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RESEARCH ARTICLE

Is mid-regional pro-atrial natriuretic peptide (MRproANP) an accurate marker of bacteremia in pyelonephritis?

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Abstract

Introduction: Mid-regional pro-atrial natriuretic peptide (MRproANP) increases during systemic infections and could possibly correlate with bacteremia.

Methods: We determined the characteristics of MRproANP for accuracy to detect positive blood culture.

Results: Bacteremia was positive in 58 (15%) of 347 patients. MRproANP levels increased in patients with bacteremia (98.4 pmol/L [interquartile range (IQR) 68.2–153.1] vs. 66.4 pmol/L [IQR 51.0-90.3], $p < 0.01$). Performance of MRproANP to predict bacteremia [AUC = 0.69, 95%CI: 0.61–0.77] was equivalent to C-reactive protein (0.66 [95%CI: 0.59–0.74], $p = 0.53$) but less accurate than procalcitonin (0.78 [95%CI: 0.72–0.84], $p < 0.001$).

Conclusion: Although MRproANP increased in bacteremic patients with acute pyelonephritis, results of likelihood ratios discarded its use at bedside to predict bacteremia.

Keywords: Bacteremia, urinary tract infection, biomarkers, procalcitonin, mid-regional pro-atrial natriuretic peptide, emergency medicine

Introduction

Phenotype resulting from the host–pathogen interaction relies on systemic changes that alter hormonal balances. In healthy volunteers, endotoxin (lipopolysaccharides, LPS) conveys release of natriuretic peptides (de Kruif et al. 2008; Vila et al. 2008). More precisely, LPS challenge leads to a 3-fold increase of circulating mid-regional pro-atrial natriuretic peptide (MRproANP) within 4 h (de Kruif et al. 2008). Existing evidence suggests that MRproANP concentrations increase with disease severity and increasing adverse medical outcome in patients with infectious diseases (Krüger et al. 2010a,b; Morgenthaler et al. 2005). Additionally, in patients with congestive heart failure, MRproANP concentration profile is similar to those of other natriuretic peptides, and therefore is considered as a marker for this condition (Chenevier-Gobeaux et al. 2010; Maisel et al. 2010). Chronic and acute heart failures, and infectious diseases

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are often observed as concomitant disorders (Dhainaut et al. 2005), and both adversely impact on patients' prognosis. Therefore, whether infection-related changes in MRproANP concentrations represent bacterial load, unstable cardiac underlying disorders or both deserves thorough consideration.

Acute pyelonephritis is a common infectious disease in emergency medicine that mostly affects young healthy women whose course is usually uncomplicated (Warren et al. 1999). It corresponds to bacterial invasion of the upper urinary tract with systemic signs and symptoms of toxemia. Gram-negative enterobacteriaceae strains are the most frequent microorganisms encountered in this context, particularly *Escherichia coli* whose cell membrane contains endotoxin. As endotoxin promotes elevation of MRproANP in healthy subject (de Kruif et al. 2008), acute pyelonephritis is an interesting clinical model to assess bacterial load impact on MRproANP concentrations. We took advantage of a large-cohort study of patients with acute uncomplicated pyelonephritis (Claessens et al. 2010a) to determine accuracy of MRproANP to predict bacteremia.

Methods

This is a secondary analysis of a multicenter, prospective, observational study conducted in 12 French emergency departments (EDs), entitled "The Biomarkers In Sepsis (BIS) Study" (Claessens et al. 2010a, 2010b). Study methods have been reported previously in details (Claessens et al. 2010a). Briefly, BIS study aimed to determine the performance of several biomarkers of inflammation including MRproANP to predict a predefined gold-standardized admission decision. We defined "gold-standard in-patients" as patients adjudicated by the experts' committee as requiring hospitalization after ED visit, corrected by the occurrence of adverse medical outcome during follow-up. Adverse medical outcomes were defined as any event (subsequent admission after initial discharge, readmission after hospitalization, death) occurring within 28 days of initial ED presentation.

Committee experts were blinded to study patient 28-day outcomes and to biomarkers under study. The study protocol and procedures complied with the principles of the Declaration of Helsinki. The review board for the protection of patients of our institution approved the study protocol and informed consent procedures to patients. Patients enrolled in the study provided written informed consent for participation.

Participants

For the specific purpose of the current study, we restricted analyses to patients enrolled in BIS study presenting to participating EDs with acute pyelonephritis. We excluded from the current analysis patients with missing value for MRproANP or for whom blood sample collection of at least one blood microbiological cultures was not performed (Figure 1). We defined acute pyelonephritis according to standard criteria, namely diagnostic of acute pyelonephritis included acute onset of at least one of the following signs or symptoms: dysuria, nausea, flank pain, costovertebral angle tenderness, body temperature over 38.5°C, and positive urinalysis with a white blood cells count exceeding 10^9

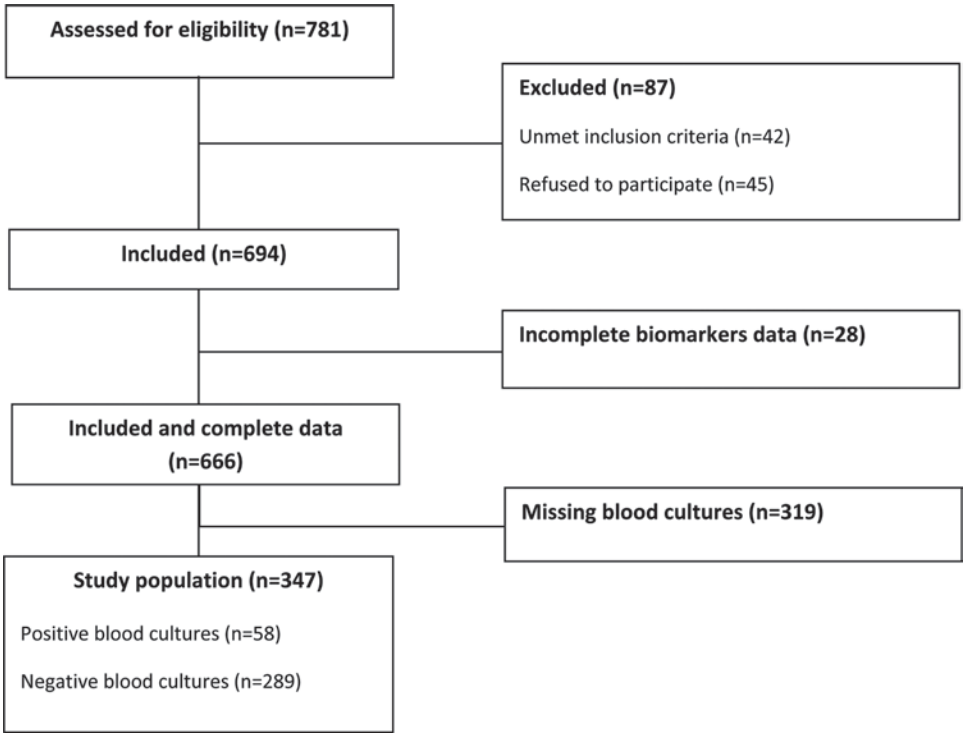

Figure 1. Flow chart of patients presenting to a participating emergency department with acute pyelonephritis and available blood cultures assessed for eligibility.

per liter. All consecutive patients with acute pyelonephritis were eligible for study enrollment if they did not present immediate signs and symptoms of severity. Therefore, we excluded patients with the following conditions: (1) history of manual or instrumental urological examination within 1 month before ED presentation; (2) history of previous antibiotic treatment, defined as antibiotic use for at least 3 days during the past month; (3) presence of an indwelling urinary catheter; (4) presence of previous structural or functional abnormalities (including bladder diverticula, cystoceles, urethral strictures, congenital abnormalities, and renal cysts, as well as functional abnormalities, such as neurogenic bladder and vesicoureteral reflux, kidney failure defined as a creatinine level >130 μmol/L); (5) lumbar or abdominal tenderness; i.e., tenderness to pressure either on the lumbar region or on the abdomen; (6) comorbid conditions that interfere with admission decision (pregnancy; human immunodeficiency virus infection or alternative immunocompromising conditions [active neoplasm, immunosuppressive therapy, prednisone >15 mg/day or equivalent]); (7) septic shock (Bone et al. 1992); (8) palliative care (precluding admission to an ICU); and (9) anticipated barriers to complete follow-up.

Baseline data consisted of demographic data (age, gender), coexisting illnesses, symptoms, clinical findings, and available laboratory tests (white blood cell count, hematocrit, blood urea nitrogen, glucose, and sodium). We performed a 28-day follow-up and afterward, patients were classified as outpatients or in-patients according to the expert committee adjudication.

Blood sample analysis

Blood cultures were processed using an automated colorimetric detection system in each participating center. We defined pyelonephritis-related bacteremia by identification of the same bacterial strain in blood culture and urinalysis within 36 h of culture. Positive samples were stained for Gram coloration and subcultured for identification. All bacterial strains were considered pathogenic but *Staphyloccocus saprophyticus* that was considered as contaminant.

Procedure to test biomarkers has been published elsewhere (Claessens et al. 2010a). Briefly, blood samples were collected in sodium heparin–treated tubes, centrifuged, and stored at –40°C in a central laboratory (CCG and SG, Department of Biochemistry, Groupe Hospitalier Broca Cochin Hôtel-Dieu) until completion of the study. The laboratory measurement process complied with French quality standards for medical laboratories. C-reactive protein (CRP) measurement was performed using an immunoturbidimetric assay (Modular analyzer; Roche Diagnostics, Meylan, France). Procalcitonin (PCT) and MRproANP (epitopes covering amino acids 53–90) concentrations were analyzed using a sandwich immunoassay based on Time Resolved Amplified Cryptate Emission measurement (Kryptor analyzer; B.R.A.H.M.S. Diagnostica, Hennigsdorf, Germany). In our laboratory, coefficients of variation for PCT were found to be <10% (9.1% at 0.28 μg/L and 7.1% at 10.8 μg/L) and for MRproANP were found to be <5%:3.4% at 419.0 pmol/L and 3.7% at 89.2 pmol/L.

Statistical analysis

Baseline and follow-up characteristics were described by means and standard deviations or by median and interquartile range (IQR) for continuous variables, as appropriate, and by percentages for categorical variables. We performed Chi^2 statistics or Fisher's exact tests when appropriate for qualitative variables, and the Wilcoxon/Mann–Whitney test for continuous variables with skewed distributions to compare baseline patient characteristics and study outcomes.

We referred to the STAndards for the Reporting of Diagnostic Accuracy recommendations for analysis of the results (Bossuyt et al. 2003). We used the area under receiver–operator characteristic curves (AUC) to assess the overall discriminatory power of CRP, PCT, and MRproANP in predicting bacteremia. The AUC and its 95% CIs were estimated for each biomarker and compared by a nonparametric method (DeLong et al. 1988). Accuracy of biomarkers to predict bacteremia was calculated for the total population. As heart failure may modify MRproANP, we specifically assessed its performance among patients with and without underlying cardiac disorder. We also estimated whether MRproANP was accurate in the subpopulation experiencing *E. coli*–related pyelonephritis to specifically evaluate the influence of LPS at bedside (Lowry 2005).

In the total population, we estimated CRP, PCT, and MRproANP cutoff values yielding 95% sensitivity and 95% specificity values, and subsequently estimated the Youden index that maximizes the sum of sensitivity and specificity (sensitivity + specificity – 1). Sensitivity, specificity, and positive and negative predictive values were calculated for each cutoff value of CRP, PCT, and MRproANP. We also calculated likelihood ratios (LRs) as a measure of the extent to which the pretest odds were altered by the test results; low LR (<0.1) and high LR (>10) are considered useful in ruling out and ruling in decisions, respectively (Deeks & Altman 2004, Irwig et al. 2002).

All tests were two-sided, and p values <0.05 were considered to denote statistical significance. All statistical analyses were performed using SAS software, V9.1 (SAS Institute, Cary, NC).

Results

Study population

Population of the main analysis has been previously described (Claessens et al. 2010a,b). Among 694 patients included in the study from November 2004 to November 2007, 347 (50%) had available data for both blood cultures and MRproANP measurements (Figure 1).

Characteristics of the study population are described in Table 1. Briefly, 320 (92%) were women and median age was 31 (24–48) years. A minority presented with significant underlying conditions. Among these, 22 (6%) suffered from chronic heart failure. Urine and blood cultures were positive in 286 (82%) and blood cultures in 58 (17%), respectively. The main pathogen was *E. coli* ($N = 273$, 79%) and other enterobacteriaceae were recognized in 12 patients (4%; Table 2). Blood cultures were positive with *E. coli* in 46 (79%), other enterobacteriaceae in 2 (3%), *Staphylococcus* in 6 (10%), other Gram-positive cocci in 3 (5%), *Haemophilus* in 1 (2%). Patients with and without positive blood cultures significantly differed in age, temperature, platelet count, urea levels, and for CRP, PCT, and CRP concentrations (Table 1). Patients with positive blood cultures were more likely to be admitted. All patients survived at 30 days.

MRproANP measurements according to results of blood cultures

Median concentrations of MRproANP were 98.4 pmol/L [IQR 68.2–153.1] in patients with positive blood culture, and 66.4 pmol/L [IQR 51.0–90.3] in those with negative blood cultures. These concentrations were significantly different ($p < 0.01$). As elevation of MRproANP has been specifically described after LPS challenge in healthy volunteers, we analyzed the subgroup of 273 patients with *E. coli*–related infection, corresponding to 46 patients with positive blood cultures and 227 with negative blood cultures and positive urine cultures. In this specific subset, MRproANP measurements were more elevated in patients with positive blood cultures (82.8 pmol/L [IQR 66.9–145.0] vs. 64.1 pmol/L [IQR 51.0–89.3], $p < 0.01$).

We obtained results for median concentrations of MRproANP according to the presence of underlying chronic cardiac disorders as heart failure may impact concentrations of natriuretic peptides. In patients with positive blood cultures, MRproANP concentrations were more elevated in patients with chronic heart failure (297.5 pmol/L [IQR 195.6–394.6] vs. 80.4 pmol/L [IQR 65–122], $p = 0.003$). This was also observed in patients without bacteremia (176.8 pmol/L [IQR 124.8–246.7] vs. 65 pmol/L [IQR 50.3–87.9], $p < 0.01$). To note, bacteremia was associated with increased MRproANP concentrations in patients without heart failure ($p = 0.001$).

We assessed whether results of blood cultures modified levels of CRP and PCT, two biomarkers usually associated with infections. We observed that CRP measurements increased in patients with positive blood cultures (161.1 mg/L [IQR 88.3–250.9] vs. 95.2 mg/L [IQR 45.7–173.7], $p < 0.01$). PCT concentrations also increased in patients with bacteremia as compared to those without

Table 1. Baseline characteristics of 347 patients presenting with acute pyelonephritis.

	Total ($n = 347$)	Positive blood culture ($n = 58$)	Negative blood culture ($n = 289$)	p value
Demographics and clinical features				
Age (years), median [IQR]	32.6 [25.1–52.6]	51.7 [31.1–69.8]	31.0 [24.6–48.7]	<0.01
Female sex, *n* (%)	320 (92.2)	50 (86.2)	270 (93.4)	0.10
Diabetes mellitus, *n* (%)	17 (4.9)	6 (10.3)	11 (3.8)	0.05
Liver disease, *n* (%)	2 (0.6)	1 (1.7)	1 (0.3)	0.31
Congestive heart failure, *n* (%)	22 (6.3)	8 (13.8)	14 (4.8)	0.02
Cerebrovascular disorder, *n* (%)	3 (0.9)	0	3 (1.0)	ND
Chronic kidney failure, *n* (%)	4 (1.2)	0	4 (1.4)	ND
Temperature (°C), median [IQR]	38.4 [37.7–39.1]	39.0 [38.2–39.6]	38.4 [37.6–39.0]	<0.01
Heart rate (bpm), median [IQR]	104.0 [90.0–115.0]	110.0 [91.0–116.0]	103.0 [90.0–114.0]	0.18
Systolic blood pressure (mmHg), median [IQR]	119.5 [108.0–134.0]	121.0 [112.0–143.0]	119.0 [108.0–133.0]	0.24
Admission, *n* (%)	112 (32.3)	30 (51.7)	82 (28.4)	<0.01
Laboratory findings				
Leucocytes (10^3/mm^3), median [IQR]	12.0 [9.8–14.9]	10.8 [9.7–15.1]	12.2 [9.8–14.9]	0.15
Hematocrit (%), median [IQR]	37.1 [35.2–39.4]	36.9 [35.0–38.8]	37.2 [35.2–39.5]	0.43
Platelets (10^3/mm^3), median [IQR]	233.5 [189.0–281.0]	186.0 [150.0–245.0]	238.5 [199.0–292.0]	<0.01
Sodium (mmol/L), median [IQR]	138.0 [136.0–139.0]	137.0 [135.0–139.0]	138.0 [136.0–140.0]	0.15
Urea (mmol/L), median [IQR]	3.9 [3.0–5.1]	4.8 [3.4–7.6]	3.8 [2.9–4.9]	<0.01
Biomarkers				
CRP (mg/L), median [IQR]	103.8 [48.1–185.9]	161.1 [88.3–250.9]	95.2 [45.7–173.7]	<0.01
PCT (ng/mL), median [IQR]	0.3 [0.1–2.3]	1.7 [0.4–7.6]	0.2 [0.1–0.8]	<0.01
MRproANP (pmol/L), median [IQR]	70.4 [52.4–99.2]	98.4 [68.2–153.1]	66.4 [51.0–90.3]	<0.01

Values are expressed as number (%) and median [IQR].

Comparisons between groups with positive and negative blood cultures were performed using the χ^2 test or Fisher's exact test for qualitative variables and the Wilcoxon/Mann–Whitney test for the Student's *t*-test or quantitative variables. p Values <0.05 were statistically significant.

CRP, C-reactive protein; IQR, interquartile range; PCT, procalcitonin; MRproANP, mid-regional pro-natriuretic peptide. ND, comparison not done because of the sample size.

bacteremia (1.7 ng/mL [IQR 0.4–7.6] vs. 0.2 ng/mL [IQR 0.1–0.8], $p < 0.01$).

Accuracy of MRproANP to predict bacteremia and comparison with CRP and PCT

The AUC of the ROC-curve of MRproANP for bacteremia was 0.689 (95% CI: 0.612–0.765) indicating a mild discriminative power (Figure 2). Accuracy of CRP to predict bacteremia was equivalent to MRproANP (AUC 0.66 [95 CI: 0.585–0.735], $p = 0.53$). PCT was more accurate than CRP and MRproANP to predict results of blood culture (AUC 0.779 [95% CI: 0.715–0.843], $p < 0.01$). In addition, we assessed whether biomarkers more accurately predicted positivity of blood culture in the subset of patients with urinary tract infection related to *E. coli*. We observed that AUC was 0.685 [0.605–0.765] for MRproANP, 0.652 [0.572–0.732] for CRP, and 0.779 [0.710–0.847] for PCT (Figure 3 and Table 3). Values of AUC did not differ for MRproANP and CRP ($p = 0.49$), but performance of PCT was significantly better as compared to MRproANP ($p = 0.02$) and CRP ($p < 0.01$).

Thresholds and corresponding characteristics (sensitivity, specificity, predictive values, and likelihood ratios) were tested for various cutoffs of MRproANP, CRP, and PCT (Table 4). As LR^+ was below 10 for each threshold, using MRproANP to rule in patients could not be safely used in daily practice. The same applied to LR^- that remained above 0.1 and was clinically irrelevant to rule out patients for bacteremia at bedside (Table 4).

Table 2. Microbiological finding in urine and blood cultures in 347 patients with acute pyelonephritis.

Microorganism	Positive urinary cultures	Positive blood cultures
Escherichia coli	264 (92.3)	46 (79.3)
Staphylococcus	2 (0.7)	6 (10.3)
Haemophilus	0	1 (1.7)
Other Gram-positive cocci	4 (1.4)	3 (5.2)
Other Gram-negative bacilli	9 (3.1)	2 (3.4)
Miscellaneous	5 (1.7)	0
Undetermined	2 (0.7)	0
Total	286 (100)	58 (100)

Values are expressed as number (%).

Discussion

In this study, we observed that (i) MRproANP was more elevated in patients with pyelonephritis that experienced bacteremia as compared to those without bacteremia, (ii) MRproANP was even more accurate to predict bacteremia in patients free from chronic heart failure that experienced infection related to enterobacteriaceae, and iii) accuracy of MRproANP was comparable to CRP for bacteremia. However, PCT remained more efficient than MRproANP whose mild performance avoided the use at bedside for prediction of positive blood culture.

Site of infection and bacterial load may impact severity of infectious diseases (Annane et al. 2005; Gerlach et al. 2003; Claessens & Dhainaut 2007). It has been postulated that biomarkers' concentrations and bacterial load could associate (van Langevelde et al. 2000). This has been recently raised for PCT that can predict positive blood stream cultures in community-acquired pneumonia (CAP) patients (Müller et al. 2010). Here, we confirm these results in pyelonephritis patients. To some extent, a strategy based on PCT concentrations could spare 12% of

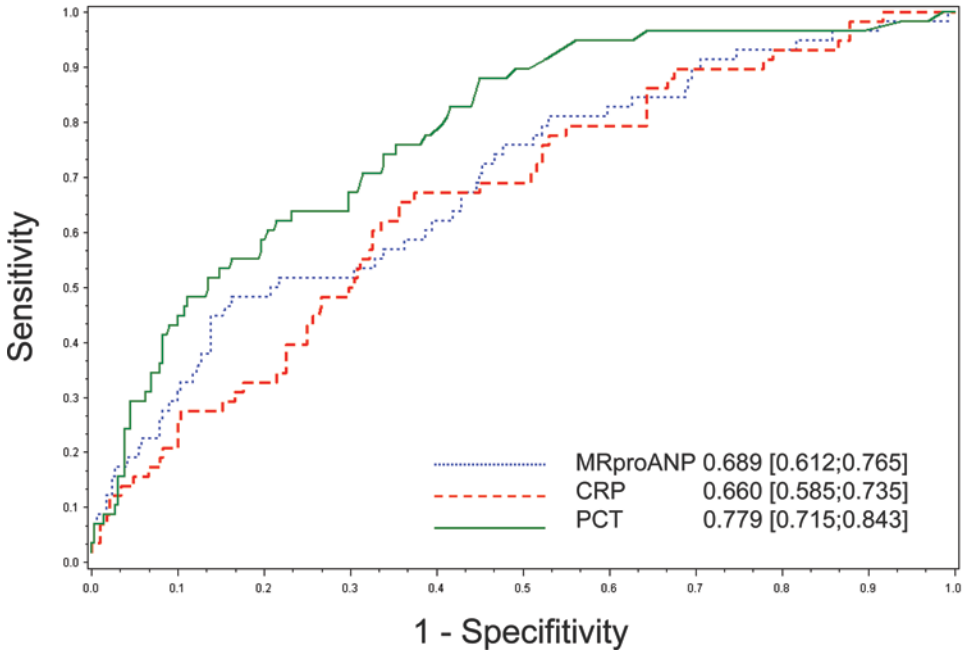

Figure 2. Receiver–operating characteristics (ROC) curves of biomarkers with respect to results of blood culture in 347 patients with acute pyelonephritis. Mid-regional pro-atrial natriuretic peptide (MRproANP): dotted blue line; C-reactive protein (CRP): dashed red line; procalcitonin (PCT): continuous green line.

negative samples (Müller et al. 2010). Therefore, PCT may reflect severity associated to burden of bacterial invasion. Although PCT levels are associated with severity scores (Müller et al. 2010; Boussekey et al. 2005), performance of PCT to predict requirement of admission is too weak to be translated in daily practice, especially in patients with pyelonephritis (Claessens et al. 2010a). Alternatively, MRproANP has emerged as a potent severity marker in sepsis. MRproANP tightly associates with risk categories in severe infections and better predicted mortality than other parameters including PCT (Morgenthaler et al. 2008). This suggests that PCT is rather a diagnosis tool while MRproANP adds information about prognosis. In healthy subjects, MRproANP concentrations increase after LPS challenge, peak at 4 h, and then decrease (de Kruif et al. 2008). Therefore, it possibly represents a marker of bacterial infection, especially in infection with enterobacteriaceae. On the other hand, changes in MRproANP concentrations are associated with diagnosis of acute heart failure and pulmonary edema (Chenevier-Gobeaux et al. 2010; Maisel et al. 2010). Consequently, MRproANP changes during infection may reflect bacterial load, unstable cardiac comorbidity, or both. Interestingly, exposure of murine macrophages to endotoxin stimulates transcription of ANP while levels of brain natriuretic peptide (BNP) mRNA do not change (Vollmar & Schulz 1995). This suggested that ANP and BNP have separate roles and functions in the host–pathogen interaction. MRproANP could be a marker of severity as it may balance proinflammatory processes (Koga et al. 2010; Xing & Birukova 2010). In addition, inflammatory mechanisms related to LPS increase in a dose-dependent manner (Sarraf et al. 1997). However, experimental data may lack medical significance. In particular, endotoxinic models deeply differ from actual infectious diseases as they consist of healthy subjects exposed to a single intravenous self-limited LPS challenge without source of infection (Lowry 2005). Therefore, characteristics of these trials limit translation of their results in human medicine. This prompted us to investigate at bedside the influence of bacteremia in patients. Results from the CAPNETZ study suggested that MRproANP predicted severity of CAP independent of the presence of underlying heart failure or disorder (Krüger et al. 2010a, 2010b). In our study, MRproANP still had a prognostic value in a subpopulation of CAP patients without chronic heart failure (Claessens et al. 2010b). However, as sepsis can impair renal and heart dysfunction, increase of MRproANP may result from instability of previously unknown underlying cardiac failure (Krüger et al. 2010a, 2010b).

In addition, it should be interrogated whether increase of MRproANP could be specific to some bacterial species. Based on observation in murine macrophages (Vollmar & Schulz 1995), it can be postulated that binding of endotoxin and zymosan may induce a targeted response for ANP transcription. Therefore, it is unsure whether a similar pattern of MRproANP levels could be observed with microorganisms other than enterobacteriaceae. In the present study, this question could not be answered as most patients had an infection related to *E. coli*, and no comparison could be done with other bacteria species.

There may be a lack of bacterial growth from peripheral blood in spite of apparently large bacterial load in the primary site of infection as results of blood cultures

Table 3. Values of AUCs for accuracy of MRproANP, PCT, and CRP to predict bacteremia in 22 patients with and 325 without congestive heart failure.

	Congestive heart failure	AUC	SD	[95% CI]
MRproANP	Present	0.750	0.120	[0.513–0.986]
	Not present	0.666	0.042	[0.584–0.749]
PCT	Present	0.928	0.054	[0.822–1.034]
	Not present	0.760	0.035	[0.691–0.829]
CRP	Present	0.696	0.125	[0.449–0.943]
	Not present	0.649	0.041	[0.569–0.730]

Table 4. Ability of MRproANP, CRP, and PCT to predict bacteremia in 347 patients with acute pyelonephritis.

Biomarkers	Cutoff	Sensitivity	Specificity	PPV	NPV	LR^+	LR^-
MRproANP (pmol/L)	48	0.94 (0.88; 1)	0.18 (0.13; 0.23)	0.21 (0.16; 0.26)	0.93 (0.85; 1)	1.14 (1.04; 3.56)	0.33 (0.11; 1.02)
	68*	0.75 (0.63; 0.87)	0.56 (0.49; 0.62)	0.28 (0.21; 0.36)	0.9 (0.86; 0.95)	1.69 (1.36; 2.75)	0.45 (0.28; 0.73)
	194	0.17 (0.07; 0.28)	0.95 (0.92; 0.98)	0.45 (0.23; 0.67)	0.83 (0.78; 0.88)	3.48 (1.52; 3.95)	0.87 (0.77; 0.99)
CRP (mg/L)	27	0.94 (0.88; 1)	0.14 (0.09; 0.18)	0.2 (0.15; 0.26)	0.91 (0.81; 1)	1.09 (1; 3.44)	0.43 (0.13; 1.34)
	136*	0.65 (0.52; 0.78)	0.65 (0.58; 0.71)	0.3 (0.22; 0.39)	0.89 (0.84; 0.94)	1.85 (1.42; 2.73)	0.53 (0.36; 0.79)
	325	0.13 (0.04; 0.23)	0.95 (0.92; 0.98)	0.39 (0.16; 0.61)	0.82 (0.78; 0.87)	2.7 (1.1; 3.02)	0.91 (0.81; 1.02)
PCT (ng/mL)	0.1	0.96 (0.91; 1)	0.28 (0.22; 0.34)	0.24 (0.18; 0.3)	0.97 (0.92; 1)	1.33 (1.2; 5.26)	0.14 (0.04; 0.55)
	0.3*	0.84 (0.75; 0.94)	0.58 (0.51; 0.64)	0.32 (0.24; 0.4)	0.94 (0.9; 0.98)	2.01 (1.66; 3.84)	0.27 (0.14; 0.51)
	8.3	0.23 (0.12; 0.35)	0.95 (0.92; 0.98)	0.52 (0.32; 0.73)	0.84 (0.79; 0.89)	4.64 (2.17; 5.4)	0.81 (0.7; 0.94)

Results are expressed as values [IQR]. *Youden index is a threshold maximizing sensitivity and specificity (sensitivity + specificity -1).
Incomplete biomarkers data: $n = 28$; assessed for eligibility: $n = 781$; included and complete data: $n = 666$; included: $n = 694$.
CRP, C-reactive protein; PCT, procalcitonin; PPV, positive predictive value; IQR, interquartile range; LR^+: positive likelihood ratio; LR^-: negative likelihood ratio; MRproANP, mid-regional pro-natriuretic peptide; NPV: negative predictive value.

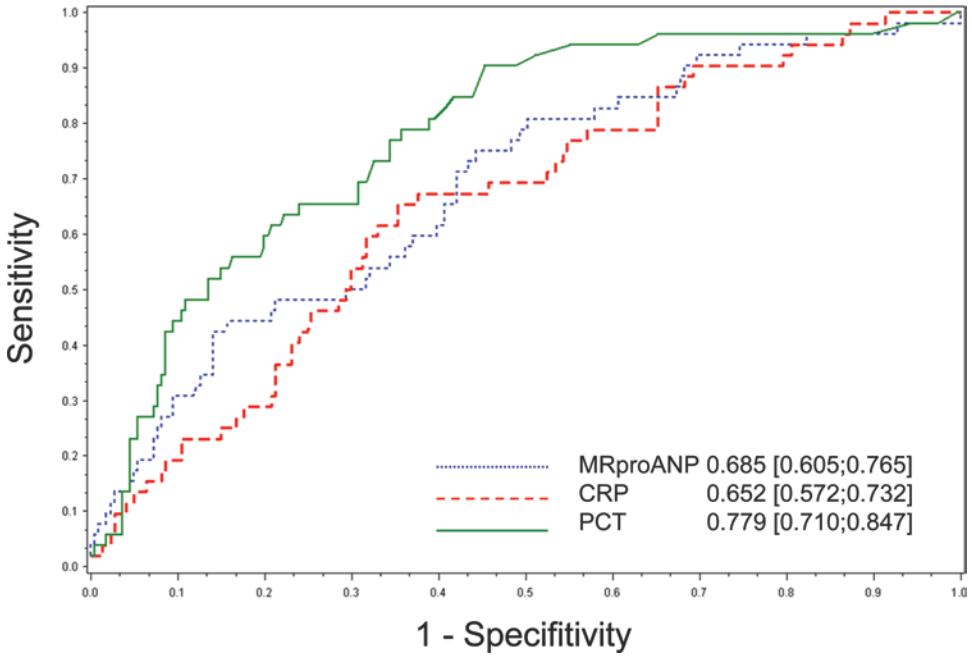

Figure 3. Receiver–operating characteristics (ROC) curves of biomarkers with respect to results of blood culture in 273 patients with *Escherichia coli*–related pyelonephritis. Mid-regional pro-atrial natriuretic peptide (MRproANP): dotted blue line; C-reactive protein (CRP): dashed red line; procalcitonin (PCT): continuous green line.

are subjected to a variety of technical barriers. Among these, bacterial load in circulating blood greatly influences detection of bacteremia. For instance, 23% cultures are positive when bacteria contained in patient's blood represent 1 colony-forming units (CFU)/mL, and positivity of blood cultures increase up to 60% if bacteremia is 10 CFU/mL (Kellog et al. 2000). Bacteremia is recorded in 7.5–30% of patients with acute pyelonephritis (Brown et al. 2005, Foxman et al. 2003). Therefore, the use of biomarkers to privilege blood tests in patients with high probability of bacteremia may be advocated. However, likelihood ratios suggested that MRproANP was clinically inefficient to identify patients with positive blood culture (Table 3).

Limitation

We acknowledge that our results should be interpreted with caution. First, this is a retrospective analysis of a prospective study. Of note, population size was not calculated to specifically address the association between biomarkers and bacteremia. In addition, obtaining blood culture was not specifically required for the study protocol. Blood cultures are not recommended to manage acute pyelonephritis with low risk of adverse outcome. It has been advocated that low rate of blood cultures' positivity limits their usefulness at bedside in patients with urinary tract infection, and that results do not alter practice and outcome (Velasco et al. 2003). Therefore, we could not specifically recommend blood culture in our study patients and this decision was left at the attending physicians' discretion. Consequently, half of the patients had blood cultures and could be analyzed here. The perceived need for blood culture could have resulted from a more severe clinical presentation. The differences between the two groups may support this hypothesis, as patients with blood culture were older (32.6 vs. 28.8 years, $p<0.01$), had higher temperature (38.4 vs. 37.5°C, $p<0.01$) and more elevated leucocytes (12,000 vs. 11,400/µL, $p=0.02$). Those patients also had higher biomarkers concentrations for CRP (103 vs. 44.7 mg/L, $p<0.01$) and PCT (0.3 vs. 0.1 mg/L, $p<0.01$). To note, MRproANP concentrations were increased in the population with blood cultures (70.4 vs. 58.9 pmol/l, $p<0.01$).

Second, patients enrolled in this study were mainly mild pyelonephritis that all survived at the end of the follow-up. We specifically excluded patients that had evidence for critical illness as we believe that these patients do not require biomarker assessment to decide site of care. However, results about blood culture cannot be translated to more severe patients.

Third, we and other previously determined that MRproANP concentrations highly depend on renal function (Chenevier-Gobeaux et al. 2010). In our trial, patients with impaired kidney function were excluded from the study. Therefore, we were unable to discuss variability of MRproANP concentrations according to renal function in the context of pyelonephritis.

Finally, some patients' categories could not enter the study, including those with severe sepsis and septic shock. Patients with critically severe urosepsis are supposed to more frequently experience bacteremia. They also have elevated concentrations of natriuretic peptides. This partly relies on enzyme activity of neutral endopeptidase 24.11 (Pirracchio et al. 2008), that was depressed with sepsis severity. Therefore, interpretation of the impact of bacteremia on natriuretic peptides

concentrations should be very cautious in critically ill patients with infection. Unfortunately, we did not measure neutral endopeptidase activity in our population. Therefore, selecting a homogenous population of patients without severe infection could limit the potential impact of MRproANP variability related to enzyme activity. However this should balance our results especially in more severely ill patients.

Conclusion

Here, we report for the first time the association of MRproANP elevation and bacteremia in patients experiencing acute uncomplicated pyelonephritis. We conclude that MRproANP is partly a marker of bacteremia in urinary tract sepsis; however, biomarkers' characteristics are too weak to recommend their use at bedside in this setting. However, we believe that this clinical model of homogeneous monomicrobial infection with Gram-negative bacilli and limited underlying conditions can help physicians understand and interpret the meaning of MRproANP increase in more complex infectious diseases including severe sepsis and CAP.

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AUC, areas under receiving–operating curves; SD, standard deviation; 95% CI, 95% confidence interval.

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S.G.B. participated in the design of the protocol, analysis of the results and drafting of the manuscript. S. Grabar participated in the design of the study and critical revision of the manuscript. C.C.G. participated in the biochemical analysis of the blood samples and critical revision of the manuscript. L.Q. performed the statistical analysis. S. Guerin was responsible for the biochemical analysis of the blood samples. J.S., G.K., P.H., B.B., C.L., A.G., E.C., and N.T. were involved in local organization of the study. B.R. participated in the critical revision of the manuscript. Y.E.C. conceived the study, participated in the design and coordination of the protocol, obtained the funding, carried out main study analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Declaration of interest

Brahms Inc. provided the Kryptor device and corresponding reagents to obtain PCT and MRproANP measurements.

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