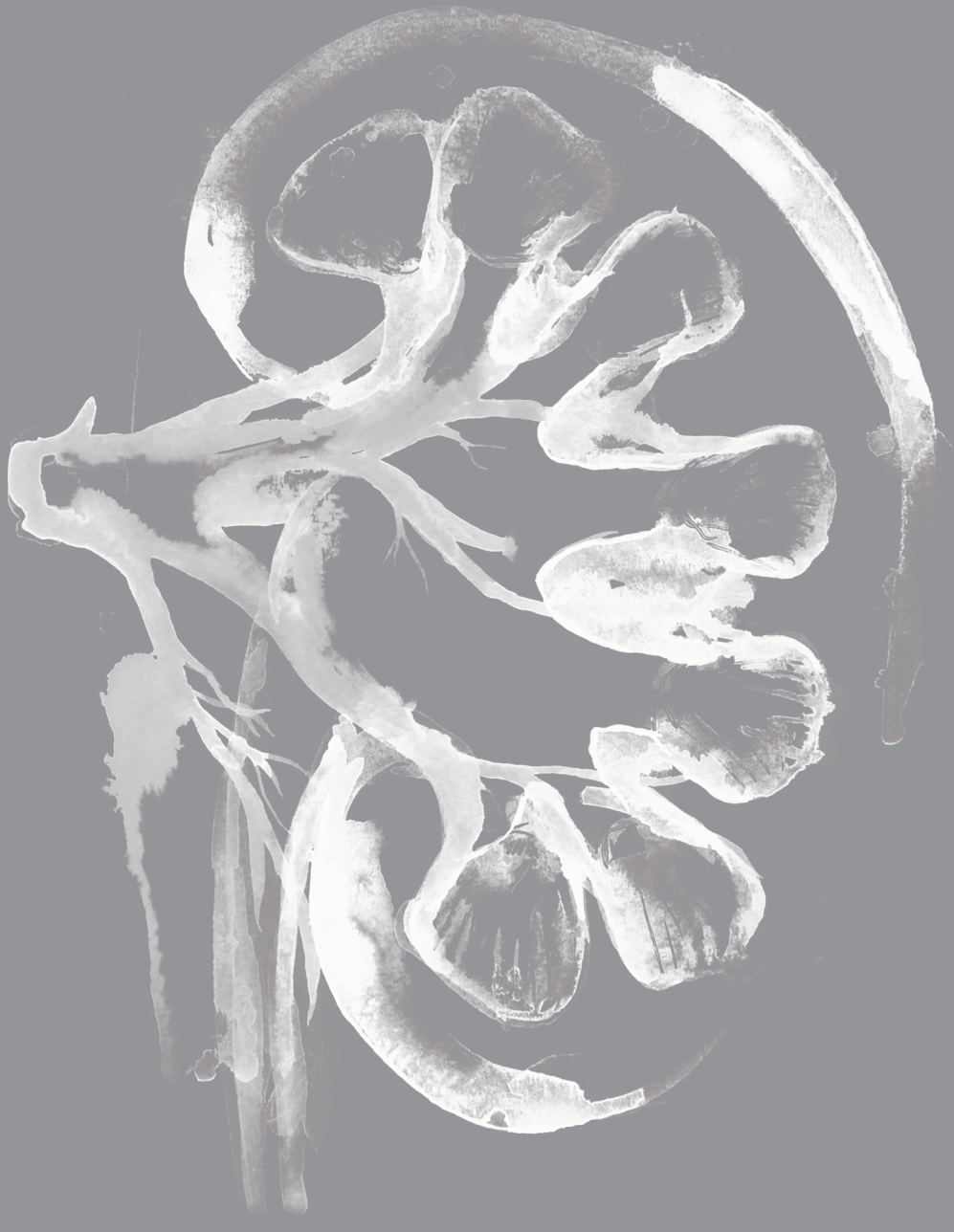

Chapter 5

Prognostic value of pro-adrenomedullin, procalcitonin and C-reactive protein in predicting outcome of febrile urinary tract infection

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Abstract

Bacterial infections such as febrile urinary tract infection (*f*UTI) may run a complicated course which is difficult to foretell on clinical evaluation only. Because the conventional biomarkers erythrocyte sedimentation rate (ESR), leukocyte count, C-reactive protein (CRP) and procalcitonin (PCT) have a limited role in the prediction of a complicated course of disease, a new biomarker - plasma midregional pro-adrenomedullin (MR-proADM) - was evaluated in patients with *f*UTI. We conducted a prospective multicentre cohort study including consecutive patients with *f*UTI at 35 primary care centres and 8 emergency departments. Clinical and microbiological data were collected and plasma biomarker levels were measured at presentation to the physician. Survival was assessed after 30 days. Of 494 *f*UTI patients, median age was 67 [IQR 49-78] years, 40% were male; two third of them had significant co-existing medical conditions. Median MR-proADM level was 1.42 [IQR 0.67-1.57] nmol/L; significantly elevated MR-proADM levels were measured in patients with bacteraemia, those admitted to the ICU, and in 30- and 90-day non-survivors, as compared to patients without these characteristics. The diagnostic accuracy for predicting 30-day mortality in *f*UTI, reflected by the area-under-the-curve of receiver operating characteristics were: MR-proADM 0.83 (95%CI: 0.71-0.94), PCT 0.71 (95%CI: 0.56-0.85); whereas CRP, ESR and leukocyte count lacked diagnostic value in this respect. This study shows that MR-proADM assessed on first contact predicts a complicated course of disease and 30-day mortality in patients with *f*UTI and in this respect has a higher discriminating accuracy than currently available biomarkers ESR, CRP, PCT and leukocyte count.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections. Febrile UTI (*f*UTI), reflecting acute pyelonephritis, prostatitis or urosepsis, is a potentially serious infection with a mortality rate of about 0.3%, but in bacteremic fUTI the mortality may be as high as 7.5-30% (1;2). Moreover, bacteraemia in *f*UTI is associated with prolonged hospitalization and a complicated course (3-5), and occurs in up to 30% of those admitted to hospital and in 15% of patients treated at home (6). Evaluation of clinical symptoms fails to provide accurate guidance to the clinician which patients have bacteraemia or who may run a complicated course, and which patients may be safely treated at home. At present, there is a lack of robust inflammatory biomarkers that may help determine severity of disease in *f*UTI (7;8). A promising new biomarker is midregional pro-adrenomedullin (MR-proADM). Adrenomedullin (ADM) has been detected in a variety of tissues including kidneys. It has immune modulating, metabolic and bactericidal activity, and is involved in regulation of complement activity (9-12). Reliable plasma measurement of ADM is challenging due to half-life time of 22 minutes (13). MR-proADM, the more stable mid-regional fragment of adrenomedullin, has been identified in plasma of patients with septic shock (13-15).
The aim of the present study is to assess the prognostic value of plasma MR-proADM in adult patients with *f*UTI with respect to bacteraemia, need for hospital admission and a complicated course, as compared to current available biomarkers like blood leukocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and procalcitonin (PCT).

Patients and methods

We conducted a prospective observational multicentre cohort study of patients presenting with a presumptive diagnosis of *f*UTI from January 2004 until March 2011. Participating centres were 35 primary care centres and 8 emergency departments (ED) in the Netherlands as described previously (6,8). The local ethics committees approved the study and all participants provided written informed consent. Over 7 years, we established a cohort of 869 patients. From this database, we randomly selected (every other patient, e.g. the 1st, 3rd,5th, etc.) frozen plasma samples of 494 patients for measurement of MR-proADM.

Inclusion criteria were age ≥18 years, fever (≥38.0 °C) and/or a history of fever or shaking chills within 24 hours before presentation, at least one symptom of UTI (dysuria, perineal pain or flank pain) and a positive nitrite dipstick test or leucocyturia. Exclusion criteria were current treatment for urolithiasis or hydronephrosis, pregnancy, hemo- or peritoneal dialysis, history of kidney transplantation or presence of polycystic kidney disease. Patients were only included once.

Procedures and definitions

Clinical data and laboratory values were collected within 24 hours of enrolment by standardized questionnaires and reviewing the medical record. All patients were empirically treated with antibiotics according to local and national policy. Blood cultures and clean midstream-catch urine cultures were obtained before starting antimicrobial therapy and analysed using standard microbiological methods. Bacteraemia was defined as growth of any pathogen in the blood culture, except coagulase-negative *staphylococci.*

Plasma ethylenediaminetetraacetic acid (EDTA) blood samples were collected, centrifuged and stored at -80 °C within two hours of patient enrolment. MR-proADM and PCT levels were measured after completion of all study enrolments, using a Time Resolved Amplified Cryptate Emission technology assay (TRACE®, Kryptor Compact, MR-proADM sensitive and PCT sensitive; Thermofisher - Brahms AG; Henningsdorf, Germany). The median concentration of MR-proADM in a cohort of healthy individuals was 0.39 nmol/L (97.5^{th} percentile: 0.55 nmol/L) (16). According to the manufacturer recommendation we tested MR-proADM levels for different cut-off values (16;17). Results of PCT measurement to predict bacteraemia have been described previously (8). Measurements of CRP, ESR and leukocyte count were only done at enrolment when indicated by the attending physician. All eight participating EDs applied similar techniques. CRP was measured using immunoturbidimetric assay, cut-off values varied from 6-10 mg/L. ESR was measured using Westergren method, cut-off values: <20mm/hour for males and females ≤50 years, 30mm/hour for females > 50 years and 15mm/hour for males >50 years. Leukocyte count was measured using flow cytometry, cut-off value: 10.0 $x10^9$/L. Data on biomarkers available in our study population were: CRP (n=319), ESR (n=158), leukocyte count (n=372) and PCT (n=321).

End points

MR-proADM values were evaluated for their predictive acumen of primary and secondary endpoints, in comparison to that of the other biomarkers. The primary endpoint was 30-day mortality. Secondary endpoints were presence of bacteraemia at admission, need for hospital admission as estimated by the Acute Pyelonephritis Severity Index score (APSI score), and need for ICU admission. The APSI score is a prediction rule allocating points to age, sex, nursing home residency, comorbidities, and vital signs at presentation (18).

Statistical analysis

The histogram of biomarker values were skewed and log-normalized before analysis. Descriptive analysis included means with confidence intervals (CIs) or medians and ranges, as appropriate. Univariate analysis was performed using ANOVA, student's *t*-test or where appropriate Mann-Whitney *U*-test for continuous variables and Chi-square tests for categorical variables. Continuous variables were added into the models as continuous variables (except for the APSI-score) and log-normalized if data were not normal distributed. The APSI-score was analysed as a binary variable, using a cut-off value of 100 points, based on previous data (18).

To assess the prognostic ability of MR-proADM compared to PCT and other conventional biomarkers in predicting the primary and secondary endpoints, AUC of ROC-curves were calculated. The main conclusion regarding the predictive ability of MR-proADM was based on this analysis. For each biomarker corresponding positive and negative predictive values and likelihood ratios were calculated for standardized cut-off values in predicting the primary endpoint. Kaplan-Meier survival curves were generated to illustrate survival probability and clinical outcome for different levels of MR-proADM. The log rank test was used to test the difference between survival curves. Survival analysis was performed on the whole cohort, ROC-analysis and sensitivity analysis on the subset of patients with data on the concerning biomarkers available. A p-value <0.05 was considered to indicate statistical significance. SPSS software (SPSS Inc., Chicago, Ill, version 20.0) was used for statistical analysis.

Results

In total 494 patients were randomly selected from our existing database resource. There were no significant differences between our study population

and the remainder of the database population, except for having a significantly older population (p=0.027) with significantly more diabetics (25%; p<0.001) in the selected study group (data not shown). Median age of our study population was 67 [IQR 49-78] years, 40% were male and 66% had co-existing medical conditions. Of 376 patients included at the Emergency Department, 329 (88%) were hospitalized. None of the patients recruited in primary care were hospitalized. (Table 1).

Table 1. Baseline characteristics of 494 patients presenting with febrile UTI

Characteristic	Febrile UTI patients n = 494
Age, median years [IQR]	67 [49-78]
Male sex	198 (40)
Antibiotic pre-treatment	171 (35)
Comorbidity	
Any	325 (66)
Diabetes mellitus	121 (25)
Malignancy	56 (11)
Heart failure	76 (15)
Cerebrovascular disease	73 (15)
Chronic obstructive pulmonary disease	77 (16)
Chronic renal insufficiency	54 (11)
Urologic history	
Urinary tract disorder[a]	126 (26)
Indwelling urinary catheter	40 (8)
Recurrent UTIs[b]	158 (32)
Presentation	
At emergency department	376 (76)
Shaking chills	290 (59)
Dysuria[c]	366 (74)
Flank pain	283 (57)
Fever duration at presentation, median hours [IQR]	32 [16-66]
Heart rate >90 beats/minute	258 (52)
Systolic blood pressure, mean mmHg ± SD	130 ± 23
Diastolic blood pressure, mean mmHg ± SD	72 ± 14

Data presented as n (%) unless otherwise stated. UTI=urinary tract infection, IQR=interquartile range, SD=standard deviation.

[a] any anatomical or functional abnormality of the urinary tract except urinary catheter and history of nephrolithiasis.

[b] defined as ≥ 3 UTIs in the past 12 months or ≥ 2 UTIs in the past 6 months.

[c] not recorded in patients with indwelling urinary catheter.

In the ED group, 30-day mortality was 3% (n=12) versus an absence of 30-day mortality in the primary care group. A total of 101 (22%) patients with blood cultures taken at presentation (n=463) presented with bacteraemia, with significantly more patients in the ED group (n=90/347, 26%, p<0.001). Nineteen (5%) of ED patients were admitted to the ICU, of these two died. (Table 2).

Table 2. Overview of primary and secondary endpoints in 494 patients with febrile UTI

Endpoint	Febrile UTI patients (n= 494)
Bacteraemia at presentation[a]	101/463 (22)
Hospitalization duration (days), median [IQR]	4 [0-7]
ICU admission	19 (4)
APSI score >100[b]	77 (16)
Mortality	
day 3	2/492 (0.4)
day 30	12/485 (3)
day 90	19/474 (4)

Data are presented in n (%) unless otherwise stated. IQR = interquartile range, ICU = Intensive Care Unit, APSI = Acute Pyelonephritis Severity Index.

[a] no blood culture performed in 31 patients.

[b] prediction rule allocating points to age, sex, nursing home residency, comorbidities and vital signs at presentation; patients with APSI score <100 can be safely treated at home without risk of readmission and mortality.

MR-proADM versus other biomarkers in predicting 30-day mortality

To define the prognostic accuracy of different biomarkers for predicting 30-day mortality, ROC analyses were performed. The AUC for MR-proADM (n=494) was 0.83 (95%CI 0.71-0.94), leukocyte count (n=372): 0.44 (95%CI 0.26-0.62), ESR (n=158): 0.60 (95%CI 0.43-0.78), CRP (n=319): 0.59 (95%CI 0.37-0.81) and PCT (n=321): 0.71 (95%CI 0.56-0.85). Based on the constructed AUCs, MR-proADM has a higher discriminating accuracy for predicting 30-day mortality as compared to the other conventional biomarkers.

The 97.5th percentile cut-off value of normal provided by the manufacturer is 0.55 nmol/L but in our target group this cut-off lacks specificity. Thus, the PPV, NPV and likelihood ratios for different MR-proADM cut-off values were calculated (Table 3). Our data indicates a plasma MR-proADM level of 1.00 nmol/L was the optimal cut-off value to stratify 30-day mortality in patients with *f*UTI. Using this cut-off, we calculated a sensitivity of 91.7% with a specificity of 48.0%; NPV 99.6%; PPV 4.3%; LR+ 1.8; LR- 0.2 (Table 3).

Table 3. Sensitivity, specificity, PPV, NPV, LR+ and LR- of different infectious biomarkers for predicting 30-day mortality

	Cut-off value	No. cases under cut-off	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	LR + (95% CI)	LR – (95% CI)
MR-proADM (nmol/L)	0.55[a]	79 (16%)	100.0 (73.4-100.0)	15.6 (12.5-19.2)	2.9 (1.5-5.1)	100.0 (95.1-100.0)	1.2 (1.1-1.2)	0.0 (0.0-0.0)
	1.00	234 (47%)	91.7 (61.5-98.6)	48.0 (43.4-52.6)	4.3 (2.2-7.5)	99.6 (97.6-99.9)	1.8 (1.5-2.1)	0.2 (0.0-1.1)
	1.50	362 (73%)	83.3 (51.6-97.4)	74.6 (70.5-78.5)	7.7 (3.8-13.7)	99.4 (98.0-99.9)	3.3 (2.4-4.4)	0.2 (0.1-0.8)
	1.88	406 (82%)	66.7 (35.0-89.9)	83.1 (79.4-86.4)	9.1 (4.0-17.1)	99.0 (97.4-99.7)	3.9 (2.5-6.2)	0.4 (0.2-0.9)
Procalcitonin (µg/mL)	0.25	129 (26%)	72.7 (39.1-93.7)	40.7 (35.1-46.3)	4.2 (1.8-8.1)	97.7 (93.3-99.5)	1.2 (0.8-1.8)	0.7 (0.3-1.8)
C-reactive protein	6	6 (1%)	100.0 (73.4-100.0)	1.3 (0.5-2.7)	2.5 (1.3-4.3)	100.0 (54.1-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
(mg/L)	8	10 (2%)	100.0 (73.4-100.0)	2.1 (1.0-3.9)	2.5 (1.3-4.3)	100.0 (69.0-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
	10	13 (3%)	100.0 (73.4-100.0)	2.8 (1.5-4.7)	2.5 (1.3-4.4)	100.0 (75.1-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
ESR (mm)	20	74 (15%)	91.7 (61.5-98.6)	15.0 (11.9-18.6)	2.7 (1.3-4.7)	98.6 (92.5-99.8)	1.1 (0.9-1.3)	0.6 (0.1-3.7)
Leukocyte count (x10^9/L)	10	106 (22%)	75.0 (42.8-94.2)	21.8 (18.1-25.8)	2.4 (1.1-4.5)	97.2 (91.9-99.4)	1.0 (0.7-1.3)	1.2 (0.4-3.1)

PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; ESR: erythrocyte sedimentation rate. Data on biomarkers available: MR-proADM (n=494), procalcitonin (n=321), C-reactive protein (n=319), ESR (n=158), leucocyte count (n=372).

[a] 97.5th percentile Brahms MR-pro-ADM Kryptor cut-off value in 144 healthy individuals.

Need for hospital admission

In the prediction of need for hospitalization, as based on an APSI score >100 points, MR-proADM outperformed PCT (n=321 patients with both data available) given the AUC for MR-proADM of 0.82 (95% CI 0.77-0.88) compared to 0.69 (95%CI 0.62-0.77) for PCT. For prediction of bacteraemia, MR-proADM and PCT performed about equally (MR-proADM: AUC 0.78 (95%CI 0.72-0.85) and PCT 0.81 (95%CI 0.75-0.87)). As the predictive values might have been influenced by antibiotic (pre)treatment (in 35% of the patients), analysis was also done separately in those with and without antibiotics on study enrolment (n=113 *vs* n=208). Corresponding AUCs for MR-proADM were 0.75 (95%CI 0.65-0.85) and 0.79 (95%CI 0.70-0.88), and for PCT 0.80 (95%CI 0.71-0.89) and 0.80 (95%CI 0.72-0.88) respectively, indicating that antibiotic pretreatment did not alter the predictive value of MR-proADM with respect to bacteraemia. In the prediction of either bacteraemia or need for hospital admission, CRP, ESR and blood leukocytes lacked predictive power (all AUC <0.60). For prediction of the need for ICU admission, pro-ADM and PCT performed almost identical (i.e., AUC of 0.77 and 0.75, respectively, n=321).

MR-proADM and clinical parameters

In addition to 30-day mortality, median MR-proADM level was significantly correlated with bacteraemia (bacteremic versus non-bacteremic patients: 1.60 [IQR 1.01-3.28] versus 0.96 [IQR 0.61-1.37] nmol/L), need for ICU-admission (ICU versus non-ICU patients: 2.01 [IQR 1.37-4.61] versus 1.04 [IQR 0.65-1.50] nmol/L) and APSI score (1.95 [IQR 1.27-2.90] nmol/L in patients with a score >100 points versus 0.94 [IQR 0.62-1.36] nmol/L in patients with a score ≤100 points). Furthermore, MR-proADM levels increased with age and were significantly higher in patients with heart failure and chronic renal insufficiency.

The Kaplan-Meier curves showed no 30- or 90-day mortality in patients with MR-proADM levels in the 1st quartile (n=123) of the whole group. In the 2nd quartile (n=124) three events occurred, four in the 3rd quartile (n=124), and eleven in the 4th quartile (n=123). All three events in the 2nd quartile occurred late in current disease episode: day 16, 27 and 33. One event in the 3rd quartile occurred on day 3, the other three occurred late after disease onset (day 41, 42 and 66). Events in the 4th quartile occurred primarily in the early stage of current disease episode. This suggests that events in the 2nd and 3rd quartile are likely due to pre-existing co-morbidity, while events

in the 4th quartile occur as result of the current active disease (Figure 1). The 30-day cumulative survival rate was 1.00 in the 1st quartile, 0.98 in the 2nd quartile (log rank *p* =0.157), 1.00 in the 3rd quartile (log rank *p* 1.00) and 0.92 in the 4th quartile (log rank *p* =0.001). The 90-day cumulative survival rate was 1.00 in the 1st quartile, 0.98 in the 2nd quartile (log rank *p* =0.083), 0.97 in the 3rd quartile (log rank *p* =0.046) and 0.91 in the 4th quartile (log rank *p* =0.001).

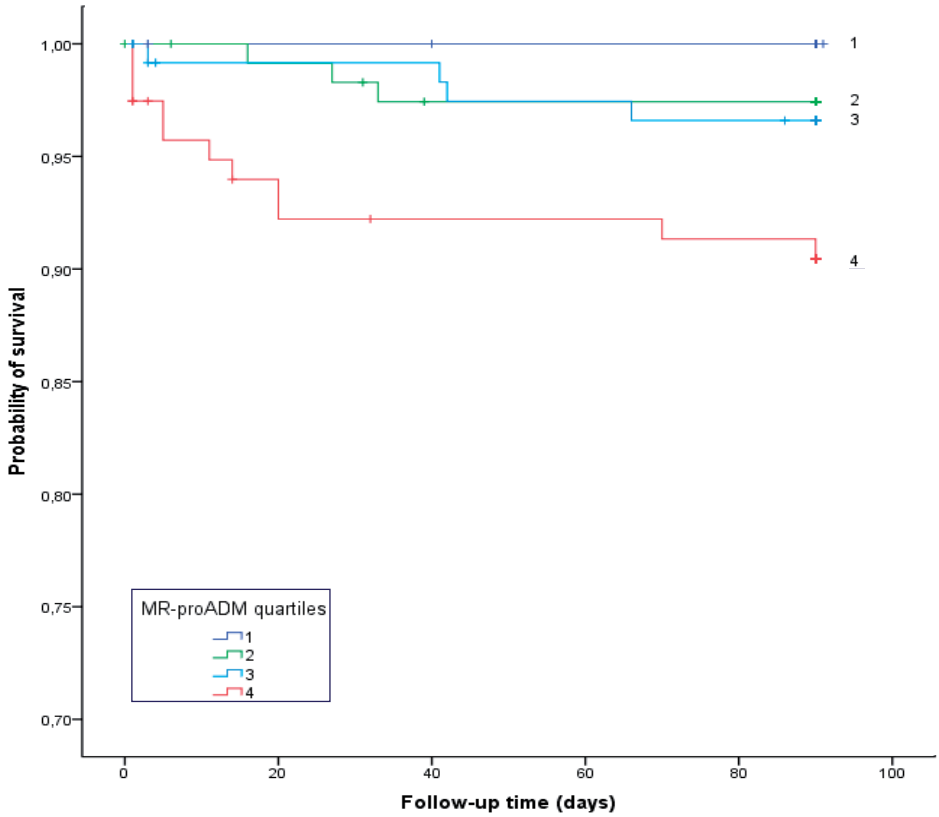

Figure 1. Kaplan-Meier curves of 90-day mortality according to quartiles of mid-regional pro-adrenomedullin (MR-proADM)

Discussion

The main finding of the present study is that MR-proADM, determined on first contact in a patient with presumptive *f*UTI, predicts a complicated course of disease necessitating hospital admission and admission to the ICU, and a worse outcome of infection as reflected by 30-day mortality. MR-proADM

more accurately predicts outcome than currently used biomarkers. Furthermore, we found significantly higher plasma MR-proADM levels in patients presenting with bacteraemia. Given these characteristics, measurement of MR-proADM in patients with a presumptive diagnosis of *f*UTI may provide the clinician more accurate guidance than currently applied biomarkers, e.g., with respect to admission of high-risk patients, and thus help focus resources to the patients that need them most.

Strengths of this study are its prospective design in which *f*UTI patients were included in both primary care and hospital ED setting, reflecting a real-life, full spectrum of invasive UTI recognizable to every clinician. Also, the large sample size of a clinically and microbiologically well characterized disease group is a strength. To our knowledge, this is the first large prospective study focusing on the predictive value of MR-proADM in adult patients with *f*UTI, and making a comparison to currently available biomarkers of inflammation like PCT and CRP. Travaglino et al. showed that in febrile ED patients, MR-proADM and PCT levels correlated with APACHE-II score and the combined use of both biomarkers might be helpful in predicting hospitaliztation (19).
There are also some limitations. We determined MR-proADM levels once, at first contact with the physician. This precludes the analysis whether a rise or decline in MR-proADM levels correlates to changes in the clinical course of disease, as has been determined for e.g. PCT (20). However, our findings show that having a single baseline value can provide clinicians guidance in predicting a complicated clinical course at the ED or primary care. This is where patients initially present and decisions have to be made regarding treatment and hospital admission. When interpreting MR-proADM, it should be taken into account that certain patient characteristics like age and heart failure may affect the plasma level of MR-proADM, as well as disease duration before presentation. A technical limitation might be that the measurement of MR-proADM and other biomarkers was done afterwards, and not immediately 'at the bedside'. However, it has been shown that frozen storage and consequent freeze-thaw cycles of blood samples has no influence on the analyte and measured concentration (15;21). Finally, it should be realized that our findings pertain to *f*UTI and need be confirmed in other infectious conditions. Of note, similar findings have been made in other infectious states albeit usually in much smaller groups of patients and rarely prospectively (14;22-25).

We hypothesize that at least two mechanisms might be responsible for the marked increase of MR-proADM in *f*UTI. *In vitro* and *in vivo* studies have shown that the onset of inflammation is accompanied by changes in both ADM and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Plasma ADM levels are markedly increased in patients with septic shock, supporting the hypothesis that pro-inflammatory cytokines augment ADM production and may increase plasma levels of ADM (11;26-28). However, ADM is also capable of upregulation of interleukine-6 in non-stimulated and LPS-stimulated macrophages, thereby suppressing LPS-induced TNF-α production (29). This suggests that ADM acts as part of a regulatory loop balancing pro-inflammatory cytokines with its anti-inflammatory actions. Since LPS and cytokine levels were not measured in our study, we cannot refute or confirm this hypothesis. Secondly, a decreased clearance of ADM by the kidneys may be – at least in part – responsible for increased proADM levels in *f*UTI. This is supported by our data with a higher median MR-proADM in patients with chronic renal insufficiency and studies that have shown a significant correlation between MR-proADM and creatinine levels (14;30).

In conclusion, we show that MR-proADM has a strong predictive value for 30-day mortality in patients with *f*UTI compared to more conventional biomarkers. Next, studies may wish to confirm the selected cut-off value as a predictor of complicated course and evaluate in daily follow up measurements of MR-proADM the relationship to treatment and clinical recovery. Such studies will establish whether MR-proADM could function as a new prognostic tool for guidance in risk stratification and clinical outcome in patients with *f*UTI.

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