

Tubular Biomarkers N-Acetyl- β -D-Glucosaminidase, Kidney-Injury-Molecule 1 and Neutrophil Gelatinase-Associated Lipocalin: Long-Term Follow-Up in a Chest Pain Cohort

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Keywords

Tubular markers · N-acetyl- β -D-glucosaminidase · Acute coronary syndrome

Abstract

Introduction: The goal of the current study was to evaluate the prognostic value of the potential cardiorenal biomarkers kidney injury molecule-1 (u-KIM-1), N-acetyl- β -D-glucosaminidase (NAG) and urinary neutrophil gelatinase-associated lipocalin (u-NGAL) regarding mortality and major adverse cardiac events (MACEs) in patients presenting with acute chest pain in the emergency department. **Methods:** The study cohort consisted of 294 patients who presented themselves with acute chest pain in the emergency department of the university hospital Regensburg. The urinary concentrations of NAG, KIM-1, and NGAL were measured alongside troponin I and NT-proBNP. The patients were followed for 60 months regarding the primary endpoints mortality of any cause and MACEs, consisting of mortality, stroke, congestive heart failure and acute coronary

syndrome with the necessity of PCI was gathered (MACE). **Results:** Fifty four patients died during the follow-up and 97 suffered from MACE. Regarding ROC-analysis NAG as well as u-KIM-1 showed promising predictive values (all-cause mortality: AUC_{NAG} 0.821, AUC_{KIM-1} 0.745, MACE: AUC_{NAG} 0.778, AUC_{KIM-1} 0.692). u-NGAL showed less promising AUCs regarding mortality (AUC 0.69) as well as MACE (AUC 0.66). According to the Kaplan-Meier analysis, patients with concentrations of urinary N-acetyl- β -D-glucosaminidase, u-KIM-1 or u-NGAL > median showed a significant worse outcome regarding MACE as well as all-cause mortality (each $p < 0.05$). Furthermore, Cox-regression analysis revealed NAG as independent predictor beside NT-proBNP and older age for mortality due to any cause and MACE (each $p < 0.001$), opposite to u-KIM-1, hypertension and diabetes (each $p = \text{n.s.}$). **Conclusion:** Urinary N-acetyl- β -D-glucosaminidase and, to a lesser extent, kidney injury

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molecule-1 showed significant value in prediction MACEs and mortality due to any cause in patients with acute chest pain. But the clinical usability is still not certain and further studies are required.

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Introduction

Acute coronary syndrome (ACS) is a syndrome consisting of three entities: ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UA) [1]. It is estimated that 30–50% of patients suffering a STEMI die before reaching the hospital and 4–6% still die in hospital [1]. Patients with NSTEMI or UA seem to have a better prognosis, but nevertheless 15% suffer from reinfarction or death within 30 days [2].

ACS may cause heart failure, but it can also trigger injuries in other organs. A common secondary organ failure during ACS is acute kidney injury (AKI), defined as an acute reduction of the renal function within 48 h, diagnosed by an absolute or relative increase in serum creatinine. An AKI caused by an acute or chronic injury of the heart is part of cardiorenal syndrome [3].

About 30% of all patients with ACS suffer from an AKI during the ACS [4] and patients with cardiorenal syndrome during ACS show an increased rate of mortality and major adverse cardiac events (MACE) [5]. Unfortunately, creatinine is a late indicator in acute changes of renal function. Serum creatinine concentrations may not increase until a significant amount of renal function has already been lost due to a large renal reserve, which may be as late as 48–72 h after the precipitating injury. However, animal studies have shown that treatment of AKI should be initiated long before serum creatinine ever rises [6]. A promising alternative would be the use of additional biomarkers. Urinary N-acetyl- β -D-glucosaminidase (u-NAG), urinary kidney-injury molecule 1 (u-KIM-1) and urinary neutrophil gelatinase-associated lipocalin (u-NGAL) are such promising markers. NAG cannot be filtered by the glomerulus because of its high molecular weight; therefore, it is released into the tubule lumen because of proximal tubule damage [7]. In animal studies, u-KIM-1 in urine reflected changes in protein expression and histopathological changes in the renal parenchyma without functional changes [8]. Several experimental and clinical studies have shown that the expression of u-NGAL in urine and serum increases significantly in AKI [9].

The tubular markers u-NAG, u-KIM-1, and u-NGAL have previously been implicated as indicators of ischemic renal injury associated with cardiorenal syndrome [10] in chronic [11–14] and acute [15–18] heart failure. The tubular markers showed prognostic value regarding renal injury, the ischemic events during cardiopulmonary bypass [19], stroke [20], and after ACS and cardiac surgery [21, 22]. With the use of those biomarkers, it was also possible to detect an AKI in patients with chronic renal insufficiency [23, 24]. In patients suffering from congestive heart failure, increased tubular markers were associated with a worse prognosis regarding cardiac and renal endpoints [10, 11]. The aim of this study was to evaluate the prognostic value of the tubular markers regarding the long-term prognosis in real-life collective of patients suffering from acute chest pain.

Methods

Study Population

From February 2015 to July 2015 and in September and October 2015, 402 patients with acute chest pain were admitted to the emergency department of the university hospital Regensburg. In August 2015, due to a lack of staff, no patients could be recruited. Patients younger than 18 years, pregnant women and patients who did not give their consent were excluded from the study. Patients with known CKD or patients on dialysis were not excluded as long as they were not anuric. All patients suffering from chest pain were included, since the goal was to evaluate the prognostic value of tubular markers in this cohort. For a further evaluation patients suffering from ACS were analyzed as a subgroup. The study design was approved by the institutional Ethics Committee and designed according to the standards established for human experimentation by the Declaration of Helsinki.

The blood and urine samples were collected as soon as possible without delaying necessary treatment. In case of patients suffering from STEMI, the urinary samples were in some cases collected immediately after the PCI. Patients without an initial measurement of NT-proBNP, u-NAG, u-KIM or u-NGAL were not included in the current analyses. Therefore, urinary biomarkers as well as cardiac biomarkers were collected in 294 patients presenting to the emergency department. Depending on the diagnosis, the patients were classified into ischemic and nonischemic chest-pain groups (online suppl. Fig. S1; for all online suppl. material, see <https://doi.org/10.1159/000552700>).

In the ischemic chest-pain group ACS was categorized according to the guidelines of the European Society of Cardiology as unstable angina pectoris, non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI) [25]. The second category included any cause of chest pain excluding unstable angina pectoris, NSTEMIs and STEMI.

According to the ESC-guidelines, patients with typical angina (at rest, new or crescendo) but no changes in troponin were classified as unstable angina. Patients with changes in troponin (up trending or down trending) or elevated levels of troponin, but without significant ST-elevation were classified as NSTEMI and patients with significant ST-elevation were classified as STEMI.

In case of a STEMI, the diagnosis of the ECG was confirmed by a cardiologist before intervention. All ECGs were retrospectively approved by members of the study team. ST-elevations were considered significant if they exceeded 0.25 mV in V2 and V3 in men younger than 40 years, 0.2 mV in men older than 40 years or 0.15 mV in women. In the remaining leads, ST-elevations exceeding 0.1 mV were considered significant. Two corresponding leads had to be elevated for the diagnosis of STEMI [26].

Follow-Up

All participants were contacted for follow-up according to an established routine. Initially, the patient's attending physician was approached by means of a standardized questionnaire. If the patient did not visit their physician in the last 30 days, the patient was contacted directly. This way, the medical history since the last follow-up including mortality and the occurrence of MACEs, defined as a combination of mortality, stroke, ACS with the necessity of PCI and congestive heart failure was gathered. If the patient was deceased, the available medical information was gathered, and the date and cause of death were documented. The aim of this study was to evaluate the correlation of the tubular markers with long-term mortality in a collective of patients suffering from chest pain. Therefore, death of any cause and at any time during the follow-up was chosen as endpoint.

Biochemical Analyses

For the evaluation of all common biomarkers, Siemens Dimension Vista 1500 autoanalyzers were used in the in-house laboratory on the day of col-

lection. Urinary creatinine concentrations (UCr) were measured in our laboratory via the enzymatic assay ECREA (Fa. Siemens Medical Solutions Diagnostik GmbH).

Only urine from spontaneous voided samples was used for the measurement of tubular biomarkers. Urine gathered from urinary catheterization was excluded. It was stored in sterile cups, centrifuged and the supernatant gathered this way was deep-frozen at -80°C . For the determination of the tubular biomarkers investigated, further processing was performed using the analytical procedure required for this purpose (u-NAG: NAG-assay [Roche, Germany], KIM-1: u-KIM ELISA KIT [R&D System, UK], u-NGAL: NGAL ELISA KIT [BioPorto, Denmark]). To offset potential bias created by difference in urinary concentration, tubular biomarkers were set in ratio to the urinary creatinine.

Statistics

Normally distributed, descriptive data are presented as mean $\pm$ standard deviation, not normally distributed as medians and IQR. Correlation coefficients were evaluated according to Spearman. The Mann-Whitney U test was performed for continuous non-normally distributed variables. The Kaplan-Meier curves were used for follow-up analysis, representing the time of follow-up and the probability of reaching the endpoint mortality and MACE. This was performed for the overall study cohort as well as for the subgroup of patients with ACS. For further evaluation of a high-risk collective, the cohort was divided in three groups. The first group included patients with u-NAG and NT-proBNP greater than the median, the second patients with u-NAG greater than the median and NT-proBNP below the median or vice versa and the third group included patients with u-NAG and NT-proBNP below the median.

ROC analysis was used to evaluate the specificity and sensitivity of the biomarkers and DeLong's test was used to assess the difference in AUC. Cox-regression analysis was used for the evaluation of possible associations between biomarker and endpoint. Furthermore, creatinine, older age, diabetes, coronary artery disease (CAD), hypertension and sex were included to the analysis. Baseline characteristics were included in Cox regression, if they were distributed in a statistically significant manner to address potential confounders. Sex was added due to its clinical relevance and CAD and the medication with diuretics, ACE-/AT-1-inhibitor or

Table 1. Baseline characteristics

	Total study cohort (n = 294)	Survived (n = 240)	Deceased (n = 54)	p value
Age (IQR), years	63 (53–74)	59 (51–71)	77.5 (69.8–82.3)	<0.001
Sex (male), n (%)	211 (71.8)	171 (71.3)	40 (74.1)	n.s.
Hypertension, n (%)	186 (63.3)	144 (60.0)	42 (77.8)	0.015
Diabetes, n (%)	76 (25.9)	54 (22.5)	22 (40.7)	0.006
HLP, n (%)	166 (56.5)	141 (58.8)	25 (46.3)	n.s.
CAD, n (%)	112 (38.1)	89 (37.1)	23 (42.6)	n.s.
AF, n (%)	40 (13.6)	21 (8.8)	19 (35.2)	<0.001
CKI, n (%)	41 (13.9)	20 (8.3)	21 (38.9)	n.s.
Congestive HF, n (%)	8 (2.7)	8 (3.3)	0 (0)	n.s.
Pacemaker, n (%)	16 (5.4)	11 (4.6)	5 (9.3)	n.s.
Apoplex/TIA, n (%)	15 (5.1)	14 (5.8)	1 (1.9)	n.s.
Final diagnosis at discharge				
STEMI, n (%)	49 (16.7)	36 (15.0)	13 (24.1)	n.s.
NSTEMI, n (%)	52 (17.7)	42 (17.5)	10 (18.5)	n.s.
Unstable angina, n (%)	71 (24.1)	61 (25.4)	10 (18.5)	n.s.
Chest wall syndrome, n (%)	56 (19.0)	50 (20.8)	6 (11.1)	n.s.
Acute treatment				
PCI, n (%)	115 (63.2)	89 (37.1)	26 (48)	n.s.
CABG, n (%)	5 (1.7)	5 (2.1)	0 (0)	n.s.
Medication at admission				
ASS, n (%)	111 (37.8)	89 (37.1)	22 (40.7)	n.s.
Beta-blocker, n (%)	129 (43.9)	101 (42.1)	28 (51.9)	n.s.
ACE/AT1-antagonists, n (%)	128 (43.5)	102 (42.5)	26 (48.1)	n.s.
Ca-antagonists, n (%)	53 (18.0)	41 (17.1)	12 (22.2)	n.s.
Diuretics, n (%)	88 (29.9)	58 (24.2)	30 (55.6)	<0.001
Clopidogrel, n (%)	18 (6.1)	13 (5.3)	5 (9.3)	n.s.
Prasugrel, n (%)	3 (1.0)	3 (1.3)	0 (0)	n.s.
Ticagrelor, n (%)	8 (2.7)	7 (2.9)	1 (1.9)	n.s.
Statin, n (%)	92 (31.3)	74 (30.8)	18 (33.3)	n.s.
Blood work				
u-NAG (IQR), U/gCr	3.23 (1.74–6.22)	2,67 (1.51–4.56)	8.31 (4.68–13.89)	<0.001
u-KIM (IQR), ng/gCr	826 (437–1,486)	765 (399–1,251)	1,609 (825–3,279)	<0.001
u-NGAL (IQR), U/gCr	28.5 (13.4–74.0)	23.6 (11.8–54.2)	67.2 (22.8–175.5)	<0.001
NT-proBNP (IQR), pg/mL	291 (69–1,506)	201 (58–838)	2,426 (1,080–8,392)	<0.001
Troponin I (IQR), ng/mL	0.01 (0.003–0.039)	0.008 (0.003–0.020)	0.037 (0.024–0.070)	0.045
CK (IQR), U/L	126.0 (73.5–254.0)	133.0 (82.0–255.0)	56.5 (85.0–242.3)	n.s.
CK-MB (IQR), U/L	2 (1–6)	2.5 (1.0–6.5)	2.0 (1.0–29.5)	n.s.
Creatinine (IQR), mg/dL	0.93 (0.79–1.15)	0.90 (0.77–1.02)	1.29 (1.01–1.70)	0.001
eGFR MDRD (IQR), mL/min/1.73 m ²	84.0 (59.0–98.8)	88.0 (67.8–100.0)	49.5 (35.8–68.3)	0.001

HPL, hyperlipoproteinemia; CAD, coronary artery disease; AF, atrial fibrillation; CKI, chronic kidney disease; CABG, coronary artery bypass grafting; CK, creatine kinase; CK-MB, creatine kinase myocardial band; eGFR, estimated glomerular filtration rate; IQR, interquartile range; PC, percutaneous coronary intervention.

aldosterone antagonists due to the relevance for the ACS-cohort and potential interactions with tubular markers. All data were analyzed by using a commer-

cially available statistical software package (SPSS 25.0, SPSS Inc., IL, USA; MedCalc 19.6.4, MedCalc Software, Mariakerke, Belgium).

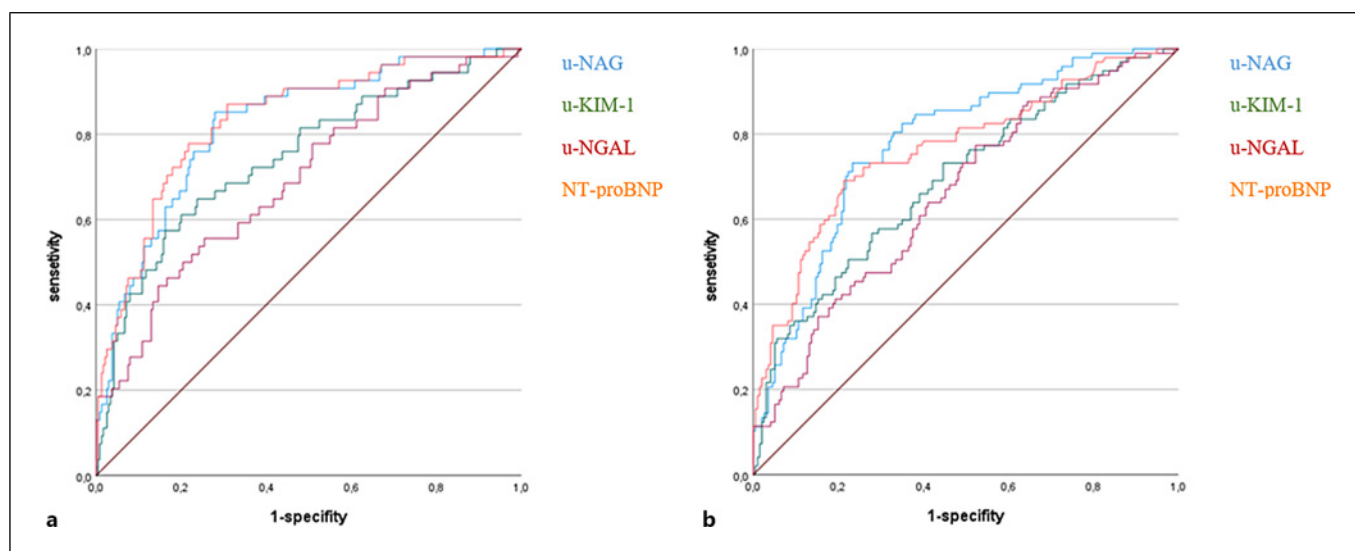


Fig. 1. **a** ROC analysis regarding mortality; u-NAG (blue), u-KIM-1 (green), u-NGAL (red), NT-proBNP (orange). **b** ROC analysis regarding MACE; u-NAG (blue), u-KIM-1 (green), u-NGAL (red), NT-proBNP (orange).

Results

Baseline Characteristics

A total of 294 patients were included in the current analysis. The baseline-characteristics are shown in Table 1. The patients were mainly male (71.8%) with a mean age of 63 years. A total of 172 patients presented with ACS in the emergency room. In total, 49 of those patients suffered from a STEMI, 52 patients from a NSTEMI and 71 patients from angina pectoris. Hypertension was evident in 63.3% and CAD in 38.5%. A total of 29.9% were obese and 14.1% suffered from CKD, including 3 patients undergoing chronic hemodialysis with residual urine output. Those 3 patients were able to provide spontaneous voided urine samples and therefore were not excluded from the analysis. 5.1% had a previous stroke in their medical history and 2.7% a history of congestive heart failure.

The measured levels of u-NAG, u-KIM and u-NGAL correlated positively and significantly with each other ($\rho = 0.75$, $p < 0.01$ for u-NAG and u-KIM, $\rho = 0.58$, $p < 0.01$ for u-NAG and u-NGAL, $\rho = 0.46$, $p < 0.01$ for u-KIM and u-NGAL). Similarly, serum NT-proBNP correlated with the tubular markers (u-NAG $\rho = 0.5$, u-KIM-1 $\rho = 0.41$, u-NGAL $\rho = 0.22$, each $p < 0.05$).

Predictive Value of the Tubular Markers and NT-proBNP

The follow-up lasted 60 months (median 55 months, IQR 52–57 months). A total of 54 patients died during follow-up and 97 patients reached MACE.

ROC Analysis in the Total Study Cohort

According to ROC analysis, u-NAG as well as NT-proBNP revealed promising predictive values regarding mortality (AUC_{u-NAG} : 0.82, sensitivity 83%, specificity 72%; $AUC_{NT-proBNP}$: 0.83 sensitivity 83%, specificity 71%) and MACE (AUC_{u-NAG} : 0.78, sensitivity 73%, specificity 76%; $AUC_{NT-proBNP}$: 0.77, sensitivity 73%, specificity 73%).

U-KIM-1 showed nonsignificant trends to lower AUCs regarding mortality (AUC_{KIM-1} : 0.75, $p = n.s.$ vs. u-NAG and vs. NT-proBNP) and a significantly lower AUC compared to u-NAG, but not to NT-proBNP regarding MACE (0.69 , $p = 0.049$ vs. u-NAG, $p = n.s.$ vs. NT-proBNP). U-NGAL showed significantly lower AUCs compared to NT-proBNP and u-NAG regarding mortality (AUC_{U-NGAL} : 0.69, $p = 0.02$ vs. u-NAG and $p = 0.01$ vs. NT-proBNP) and MACE (AUC_{U-NGAL} : 0.66, $p = 0.009$ vs. u-NAG and $p = 0.02$ vs. NT-proBNP) (Fig. 1).

Prognostic Value of Tubular Markers in the Total Study Cohort

In Kaplan-Meier analysis u-NAG $\geq$ median was a significant predictor for mortality, as well as u-KIM-1, u-NGAL or NT-proBNP $\geq$ median (each $p < 0.05$). U-NAG, u-KIM-1, u-NGAL as well as serum NT-proBNP $\geq$ median were also significant predictors for MACE (each $p < 0.05$) (online suppl. Table S1).

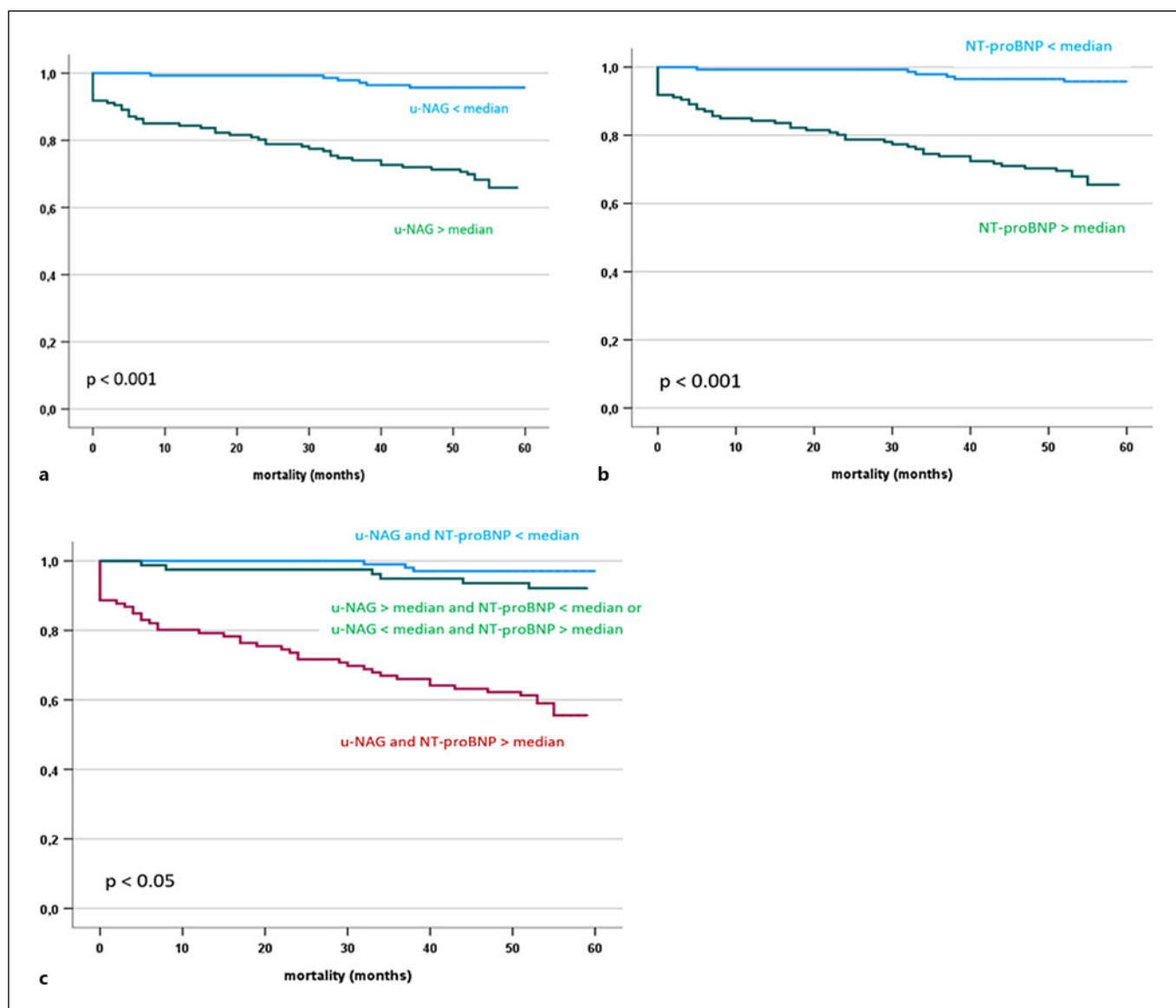


Fig. 2. **a** Kaplan-Meier analysis regarding mortality; u-NAG < median (blue), u-NAG > median (green). **b** Kaplan-Meier analysis regarding mortality; NT-proBNP < median (blue), NT-proBNP > median (green). **c** Kaplan-Meier analysis regarding mortality; u-NAG and NT-proBNP < median (3/106; blue), u-NAG > median and NT-proBNP < median or u-NAG < median and NT-proBNP > median (6/82; green), u-NAG and NT-proBNP > median (45/106; red).

Patients with both u-NAG and NT-proBNP greater than the median showed a significantly higher event rate than patients with only one of those markers greater than the median or patients with none of those markers greater than the median (3/106 vs. 6/82 vs. 45/106, $p < 0.05$ for all-cause-mortality 12/106 vs. 19/82 vs. 66/106, $p < 0.05$ for MACE) (Fig. 2c, 3). Cox-regression analysis, including only u-NAG, u-KIM-1, u-NGAL, and NT-proBNP and creatinine, revealed u-NAG and

NT-pro-BNP as independent and significant predictors for all-cause mortality (online suppl. Table S2).

Based on this, u-NAG and NT-proBNP were included in the Cox-regression analysis containing clinical parameters. Cox-regression analysis confirmed u-NAG, NT-proBNP as well as older age as independent predictors for all-cause-mortality and MACE. Creatinine and preexisting medication with diuretics were shown as significant independent

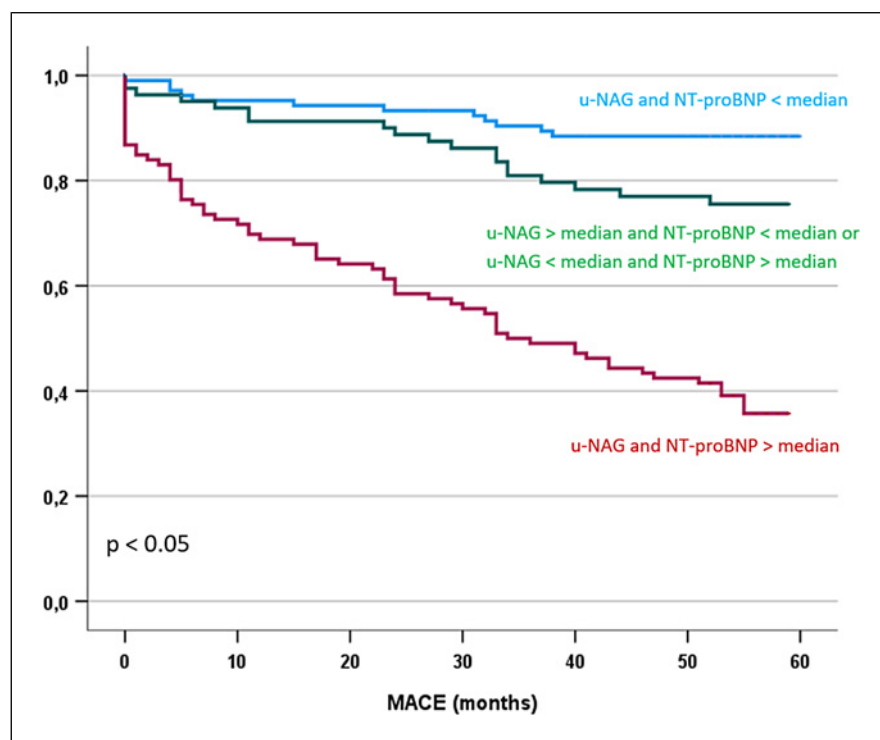


Fig. 3. Kaplan-Meier analysis regarding MACE; u-NAG and NT-proBNP < median (12/106; blue), u-NAG > median and NT-proBNP < median or u-NAG < median and NT-proBNP > median (19/82; green), u-NAG and NT-proBNP > median (66/106; red).

predictors of MACE, but not mortality. In contrast, hypertension, diabetes, preexisting CAD, premedication with an aldosterone antagonist or an ACE-/AT-1-inhibitor or the received volume of contrast agents were no significantly independent predictors for mortality or MACE (each $p = \text{n.s.}$; Table 2). The logarithm of u-NAG, u-KIM-1, u-NGAL and NT-proBNP was used for cox-regression analysis due to the wide range of their unmodified values.

ACS Patients

In the total study cohort, 172 patients suffered from ACS. In total, 33 deceased during follow-up and 67 suffered from MACE.

ROC Analysis in ACS Patients

Regarding patients suffering from ACS, ROC analysis showed similar AUCs compared to the total study cohort for all-cause mortality (u-NAG: AUC: 0.755, sensitivity 73%, specificity 61%; NT-proBNP: AUC: 0.801 sensitivity 73%, specificity 79%; u-KIM-1: AUC: 0.753; u-NGAL AUC: 0.692; each $p = \text{n.s.}$) and slightly lower AUCs for MACE (u-NAG: AUC: 0.676, sensitivity 72%, specificity 63%; NT-proBNP: AUC: 0.684, sensitivity 72%, specificity 51%; u-KIM-1: AUC: 0.625; u-NGAL AUC: 0.659; $p = \text{n.s.}$ for significant difference) (Fig. 4).

Prognostic Value of Tubularly Markers ACS Patients

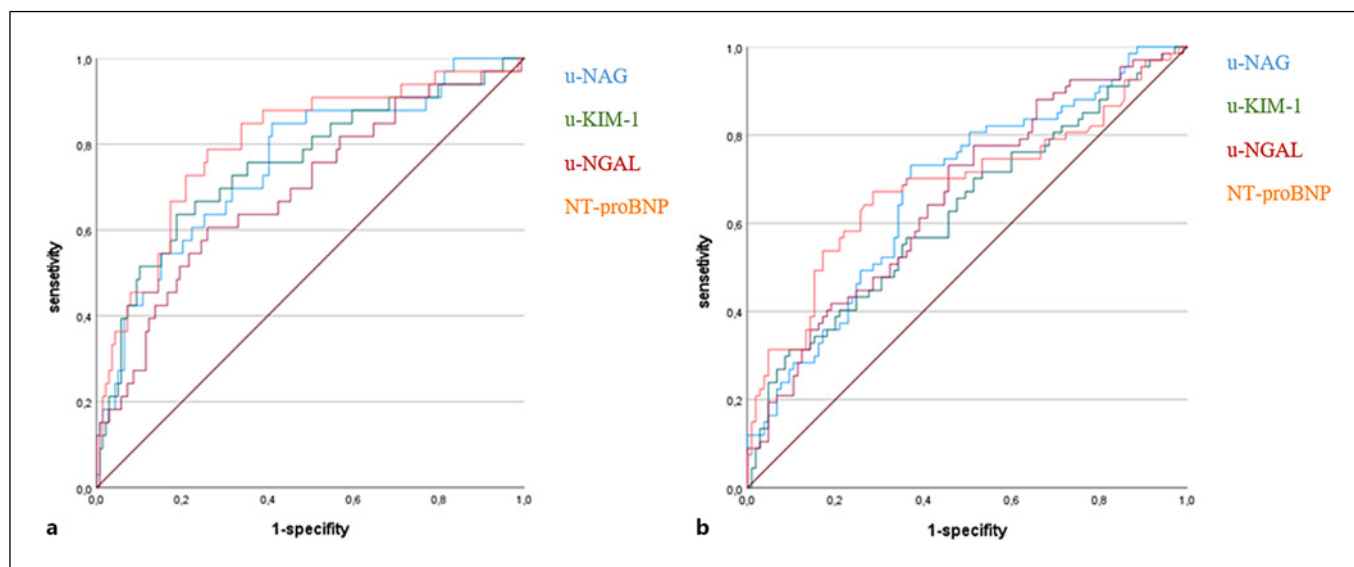
In Kaplan-Meier analysis patients with u-NAG, u-KIM-1, u-NGAL, and NT-proBNP greater than median showed a lower rate of mortality ($p < 0.05$ each). u-NAG, u-NGAL, and NT-proBNP had the same predictive value regarding MACE, but u-KIM-1 did not ($p < 0.05$ for u-NAG, u-NGAL and NT-proBNP; $p = \text{n.s.}$ for KIM-1) (Fig. 5; Table 3; online suppl. Table S3). In Cox-regression analysis u-NAG, hypertension, creatinine, preexisting CAD and older age were independent predictors for mortality opposite to NT-proBNP, sex, diabetes the volume of contrast agents received, premedication with diuretics, an aldosterone antagonist or an ACE-/AT-1-inhibitor (each $p = \text{n.s.}$). Regarding MACE, u-NAG, and older age were independent predictors opposite to NT-proBNP, sex hypertension, creatinine, preexisting CAD, diabetes, the volume of contrast agents received premedication with diuretics, an aldosterone antagonist or an ACE-/AT-1-inhibitor (each $p = \text{n.s.}$).

Discussion

The aim of the current study was the evaluation of the tubular markers u-NAG, u-KIM-1, and u-NGAL as potential predictors of adverse events in a cohort

Table 2. Cox-regression analysis regarding mortality and MACE

	Mortality			MACE		
	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
Ig u-NAG	5.176	2.119–12.643	<0.001	3.547	1.876–6.708	<0.001
Ig NT-proBNP	1.917	1.181–3.112	0.008	1.479	1.048–2.087	0.026
Creatinine	1.474	0.959–2.265	0.077	1.454	1.049–2.015	0.025
Older age	1.060	1.032–1.089	<0.001	1.028	1.010–1.046	0.003
Sex	0.707	0.368–1.356	0.297	0.715	0.442–1.157	0.172
Hypertension	0.641	0.309–1.328	0.231	0.703	0.426–1.161	0.169
Diabetes	1.260	0.681–2.331	0.461	1.244	0.791–1.957	0.344
CAD	1.288	0.740–2.241	0.370	0.817	0.531–1.258	0.358
Diuretics	1.516	0.874–2.629	0.138	1.697	1.120–2.571	0.013
ACE-/AT-1-inhibitor	0.724	0.365–1.438	0.357	0.839	0.519–1.356	0.473
Aldosterone antagonists	1.392	0.560–3.460	0.476	1.189	0.555–2.547	0.657
Volume of contrast agents received	0.998	0.994–1.001	0.204	1.001	0.999–1.004	0.210

**Fig. 4.** **a** ROC analysis in ACS subgroup regarding mortality; u-NAG (blue), u-KIM-1 (green), u-NGAL (red), NT-proBNP (orange). **b** ROC analysis in ACS subgroup regarding MACE; u-NAG (blue), u-KIM-1 (green), u-NGAL (red), NT-proBNP (orange).

of chest-pain-patients. u-NAG was presented as a significant and independent predictor for all-cause mortality and MACE in chest-pain-patients as well as in patients suffering from ACS, as opposed to u-KIM-1 and u-NGAL. Deceased patients showed significantly higher levels of tubular markers but were also

significantly older than surviving patients. This might hint to a confounder, but in cox regression, NAG was shown as a significantly independent predictor for mortality and MACE alongside older age. Therefore, elevated levels of NAG seem to show predictive value independent from older age.

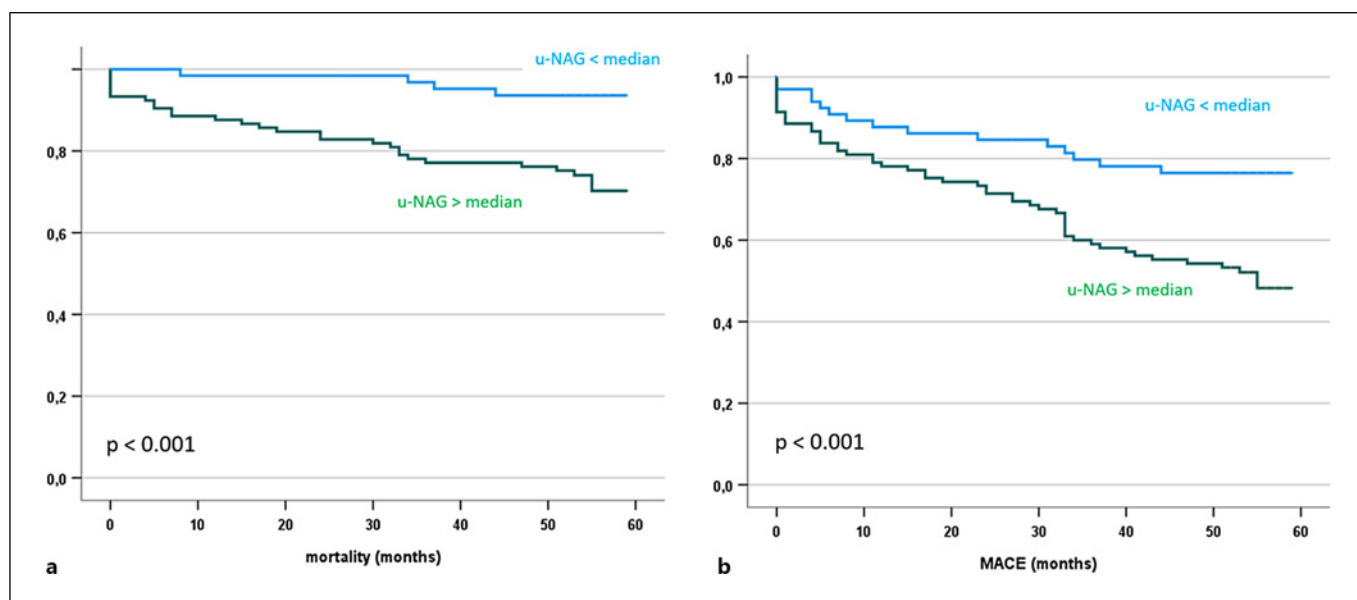


Fig. 5. **a** Kaplan-Meier analysis regarding mortality in ACS subgroup; u-NAG < median (blue), u-NAG > median (green). **b** Kaplan-Meier analysis regarding MACE in ACS subgroup; u-NAG < median (blue), u-NAG > median (green).

Table 3. Cox-regression analysis in ACS subgroup regarding mortality and MACE

	Mortality			MACE		
	HR	95% CI	p value	HR	95% CI	p value
Ig u-NAG	11.240	3.740–33.786	0.001	3.413	1.667–6.988	<0.001
Creatinine	2.348	1.332–4.139	0.003	1.846	1.307–2.605	<0.001
Older age	1.069	1.028–1.111	0.001	1.030	1.009–1.052	0.006
Hypertension	0.243	0.065–0.912	0.036	0.551	0.265–1.144	0.110
CAD	2.534	1.192–5.383	0.016	0.999	0.585–1.706	0.997
Ig NT-proBNP	1.738	0.848–3.562	0.131	1.375	0.889–2.128	0.153
Sex	0.651	0.292–1.453	0.295	0.678	0.373–1.232	0.202
Diabetes	1.710	0.783–3.732	0.178	1.565	0.907–2.700	0.108
Diuretics	1.211	0.487–3.012	0.680	1.307	0.780–2.190	0.309
ACE-/AT-1-inhibitor	1.104	0.430–2.837	0.837	1.152	0.639–2.078	0.638
Aldosterone antagonists	2.586	0.949–7.052	0.063	0.879	0.336–2.304	0.794
Volume of contrast agents received	0.998	0.993–1.002	0.274	1.002	0.999–1.005	0.147

A total of 41 patients suffered from chronic kidney disease. Those patients showed significantly higher levels of all four markers compared to the remaining patients. To exclude this as a confounder, we analyzed the subgroup of patients without CKD in the total study

cohort, as well as the ACS subgroup. The remaining study cohort showed the same results regarding Kaplan-Meier analysis as well as Cox-regression analysis for both endpoints (online suppl. Fig. S2; online suppl. Tables S4, S5).

Premedication with diuretics was also shown as an independent predictor for mortality and MACE. This is most likely driven by hospitalizations due to congestive heart failure and a surrogate parameter for prior hospitalizations due to congestive heart failure.

Physiological and Pathophysiological Appearance of Tubular Markers

Tubular markers are proteins excreted in the proximal tubules. In case of an acute injury, the urinary concentration of these markers increases due to epithelial damage [27].

NAG is a lysosomal enzyme produced in the cells of the proximal and distal tubules. It can also be found in various other organs such as thymus, adrenal glands, testis, liver, spleen and pancreas and parts β -glycosidic bonds in the catabolism of glycoconjugates [28]. Elevated levels of u-NAG can be found 12 h after renal damage [29].

u-KIM-1 is a glycoprotein bound to the cellular membrane which can be hardly found in a healthy kidney but is built in the proximal tubules after ischemic damage [30]. Physiologically, it takes part in the phagocytosis of apoptotic cells by directing them to lysosomes [31].

u-NGAL is a member of the lipocalin group. It is found in several tissues such as the kidney, the lung and the intestinal system [32]. In case of a kidney injury, it is produced in the damaged nephron [33]. Physiologically, it takes part in angiogenesis [34].

Prognostic Value of Tubular Markers

Data on the tubular markers studied in the context of acute cardiovascular events in patients at the emergency department are still a rarity. Tubular markers have been studied in the context of acute and chronic heart failure and cardiorenal syndrome [10, 11, 14, 20, 35, 36] and less in the context of ACS [37, 38]. The tubular markers may incorporate early information regarding the occurrence of adverse events beside a decreasing renal function. Tubular markers were already evaluated in different cohorts and several studies already showed a correlation between them and adverse events in different disease entities.

Allgaier et al. [36, 39] examined patients with an ICD and demonstrated an association between elevated u-NAG-levels and an increased occurrence of mortality, rehospitalization, appropriate ICD-therapies and a worsening in renal function. In 708 patients with acute heart failure without an acute ischemic cause, patients with elevated u-NAG levels

suffered from a significantly increased rate of mortality and worsened cardiac function [15] and a correlation between elevated levels of u-NAG and u-KIM-1 and long-term progression of chronic kidney disease in patients suffering from chronic heart failure has already been demonstrated [11]. The current study was able to extend these findings to patients with acute cardiac ischemia regarding mortality and MACE. It evaluated u-NAG for the first time in a collective of ACS patients and demonstrated promising potential as a cardiorenal marker.

Torregrosa et al. [21] presented the clinical utility of u-KIM-1 through its predictive power for renal injury in patients with AKI after cardiac surgery and coronary angiography. Except the current study, there is hardly any data regarding u-KIM-1 in cardiac patients, especially in ACS patients. Therefore, the current study was able to extend the existing knowledge as u-KIM-1 showed some potential in the Kaplan-Meier analysis, although u-NAG seems to be the most promising among the tubular markers.

A total 141 patients suffering from congestive heart failure showed increasing levels of u-NAGL during diuretic therapy, but so far there is limited data available regarding u-NGAL in ACS [18]. Menzorov et al. [40] examined a collective of 122 patients with ACS and was able to show an association between elevated levels of u-NGAL and the occurrence of acute kidney injuries. Shalenkova et al. [38] demonstrated a correlation between increased levels of u-NGAL and the occurrence of acute heart failure in 110 ACS patients. The current study was in line with these findings, as there was a correlation between elevated levels of u-NGAL and adverse event. In extension to those findings, the current study showed the superior predictive value of u-NAG in comparison to u-NAGL.

NT-proBNP is a well-established marker in cardiac diagnostic(s) and has already proven its value in patients with ACS. Galvani et al. [41] demonstrated an association between increased levels of NT-proBNP and a higher rate of mortality in the first 30 days after myocardial infarction and Omland et al. [42] showed NT-proBNP as an independent predictor for mortality in 609 patients suffering from ACS. The current study confirmed these findings, as NT-proBNP showed promising results in the Kaplan-Meier analysis. Additionally, in extension the combination of NT-proBNP and u-NAG revealed greater predictive value than NT-proBNP alone.

Tubular markers may help identify a high-risk collective among patients with chest pain and with ACS in particular. Thus, these markers may be used to identify patients early who need a close outpatient follow-up. Furthermore, they provide a noninvasive diagnostic tool with predictive value about mortality and MACE. But this study shows a relatively small cohort and moreover, in COX-regression u-KIM-1 and u-NAGL had no additional value compared to u-NAG and NT-proBNP. Therefore, the predictive value and clinical usefulness of tubular markers need further evaluation. Additional studies with larger cohorts are necessary to further evaluate the possible clinical usage of u-NAG. Nevertheless, U-NAG showed promising results and might show its worth as a point of care tool e.g., in a test strip for urine in rural areas without a laboratory at night.

Limitations

A total 402 patients were originally recruited, but only 294 patients could be included in the current study due to the absence of blood and/or urine samples. In most cases, the urine sample was absent. In August 2015, due to a lack of staff, no patients could be recruited, and the recruitment was not prolonged beyond October 2015. Therefore, the small cohort limits the informative value of this study.

The blood and urine sample had to be collected in the chest pain unit according to the study protocol. We were not able to collect urine samples from some patients prior or directly after the coronary angiography. This group especially includes patients with the need for prompt treatment, like PCI. In those cases, the already applied contrast agent might affect the tubular markers and impair their informative value. The failure to obtain a blood and urine sample in these most severe patients might limit the analysis of the prognostic value of the tubular markers due to a potential selection bias.

The posterior leads are done regularly in patients with ACS. Two patients with RCX occlusions in coronary angiography showed no elevations in the conventional leads and were therefore documented as NSTEMI.

Conclusion

In summary, the current study evaluated the prognostic value of the tubular markers u-NAG, u-KIM-1, and u-NGAL alongside NT-proBNP in a

cohort of patients suffering from chest-pain and a subgroup of patients with ACS. U-NAG was shown as the most promising tubular marker and as an independent predictor for mortality and MACE. The combination of u-NAG and NT-proBNP was able to identify a high-risk collective among those patients. These findings could be used in the emergency department as part of a strategy to stratify ACS patients in a high-risk cohort and a low-risk cohort. The high-risk cohort might need closer monitoring, may be in an ICU or an IMC-unit, in a closely monitored setting. The low-risk cohort could be treated in a regular ward in cases of an NSTEMI or unstable angina. But further data must be gathered before such decisions could be supported by tubular markers.

Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki. This human study was approved by Ethics Committee of the university hospital Regensburg-approval: 14-101-0311. All adult participants provided written informed consent to participate in this study.

Conflict of Interest Statement

There are no conflicts of interest to declare.

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Author Contributions

Andreas Leonhard Schober: formal analysis, investigation, and writing the original draft. Julia Bindig: formal analysis and writing the original draft. Michael Philipp Schober: validation, review, and editing. Alexander Daniel Schober: validation, review, and editing. Ute Hubauer: data curation and project administration. Andreas Luchner: validation, review, and editing. Lars Siegfried Maier: validation and supervision. Carsten Jungbauer: conceptualization, supervision, validation, review, and editing.

Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request.

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