

Aortic-related re-interventions, as reported in these studies, may be caused by failure of the open TAAA repair, namely stenotic target vessels or life-threatening infection of the aortic graft. Yet, as reported here, the majority of re-interventions and subsequent aortic procedures were related to disease progression, as non-surgically repaired segments of the aorta were treated. Compared to endovascular aortic repair, re-intervention after open TAAA repair (Chapter 3) during follow-up was favourably low (8). Furthermore, an aortic-related mortality rate of 2.9% was observed after discharge from the hospital, indicating a low aortic-related mortality rate and highlighting the favourable robust results of open TAAA repair (24, 25).

During follow-up, a long-term effect of peri-operative acute renal failure was observed, with a decreased survival rate in patients suffering from peri-operative AKI, as described in the literature (26). Again, these findings underline the relevance of peri-operative organ failure and the importance of each measure attenuating the severity of peri-operative complications.

The relevant mortality rate in the peri-operative period in case of open TAAA repair raises the question of whether an endovascular approach would have led to better outcomes, as a favourable low complication rate has been reported (10, 27). Yet, as described, the

interdisciplinary assessment of the treated aortic pathology, even in an elective setting, led to open repair, as endovascular options were limited. In general, the mortality rate of TAAA repair following FET is high, especially if compared with non–type II TAAA repair. Given that the mortality and morbidity rate following TAAA repair, even in specialized centres such as Aachen-Maastricht, is of utmost relevance, each aspect of treatment should be considered carefully to identify openings for improvement in survival. Against the background of the striking dominance of endovascular aortic solutions, this need has become even clearer. Yet, a large group of patients cannot be treated by endovascular means, whether they have aortic graft infection or connective tissue disease (11). Even in experienced centres, the results of endovascular aortic repair in CTD patients are moderate, and unfavourable complication rates have been reported (28, 29).

Strategies such as distal aortic perfusion during aortic cross-clamping, selective aortic clamping, and neuromonitoring including MEPs have already improved intraoperative outcomes, yet several factors could be leveraged peri-operatively. Below, we discuss one of these factors: application of novel biomarkers to detect organ dysfunction.

Confirmed in retrospective and prospective settings that biomarkers of patient outcomes in general after open and endovascular TAAA repair are predictive of mortality and peri-operative adverse events (Chapters 4–6)

The first prospective single-centre study discussed in this chapter included 33 patients (51.5% women, mean age 63.0 ± 16.2 years) undergoing elective complex open and endovascular aortic aneurysm repair. The biomarker bioactive adrenomedullin (bio-ADM) was measured for 72 h peri-operatively and an association with shock, death, and the combined endpoint of both was assessed. Furthermore, the association between bio-ADM and baseline characteristics and peri-operative details including sepsis biomarkers score was assessed. In this study, more than 50% of the patients developed postoperative AKI. Shock was observed in 12.1% (n = 4). The in-hospital mortality rate was 18.2% (n = 6), and 24.2% (n = 8) developed shock and/or died in the hospital. A significant correlation of bio-ADM concentrations from all available time points was observed with leucocytes ($P < 0.001$), C-reactive protein ($P < 0.001$), and serum creatinine ($P < 0.001$).

Increased bio-ADM at 12, 24, 48, and 72 h after admission to the ICU was associated with both in-hospital death and cardiogenic shock, with AUCs for the combined endpoint of 0.598, 0.720, 0.880, and 0.967, respectively, for each time point. Bio-ADM concentrations at 48 h and 72 h after admission to ICU were predictive for in-hospital death and shock (both $P < 0.01$). Accordingly, bio-ADM may serve as a postoperative biomarker for shock and death after complex open and endovascular aortic aneurysm repair, potentially enabling earlier and thus more adequate treatment of an adverse outcome after major surgery.

The second retrospective study focussed on peri-operative zinc levels in patients undergoing TAAA repair. For 33 patients, the relationship between zinc and outcome after TAAA repair was investigated. Patients who died (n = 6) had significantly decreased zinc levels before the intervention (zinc levels before intervention: 60.09 µg/dL for survivors vs. 45.92 µg/dL among non-survivors). Consistently, the post-interventional intensive care SOFA (Sepsis-related Organ Failure Assessment) score at day 2 and the SAPS (Simplified Acute Physiology Score) at day 2 were higher with low pre-interventional zinc levels. We found no significant correlation between patient comorbidities and zinc level before the intervention, except for peripheral arterial disease (PAD), which was significantly inversely correlated with reduced baseline zinc. Septic shock, pneumonia, and urinary tract infections were not associated with preoperative zinc levels or with zinc values during therapy. We infer from these findings that

decreased zinc levels before the intervention may be related to a poorer outcome because of poorer general physical status, along with possibly negatively altering the peri-operative inflammatory response.

In the third study, we assessed circulating dipeptidyl peptidase-3 ([c]DPP3) postoperatively in a prospective observational single-centre investigation and found postoperative organ failure in 17 patients (51.5%) and six deaths. At 12 h after ICU admission, cDPP3 levels were significantly increased in patients who later died or developed organ failure ($P < 0.001$). cDPP3 levels after surgery demonstrated a remarkable predictive accuracy for in-hospital mortality (12-h AUC: 0.907, $P < 0.001$; 24-h AUC: 0.815, $P = 0.016$; 48-h AUC: 0.914, $P = 0.003$) and development of organ failure (12-h AUC 0.882, $P < 0.001$; 24-h AUC: 0.850, $P < 0.001$; 48-h AUC 0.846, $P < 0.001$). Additionally, we found a significant correlation between cDPP3 and SOFA score ($P < 0.001$), procalcitonin ($P < 0.001$), C-reactive protein ($P = 0.011$), and IL-6 ($P = 0.007$) from all available measurements and time points.

These findings highlight the role of cDPP3 as an early, highly specific post-operative biomarker for in-hospital mortality and organ failure after TAAA repair.

Across the studies, we found a correlation of peri-operatively elevated biomarker levels in the case of adverse outcomes after open and endovascular TAAA repair. All but one of the assessed biomarkers were validated previously in the setting of major surgeries or with critically ill patients. The exception was cDPP3, which is a new biomarker that had not been assessed in this setting before our work (30, 31).

A limitation of all included studies that is relevant for this key message is the small number of included patients, which means that our studies on biomarkers remain rather hypothesis-generating despite promising results and appropriate test quality. In the cDPP3 study, the category “any organ failure” is somewhat non-specific and does not enable targeted measures to prevent organ failure, but it could be used as a predictive surrogate parameter for a patient’s risk assessment and outcome. Further clinical studies are required to verify the relevance of cDPP3, zinc, and bio-ADM as biomarkers for early detection of in-hospital mortality and organ failure after open and endovascular TAAA surgery.

In these studies, AKI and pneumonia were the most frequent complications (32, 33). It is widely known that a high incidence of organ failure ultimately results in a prolonged ICU stay. In this scenario, the use of biomarkers for risk assessment may have a positive impact on early patient treatment and potentially reduce complication severity and ICU hospitalisation.

Regarding bio-ADM, the present findings highlight for the first time the role of peri-operatively measured bio-ADM as a potential biomarker for the prediction of shock and mortality in patients after open or endovascular TAAA. The biomarker could be used for the early assessment of postoperative risk, as suggested by several trials (30, 34).

Decreased serum zinc levels before surgery, which can be interpreted as a concomitant effect of pre-existing diseases and an altered nutritional status, were observed in patients who died. Of note, we observed no association between preoperative zinc levels and most comorbidities, such as hypertension, coronary heart disease, chronic obstructive pulmonary disease, diabetes mellitus, medication (antihypertensives, beta-blockers, anticoagulants, diuretics, and opiates), or prior operations of the aorta. Decreased zinc levels before the intervention may be related to a poorer outcome because of a negatively altered peri-operative inflammation reaction, which could be significantly correlated with two intensive care risk scores (SOFA and SAPS) for patient survival. Therefore, a preoperative screening of zinc status could be considered for patients undergoing TAAA repair.

High cDPP3 blood levels in patients in cardiogenic shock at admission were associated with severe organ dysfunction, refractory shock, and high short-term mortality, whereas a reduction in cDPP3 levels within 24 h of admission was associated with improved outcomes (35-37).

Invasive surgery induces release of a flood of DPP3 into the bloodstream because of massive cell death, followed by systemic inflammation. The present study is the first to address the time course of cDPP3 levels and its clinical significance after TAAA repair. Here, cDPP3 levels were characterised by a rapid postoperative increase, followed by a decrease until 72 h after surgery, and postoperatively elevated cDPP3 levels were closely associated with poor outcomes, as assessed by SOFA scores after surgery. The significant correlation between cDPP3 levels and procalcitonin and IL-6 further supports its relevance as a potential biomarker in this setting, in association with the extent of surgery-related inflammation and the severity of the underlying disease (38).

The present findings further demonstrate that elevated cDPP3 levels postoperatively measured early after ICU admission showed remarkable predictive accuracy for the development of organ failure and, ultimately, in-hospital mortality. This result highlights cDPP3 as a promising predictive biomarker for detecting organ dysfunction in patients undergoing open

or endovascular TAAA repair, which are both associated with a sizable risk of severe complications (39).

The early prediction of adverse outcomes after these extensive surgical procedures based on biomarkers may be challenging and not well established, yet it can still be clinically relevant. The postoperative course after major surgical procedures, such as TAAA, is characterised by an overwhelming release of inflammatory markers and associated with complications during the first 48 h after surgery. As noted, identifying adequate biomarkers in this early time window has been a challenge. Yet biomarkers for risk assessment and outcome prediction, especially for in-hospital mortality and organ failure, would be desirable. Such a tool could be a useful addition to established surrogate parameters such as inotrope dosage, urinary output, and lactate for adequate initiation of treatment bundles leading to improved patient outcomes. In general, the identification of new blood-based biomarkers might enable their integration into postoperative surveillance, leading to an ameliorated diagnostic routine. In a comparison with more clinically established parameters, in the case of adrenomedullin, we even identified an increased specificity for pre-defined clinical endpoints such as cardiac shock. DPP3 showed a good predictive ability within the first 24 hours for the endpoints of in-hospital mortality (12-h AUC: 0.907, $P < 0.001$) and organ failure (12-h AUC 0.882, $P < 0.001$). By using these biomarkers, measures in intensive care such as fluid management and antibiotic therapy could be adopted in a timelier fashion.

Trials focussed on critically ill patients have shown results similar to ours for cDPP3, especially considering the limitations of this study, namely the small cohort of patients and non-randomised design (35, 36).

Biomarkers assessing AKI were successfully assessed as a reliable source of information, delivering fast, timely, and appropriate information for post-operative kidney function after open TAAA repair (Chapters 7–10)

Chapter 7 is formed by our retrospective study including 52 patients. Here UNGAL was measured peri-operatively (48 h) and correlated with AKI requiring dialysis, tracheotomy, and other adverse outcomes. A total of 26.9% (n = 14) developed AKI, 21.1% (n = 11) required renal replacement therapy (RRT), the tracheotomy rate was 19.2% (n = 10), and the in-hospital mortality rate was 7.6% (n = 4). uNGAL levels were related to AKI requiring dialysis in the ICU (p = .0002), need for tracheotomy at baseline (p = .0222), and admission to ICU (p = .0028), as well as adverse discharge modality, i.e., discharge via the weaning ward (p = .0051) or ICU (p = 0.0048). The diagnostic quality was good for uNGAL levels at admission to ICU regarding AKI requiring RRT (sensitivity: 81.8%, 95% CI 48.2%–97.7%; specificity: 87.8%, 73.8%–95.9%; AUC: 0.874, 0.752–0.949). The diagnostic quality of uNGAL was favourable for the prediction of tracheotomy (sensitivity: 70.0%, 34.8%–93.3%; specificity: 83.3%, 68.6%–93.0%; AUC: 0.807, 0.674–0.903) and adverse discharge (sensitivity: 77.8%, 40.0%–97.2%; specificity: 83.7%, 69.3%–93.2%; AUC: 0.817, 0.685–0.910). Accordingly, uNGAL may be valuable as a post-operative predictor of AKI and adverse outcomes after open and endovascular TAAA repair.

The second study of this sub-chapter evaluated the predictive abilities of RNase 1 and its antagonist RNH1 in patients undergoing TAAA repair. RNase 1 belongs to the group of antimicrobial peptides elevated in septic patients and indicates the prediction of two or more organ failures. In this study, we analysed RNase 1 and RNH1 serum levels in patients undergoing open (n = 14) or endovascular (n = 19) TAAA repair. The role of RNase 1 and RNH1 in post-operative AKI and in-hospital mortality prediction was assessed.

We found increased RNH1 serum levels after open TAAA repair compared with endovascular TAAA repair immediately after surgery and at 12, 48, and 72 h after surgery (all $p < 0.05$). Additionally, elevated RNase 1 and RNH1 serum levels at 12, 24, and 48 h after surgery were significantly associated with AKI (all $p < 0.05$). RNH1 serum levels before and RNase 1 serum levels 12 h after TAAA repair were significantly correlated with in-hospital mortality

(both $p < 0.05$). Based on these findings, RNase 1 and RNH1 may be therapeutically relevant and represent biomarkers for post-operative AKI and, to a lesser extent, in-hospital mortality.

In the third study, which focussed on AKI following TAAA repair, secretory leucocyte peptidase inhibitor (SLPI) was assessed. We tested whether SLPI measured in serum is an appropriate biomarker of AKI after TAAA. In a prospective observational single-centre study including 33 patients (51.5% women, mean age 63.0 ± 16.2 years) undergoing open and endovascular aortic aneurysm repair, SLPI was measured peri-operatively (until 72 h after surgery). After surgery, postoperative AKI was assessed, defined according to the KDIGO diagnostic criteria, sepsis, death, MACE (major cardiovascular events), and pneumonia. In a subgroup analysis, patients with pre-existing kidney disease were excluded. Of 33 patients, 51.5% (n = 17) of patients developed AKI. Twelve hours after admission to the ICU, SLPI serum levels were significantly increased in patients who developed AKI. Multivariable logistic regression revealed a significant association between SLPI at 12 hours after ICU admission and AKI ($P = 0.0181$, OR 1.055, 95% CI 1.009–1.103). The sensitivity of SLPI for AKI prediction was 76.47% (95% CI 50.1%–93.2%) and the specificity was 87.5% (61.7%–98.4%), with an AUC 0.838 (0.7–0.976) for an optimal cut-off of 70.03 ng/mL at 12 hours after surgery. In patients without pre-existing impaired renal function, an improved diagnostic quality of SLPI for AKI was observed (sensitivities of 45.45%–91.67%, specificities of 77.7%–100%, AUCs of 0.716–0.932). There was no association between peri-operative SLPI and the incidence of sepsis, death, MACE, or pneumonia. Based on our studies, SLPI might be a very suitable post-operative biomarker of AKI after TAAA repair, with a superior diagnostic accuracy for patients without pre-existing impaired renal function.

In the fourth study, we assessed the combination of two tissue biomarkers, inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7), which have been described as predictive for AKI in different settings but not in patients undergoing TAAA repair. A total of 40.4% (n = 21) of the patients in this retrospective observational study (N = 52) developed AKI, and 21.2% (n = 11) required renal replacement therapy. In-hospital and total mortality rates were 7.7% (n = 4) and 9.6% (n = 5), respectively. We never observed a significant difference in TIMP2*IGFB7 levels between patients undergoing open or endovascular surgery. The predictive potential of the TIMP2*IGFBP7 value on ICU admission was good regarding AKI requiring temporary renal replacement therapy (sensitivity: 55.56%, 95% CI 38.1%–72.1%; specificity 90.91%, 58.7%–99.8%) with

an AUC of 0.694 (95% CI 0.543–0.820). Mean follow-up was 13.2 months (min–max: 2–20). For 90-day mortality, the predictive potential of the TIMP2*IGFBP7 value was not sufficient (sensitivity: 80%, 28.4%–99.5%; specificity: 52.38%, 36.4%–68%), with an AUC of 0.607 (0.454–0.746). The combination of TIMP2*IGFBP7 level measured early after TAAA repair may be able to detect AKI requiring RRT. Yet, compared with biomarkers assessed in the other studies in this thesis, the diagnostic accuracy is moderate.

Based on the preliminary results that we summarized in the perspective, the assessment of the bedside testing kit Nephrocheck™, which includes TIMP2*IGFBP7 as a biomarker combination, seems to enable timely and appropriate detection of AKI in the field of complex open aortic surgery. In this prospective, multicentre study including more than 50 patents undergoing open TAAA repair according to an *a priori* protocol, patients were observed in the ICU for 72 h using Nephrocheck™. Based on an interim analysis including 24 patients, AKI occurred in 19 (17 men). Fourteen patients (58.3%) developed an AKI stage 3 (KDIGO) and required RRT. In five cases (20.8%), AKI stage 1 (KDIGO) was diagnosed. The diagnosis was made in most cases on the first postoperative day (time to diagnosis: 0–11 days, median 1 day). Although postoperative complications such as pneumonia and systemic sepsis were associated with AKI ($p = .007$ and $p = .023$, respectively), we found no correlation between duration of surgery and the occurrence of AKI ($p = .777$).
Nephrocheck™ measurements showed a significant increase at 12 hours ($t (14.776) = -2.485$, $p = .025$), 24 hours ($t (17.419) = -2.152$, $p = .046$), and 48 hours ($t (12.032) = -2.565$, $p = .025$). Direct postoperative measurements did not significantly differ from the baseline. ROC curve analysis showed a reliable diagnostic value of the Nephrocheck™ system starting at the 12-hour mark (sensitivity 84.6%, specificity 77.8%, AUC 0.833). Based on the robust design of this study, supporting the previous findings of our research projects, timely detection of AKI by application of novel biomarkers after TAAA repair is possible and useful, enabling more appropriate treatment strategies including RRT on ICU.

In our continued efforts to understand the pathophysiology of AKI after TAAA repair, we assessed several partially established, partially novel biomarkers for AKI.

We found that *uNGAL* can be used as a postoperative biomarker of AKI leading to dialysis, tracheotomy, and adverse discharge modality, i.e., discharge via the weaning ward or ICU, after open and endovascular TAAA repair, with a surprisingly adequate statistical significance for a retrospective study. Our study of TIMP-2/IGFBP7 showed similar potential

findings, but not with the same diagnostic quality. The same applies for RNase 1 and RNH1, whereas SLPI outmatched the diagnostic abilities of uNGAL. The relevant limitations of serum creatinine, such as lack of specificity and late and nonspecific increase after kidney damage, as an indirect marker of impaired renal function have been reported previously (40, 41). Only a timely initiation of treatment bundles including renal replacement therapy could improve the long-term survival of patients who suffered from AKI (42). Few biomarkers of postoperative complications have been investigated in the setting of TAAA. Therefore, it may be beneficial to identify appropriate serum markers of kidney injury that could be used in routine diagnostics for AKI. Here, the best predictive accuracy during the first 12 to 24 hours after surgery was attributed to SLPI, suggesting that it is an early biomarker.

The lack of relevant differences in serum SLPI levels in the endovascular and open repair groups is an interesting finding. Twelve hours after a complex aortic intervention, patients with AKI had significantly increased serum SLPI levels, and SLPI negatively correlated with urine output. Serum SLPI performed well at predicting AKI, with a promising diagnostic accuracy at 12 and 24 hours after ICU admission, which was confirmed in multivariable analysis.

Regarding risk assessment, pre-operatively increased serum creatinine >1.25 mg/dL could be applied in risk stratification for AKI after major surgery, with increasing accuracy after exclusion of patients suffering from pre-existing renal failure (43, 44). Dysregulated inflammation may be involved in the pathogenesis of AKI after TAAA repair. Thus, the extensive release of SLPI during aortic surgery may be part of an inflammatory response and compensatory mechanism to balance the inflammatory reaction (45). This hypothesis is supported by our observation of a significant correlation between serum SLPI and procalcitonin, an early inflammatory marker of the immune response, 24 and 72 hours after surgery. Yet, based on the statistical analysis, we found a clear correlation of impaired renal function, acute kidney injury, and elevated biomarker levels for all three described biomarkers.

Our studies confirmed several biomarkers as predictors of postoperative AKI after open and endovascular TAAA repair. In particular, SLPI can be highlighted as a promising new biomarker for detecting postoperative AKI within 72 hours after open and endovascular TAAA repair, which may enable early initiation of renal replacement therapy, potentially leading to better survival, as suggested previously (42). Early decision-making leading to renal replacement therapy cannot be accurately performed based on biomarkers such as serum creatinine; even if renal function limitation of more than 50% leads to increased serum

creatinine levels, which may be associated with oliguria or polyuria, these levels are not specific for impaired renal function (46-48).

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Chapter 13

Scientific and Social Impact

Open TAAA repair remains a relevant and necessary surgical approach for treating potentially life-threatening aortic pathologies. Open repair is especially the only option for surgical therapy and cure in young patients suffering from connective tissue disease (e.g., Marfan syndrome), in patients with infected aortic aneurysms, or after failed endovascular procedures. Despite improvements in procedural techniques and moderation of the intensity of aortic cross-clamping–induced ischaemia-reperfusion damage, the relevant rate of perioperative complications, such as AKI, SCI, and pulmonary failure, as well as long-term ventilation remains unclear. Even in experienced centres such as Maastricht and Aachen, where distal aortic perfusion and selective perfusion of the viscero-renal vessels are part of the standard procedure during open TAAA repair, major complication rates of more than 20% are common.

Today, endovascular TAAA repair is an adequate treatment alternative in most cases. This observation emphasizes the importance of scientific analysis of outcomes with open TAAA repair, with the aim of improving surgical and perioperative modalities. Even if patients receive adequate treatment in the intensive care unit (ICU), assessment during the first 24 hours remains a challenge, and established tools enabling detection of adverse outcomes and organ failure may not work. Novel biomarkers could enable timely assessment of clinical but undetectable organ failure, leading to earlier treatment and potentially better patient outcomes. Based on the findings described in this thesis, the evaluated biomarkers, alone or in combination, could be entered into clinical practise and be applied in ICU monitoring after major surgery. Such a tool could potentially lead to more precise detection and treatment of adverse outcomes. In the 21st century, biomarkers could strengthen the application of artificial intelligence in ICU wards, which relies on clinical surrogate parameters such as laboratory findings, enabling early detection of organ failure. The close scientific cooperation of the PhD candidate's working group with companies such as Sphingotec® (Berlin) will be helpful in assessing the reasonable application of biomarkers after major surgery in general.

Insights from these studies focussing on complex aortic surgery may be transferred to or re-evaluated in other surgical settings. The perspective from a prospective, multicentre TAAA study focussing on early detection of AKI is unique in Germany and, as far as the author is aware, possibly Europe. The candidate hopes that the findings described in this PhD thesis

will one day be seen as a step toward a better understanding of pathophysiological changes following open TAAA repair.

Our studies underline the consistently relevant rate of major complications following open (and to a lesser extent, endovascular) surgery. Although surgical techniques such as open TAAA repair have evolved to improve patient outcomes, a more accurate and timely postoperative assessment based on early use of biomarkers of organ damage, e.g., AKI, seems necessary. A prospective, multicentre assessment of outcomes after open TAAA repair in combination with a biomarker panel could advance understanding of pathophysiological changes, such as the dysregulated inflammation reaction following aortic cross-clamping.

Finally, biomarkers could reduce therapy costs for patients undergoing emergency and elective major surgery by leading to a shorter stay in the ICU, shorter artificial ventilation time, and faster recovery after surgery.

The biomarkers evaluated here are relevant to postoperative or postinterventional adverse effects, and their potential role as prognostic indicators was not assessed in the included studies.

Chapter 14:

Summary:

Aortic aneurysms are most often caused by degenerative changes of the aortic wall involving all three layers: the intima, media, and adventitia. Thoracoabdominal aortic aneurysms (TAAAs) involve both the thoracic and abdominal parts of the aorta. Although the disease is slowly progressive, with an average annual growth rate of 3–5 mm, the related morbidity and mortality are important, especially because mortality in case of aneurysm rupture is dramatically high. The final purpose of aortic aneurysm treatment is preventing aneurysm rupture, a fatal condition with high morbidity and mortality rates even after successful emergency repair. The development of endovascular techniques has increased safety and improved outcomes after surgery for aortic pathologies such as thoracic aortic aneurysm and infrarenal aortic aneurysm, which can be treated with an endovascular approach in many cases. In case of more complex aneurysms involving the thoracoabdominal aorta or the juxtarenal aorta, however, morbidity and mortality rates remain troubling, even in experienced vascular surgical departments with the necessary expertise to treat these complex cases. Even if the surgical therapy, whether open or endovascular, is uneventful, the impact of the surgical approach and certain specific requirements have a relevant and potentially negative impact on patient outcomes. In endovascular repair, the length of a covered aortic segment may lead to dysregulated activation of the endothelium-related inflammation reaction. If TAAA repair is performed in an open procedure, aortic cross-clamping, distal aortic and selective organ perfusion using a heart-lung machine, and systemic anticoagulation are requirements. At the same time, however, there are well-known risk factors for adverse outcomes induced by ischemia-reperfusion damage and upregulated inflammation cascade. The perioperative changes induced by activation of the inflammation system have an unknown aetiology and may lead to an unpredictable and adverse course after an initially successful surgical treatment.

My motivation in this work was to improve understanding of these pathophysiological processes, enabling earlier detection and thus timelier treatment in critically ill patents. My genuine interest in clinical assessment of patients pre-, peri-, and postoperatively is in keeping with my chosen profession as a vascular surgeon dealing daily with complex aortic aneurysm patients in the operating room and the intensive care unit. Based on the high risk of

complications, especially in the first postoperative days, a rapid and more appropriate surveillance of critically ill patients would be favourable. Physicians are trained to use different, clinically established biomarkers that could improve patient assessment. In these cases, the biomarkers are signal and effector molecules of different tissue origins that could be used as indicators for adverse events such as organ failure, chronic and acute processes such as bacterial infection, and deteriorated general condition. By applying this "early warning system", physicians working with critically ill patients might be able to recognize and treat them in a more timely fashion, potentially leading to improved outcomes.

In the fields of open and endovascular TAAA repair, despite an urgent clinical need, the use of specific biomarkers for organ damage and poor general condition has not been established to date. In my doctoral thesis, "The assessment of biomarkers focusing on organ-specific and general outcomes in patients undergoing open and endovascular TAAA repair", my aim is to contribute to improved outcomes for patients who have undergone major aortic surgery. Furthermore, my findings in this specific aortic field could be transferred to other surgical disciplines, facilitating comparable studies in the future. Based on the results described in my thesis, several biomarkers could be used to establish a more appropriate postoperative assessment of patients undergoing major surgery such as open and endovascular TAAA repair. Especially, early detection of postoperative acute kidney injury is of utmost importance as it may lead to timelier treatment, which could be related to better outcomes for the patients.

Chapter 15

Nederlandse Samenvatting

Aorta aneurysmata worden meestal veroorzaakt door degeneratieve veranderingen van de aortawand waarbij alle drie lagen van de vaatwand betrokken zijn: de intima, media en adventitia. Dit proefschrift beschrijft thoracoabdominale aorta aneurysmata (TAAA's) die zowel de thoracale als de abdominale aorta omvatten en hoewel langzaam progressief, met een gemiddelde jaarlijkse groei van 3-5 mm, een aanzienlijke gerelateerde morbiditeit en mortaliteit hebben. Het uiteindelijke doel van de behandeling van een aorta aneurysma is het voorkomen van een ruptuur van het aneurysma. Een ruptuur is een vaak fatale aandoening met hoge morbiditeit indien niet fataal, zelfs na initieel succesvolle spoedoperatie. De ontwikkeling van endovasculaire technieken heeft de resultaten van chirurgie voor aortapathologieën zoals thoracale aorta aneurysma en infrarenaal aorta aneurysma (AAA) verbeterd. In het geval van complexere aneurysmata waarbij de thoracoabdominale aorta of de juxtarenale aorta betrokken zijn, blijven de morbiditeits- en mortaliteitscijfers van chirurgie echter hoog, ook op ervaren vaatchirurgische afdelingen met veel expertise in deze complexe gevallen. Zelfs als de chirurgische aneurysmavervanging, open of endovasculair, probleemloos verloopt, hebben de impact van de chirurgische benadering en bepaalde andere karakteristieken van de behandeling een relevante en mogelijk negatieve impact op de uitkomst. Bij endovasculair herstel van een TAAA kan de uitgebreide bedekking van de aorta leiden tot activering van een endotheelgerelateerde ontstekingsreactie. Als open TAAA herstel wordt uitgevoerd, zijn het klemmen van de aorta, distale aorta en selectieve orgaanperfusie met behulp van een hart-longmachine en systemische antistolling vereist. Tegelijkertijd zijn dit echter bekende risicofactoren voor nadelige uitkomsten van aorta operaties veroorzaakt door ischemie-reperfusieschade en een opgereguleerde ontstekingscascade. De perioperatieve veranderingen die worden veroorzaakt door activering van het ontstekingssysteem hebben een onbekende etiologie en kunnen leiden tot een onvoorspelbaar en ongunstig beloop na een aanvankelijk succesvolle chirurgische TAAA behandeling.

Mijn motivatie in dit werk was om het begrip van deze pathofysiologische processen te verbeteren, waardoor eerdere detectie en dus tijdigere behandeling van ernstig zieke patienten mogelijk zou worden. Mijn oprechte interesse in de klinische beoordeling van patiënten,

zowel pre-, peri- en postoperatief, past bij mijn beroep als vaatchirurg die dagelijks te maken heeft met complexe aorta aneurysmapatiënten in de operatiekamer en op de intensive care. Gezien het hoge risico op complicaties, vooral in de eerste postoperatieve dagen, zou een snelle en meer geschikte beoordeling van ernstig zieke patiënten gunstig zijn. Artsen zijn opgeleid om verschillende biomarkers te gebruiken om de status van een patient te beoordelen. In deze gevallen zijn de biomarkers signaal- en effectormoleculen van verschillende weefsels die kunnen worden gebruikt als indicatoren voor complicaties zoals orgaanfalen, chronische en acute processen zoals bacteriële infectie en een verslechterde algemene toestand. Door dit "systeem voor vroegtijdige waarschuwing" toe te passen, kunnen artsen die met ernstig zieke patiënten werken, deze mogelijk sneller herkennen en behandelen, wat mogelijk tot betere resultaten leidt.

Op het gebied van open en endovasculaire TAAA herstel is het gebruik van specifieke biomarkers voor orgaanschade en een slechte algemene conditie, ondanks een dringende klinische behoefte, tot op heden niet standaard. In dit proefschrift, "Biomarkers voor orgaanspecifieke en algemene uitkomsten bij patiënten die open en endovasculaire TAAA chirurgie ondergaan", is mijn doel om bij te dragen aan betere resultaten voor patiënten die een grote aorta operatie hebben ondergaan. Bovendien kunnen mijn bevindingen op dit specifieke aortagebied worden overgedragen naar andere chirurgische disciplines, waardoor vergelijkbare studies in de toekomst mogelijk worden. Op basis van de resultaten beschreven in mijn proefschrift, zouden verschillende biomarkers kunnen worden gebruikt om patiënten die een grote operatie ondergaan, zoals open en endovasculair TAAA herstel, adequater te kunnen beoordelen in de postoperatieve fase. Vooral vroege detectie van postoperatief acuut nierletsel is van het grootste belang, omdat snelle behandeling hiervan kan leiden tot betere resultaten voor de patiënten.

Appendix

About the Author

Alexander Gombert was born on 12 July 1983 in Aachen, Germany.

He completed his early education in 2002 in Monschau, Germany, and studied medicine in Aachen from 2004 until 2010, after one year of civilian service. Starting his medical education in 2011 in the Marienhospital Aachen, he continued his medical education in the Department of Vascular Surgery of the University Hospital RWTH Aachen in 2013. He received his doctoral degree in the Department of Plastic and Reconstructive Surgery, under leading surgeon Univ.-Prof. Dr. med. Pallua in 2013.

He completed his vascular surgical training in 2017, becoming a Fellow of the European Board of Vascular Surgery in 2018.

He received his postdoctoral lecture qualification/Venia Legendi in 2019, focusing on outcomes after thoracoabdominal aortic surgery:

“Der Stellenwert organspezifischer Biomarker und modifizierter Behandlungskonzepte zur Verbesserung des Outcomes nach offener und endovaskulärer thorakoabdomineller Aortenchirurgie”.

Since 2018, he has served as a reviewer for more than 35 peer-reviewed journals, with regular review activity for the European Journal of Vascular Surgery (EJVES), British Journal of Surgery (BJS), and Journal of Vascular Surgery (JVS). Since 2019, he has been on the editorial board of Scientific Reports and EJVES, and since 2020, he has been a key reviewer for JVS. Furthermore, he is guidelines co-director of the European Society of Vascular Surgery (ESVS) Guideline Update for Descending Thoracic Aortic Aneurysm, set for online publication in 2022. Since 2021, he has been an ESVS Fellow, an honour membership for ESVS members with distinguished status within the vascular community. In 2021, he began contributing as an assistant editor of EJVES – Vascular Forum and was elected an International Member of the Society of Vascular Surgery.

He is involved in multiple national and international research projects focusing on basic research in the fields of aortic surgery and perioperative outcomes. Up to Fall 2021, he had received grants in the amount of 223,000 € for his research projects. A grant application for 680,000 € for the cooperative project “LowProTex2” between the Department of Vascular Surgery and the Institut für Textiltechnik, RWTH Aachen University, is pending.

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