Original Paper

Comparison of the Diagnostic Performance of Three Natriuretic Peptides in Hemodialysis Patients: Which is the Appropriate Biomarker?

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Key Words

Natriuretic peptides • Hemodialysis • Mortality

Abstract

Background: Plasma concentrations of natriuretic peptides are often elevated in chronic hemodialysis patients and difficult to interpret due to accumulation, high incidence of cardiac disease and changes in volume status. Mid-regional pro-ANP is a newly developed assay whereas BNP and its fragment NT-pro-BNP are available for a longer time. In this cross-sectional study, we compared the plasma concentration of MR-pro-ANP, BNP and NT-pro-BNP in stable ambulatory hemodialysis patients (n = 239) and investigated their associations with clinical factors such as residual diuresis, cardiac status and interdialytic weight gain and with mortality.

Methods and Results: In all patients enrolled, the plasma concentration of all natriuretic peptides were largely elevated with a median concentration of 337 pg/ml (interquartile range 146-684) for BNP, 4435 pg/ml (1687-16228) for NT-proBNP and 907 pmol/L (650-1298) for MR-pro-ANP. Plasma concentration of all natriuretic peptides correlated independently with age, degree of systolic dysfunction and negatively with residual diuresis. Dependency on residual renal clearance was strongest for the fragments MR-pro-ANP and NT-pro-BNP. The plasma concentration of all natriuretic peptides was associated with mortality within 2 years of follow-up. Receiver-operated curves revealed a low sensitivity (32-45%), but high specificity for all natriuretic peptides (85-93%) resulting in a high negative predictive (82-87%). Best cut-off values obtained from were 18 611 pg/ml for NT-pro-BNP, 958 pg/ml for BNP and 1684 pmol/L for MR-pro-ANP. **Conclusions:** In hemodialysis patients, the fragments NT-proBNP and MR-pro-ANP are largely elevated compared to BNP which is explained by accumulation. The prognostic performance of MR-pro-ANP is similar to that of NT-pro-BNP or BNP.

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Introduction

Elevations of the plasma concentration of natriuretic peptides are commonly found in chronic hemodialysis patients and are associated with increased mortality [1-5]. Physiologically, natriuretic peptides such as atrial and B-type natriuretic peptide (ANP and BNP) are secreted from cardiac muscle in response to an elevated filling pressure and reflect volume overload [6, 7]. In patients with heart failure, this response is highly stimulated and results in sometimes largely elevated plasma levels which can be utilized for diagnostic purposes [8, 9]. After secretion of precursor peptides, biologically active ANP and BNP is formed after cleavage of N- and C-terminal fragments leaving peptides of 28 and 32 amino acids in length and 3 and 3.5 kDa in weight, respectively. The clearance of ANP and BNP is achieved by the serum protease neutral endopeptidase and after receptor binding [6]. The most common markers measured in clinical practice are BNP and the N-terminal fragment of pro-BNP (NT-pro-BNP) whereas the determination of ANP is hampered by its instability *ex vivo* [10]. Therefore, assays measuring the concentration of stable precursor pro-ANP were developed [11, 12]. In 2004, a novel assay detecting mid-regional pro-ANP (MR-pro-ANP) was developed [13]. Up to the present, only one study evaluated its value in hemodialysis patients showing an association with mortality [14]. In predialysis patients, MR-pro-ANP was predictive of progression of renal failure [15].

In patients with end-stage renal disease, largely elevated plasma natriuretic peptide concentrations are difficult to interpret due to possibly reduced renal clearance and accumulation on the one hand and a high prevalence of cardiac disease and heart failure, even in the presence of preserved ejection fraction, on the other [16, 17]. In addition, plasma natriuretic peptide concentrations are affected by the volume status [18], which varies particularly between the hemodialysis sessions. Still, plasma natriuretic peptide concentrations have proven to be a strong predictor of mortality in ESRD patients [4, 19].

In our study, we analyzed factors influencing the plasma concentration of MR-pro-ANP, BNP and NT-pro-BNP in a comparative approach in stable ambulatory hemodialysis patients in order to investigate the contribution of accumulation, cardiac disease or volume status. In addition we analyzed the prognostic value of each natriuretic peptide.

Materials and Methods

Patients and cohort

This cross-sectional prospective multicentre study was conducted in stable ambulatory hemodialysis patients from four dialysis centers in Southwest Germany between September 2009 and April 2010. Patients were included after obtaining informed consent, when there was no evidence of an acute illness such as infection and a cardiac event such as myocardial infarction or percutaneous coronary procedure within the previous two months. Patients with cardiac diseases leading to increased plasma natriuretic peptide concentration independent of ESRD such as amyloidosis were excluded. The study was approved by the local ethics committee.

Laboratory assays

Plasma concentration of MR-pro-ANP, NT-pro-BNP and BNP was measured in three independent samples taken within 2 weeks, each prior to the start of a dialysis session. Blood was collected in lithium-heparinized tubes (Fa. Sarstedt, Nuembrecht, Germany), cooled at 4°C, centrifuged within 4 hours and the sera stored at -80°C for further analysis. Plasma concentration of MR-pro-ANP was measured using an automated immunoluminometric assay on a Kryptor system (B.R.A.H.M.S AG, Henningsdorf, Germany) as described in [13]. In healthy persons, the range of MR-pro-ANP concentrations was 9.6–313 pmol/L with a median of 45 pmol/L and a 99th percentile of 197.5 pmol/L [13]. Plasma concentration of BNP and NT-pro-BNP were measured on a Siemens ADVIA Centaur and Siemens Immulite 2000 XPI system, respectively, as specified by the manufacturer (Siemens Healthcare Diagnostics, Eschborn, Germany).

Plasma beta-2-microglobulin concentration was measured using a turbidimetric assay (Randox Laboratories, Antrim, United Kingdom). All other laboratory values (parathormone, hemoglobin, albumin and C-reactive protein) were extracted from the patients' medical records and averaged from the available values of the previous year (4 – 12 values).

Clinical data

From each patient data on residual diuresis (measured by 24 h urine collection), single pool Kt/V (mean of last 4 values), interdialytic weight gain, predialytic systolic and diastolic blood pressure (means from the last 12 values, resp.), dialysis access and membrane, time on dialysis, blood pump flow and finally shunt flow (measured with a Transonic system, Ithaca, NY, USA) were extracted. Left ventricular (LV) systolic function was classified from available echocardiography examinations whereby class 1 denoted normal, 2 mildly reduced, 3 moderately or severely reduced systolic function. Determination of the LV systolic function was done at the discretion of the cardiologist and not standardized. Echocardiography was available in 84% of all patients within one year (plus-minus) relative to study enrollment.

Statistical analysis

Three samples were available in 87% of the patients and were averaged to calculate the arithmetic mean without excluding possible outliers. Arithmetic means of the plasma natriuretic peptide concentration and continuous clinical data were log transformed to approximate normal distribution. The association of the plasma natriuretic peptide concentration with clinical or dialysis-related factors was analyzed by univariate parametric correlation. To identify independent determinants of the plasma natriuretic peptide concentration, multivariate linear regression analyses were performed. Selection of the variables entering the model were derived from forward-stepwise multiple linear regression, and all variables with a p-value < 0.05 were subsequently included in the multivariate linear regression models. Finally, the residuals of each model were tested for normality. Averaged values of the deceased patients were compared to those from the surviving patients using t-Test or Wilcoxon's test. Kaplan-Meier curves were generated after stratification into tertiles of the variable according to its distribution. The follow-up period started on the first day of blood draw and was censored as of January 2012. The diagnostic performance was analyzed using receiver-operator curves (ROC or c-index) and the best cut-off value was considered as the maximal difference of sensitivity and 1-specificity (Youden index). Univariate and multivariate proportional hazards were calculated to analyze the risk ratios of each predictor and the independence of the predictors. Data analysis was done using the statistical software package JMP 8.0.1 (SAS Institute, Cary, NC).

Results

Patients

From a total of n = 250 available patients treated in the participating centers, n = 239 were included in the study. N = 11 patients were excluded due to decline to participate (n = 6), death within the period of blood sampling (n = 2), cardiac amyloidosis (n = 2) and recent enrollment to dialysis (n = 1). The characteristics of the study cohort are given in table 1.

Plasma natriuretic peptide concentration in the cohort

In all participating patients, the plasma concentration of MR-pro-ANP was elevated with a median concentration of 907 pmol/L (650-1298) and exceeded the upper limit used to diagnose heart failure at 130 pmol/L [20] (Figure 1A). This proportion was 99% for NT-pro-BNP > 300 pg/mL [21] and 81% for BNP >100 pg/mL [9]. Plasma natriuretic peptide concentrations were highly correlated to each other, with the highest correlation between MR-pro-ANP and BNP (0.86, p<0.0001), followed by BNP and NT-pro-BNP (0.51, p<0.0001) and MR-pro-ANP and NT-pro-BNP (0.47, p<0.0001). Within three samples, the variability was least for MR-pro-ANP (10 ± 9 %), followed by NT-pro-BNP (23 ± 16 %) and BNP (32 ± 21%). The plasma concentration of MR-pro-ANP was significantly higher after an interdialytic interval of 3 days than of 2 days (1027 [702; 1370] vs. 802 [536; 1232] pmol/l).

Table 1

Patient characteristics of the cohort (n=239)		Values shown are the median and interquartile range
median age		70 (61; 77) years (n= 239)
gender distribution		64 % male (n= 153) / 36 % female (n= 86)
renal disease	diabetic nephropathy	26 % (n= 63)
	hypertension	8 % (n= 19)
	glomerulonephritis	30 % (n= 71)
	polycystic disease	5 % (n= 11)
	other / unknown	31 % (n= 75)
cardiac comorbidities	coronary heart disease	31 % (n= 74)
	revascularized	19 % (n= 31)
	valvular heart disease	26 % (n= 61)
	atrial fibrillation	23 % (n= 55)
	pulmonary hypertension	7 % (n= 16)
	AICD carrier	2 % (n= 4)
other comorbidities	diabetes mellitus	38 % (n= 90)
	peripheral vascular disease	33 % (n= 80)
	stroke	16 % (n= 38)
	vasculitis	3 % (n= 8)
	malignoma	14 % (n= 34)
	COPD	8 % (n= 19)
length of time on dialysis		46 (19; 85) months (n= 239)
duration of dialysis session		4.0 (4.0; 4.5) hours (n= 239)
dialysis access	arteriovenous fistula	71 % (n= 169)
	PTFE graft	13 % (n= 31)
	tunneled catheter	16 % (n= 38)
dialysis membrane	high-flux	92 % (n= 219)
	low-flux	8 % (n= 20)
residual diuresis		250 (0; 1000) ml / day (n= 239)
	anuric patients	39 % (n= 93)
interdialytic weight gain		1.85 (1.29; 2.47) kg (n= 239)
blood pump speed		300 (280; 340) ml / min (n= 239)
shunt flow		1080 (733; 1475) ml / min (n= 187)
blood pressure		134 (122; 144) / 69 (63; 74) mm Hg (n= 239)
pulse pressure		65 (54; 74) mm Hg (n= 239)
singe pool Kt / V		1.55 (1.40; 1.73) (n= 239)
laboratory data	hemoglobin	11.5 (11.1; 12.0) g / dl (n= 239)
	C-reactive protein	8.6 (4.6; 15.0) mg / l (n= 239)
	albumin	37.1 (35.4; 39.3) g / l (n= 239)
	parathormone	204 (130; 348) pg / ml (n= 239)
	β2-microglobulin	23.4 (19.4; 25.4) mg / l (n= 239)
LV systolic function	normal	59 % (n= 140)
	mildly reduced	13 % (n= 32)
	moderately or severely reduced	12 % (n= 29)
	unknown	16 % (n= 38)

N indicates number of patients from which data were available, abbreviations: PTFE polytetrafluorethylen, LV left ventricle, AICD automated implantable cardioverter-defibrillator

The same was true for BNP (435 [186; 888] vs. 274 [86; 532] pg/ml) and NT-pro-BNP (5407 [2044; 19401] vs. 2355 [1115; 8229] pg/ml).

Univariate analyses

Table 2 lists the results of univariate correlation analyses of the plasma natriuretic peptide concentration with the collected parameters. MR-pro-ANP as well as BNP and NT-pro-BNP showed a significant positive correlation to age, degree of systolic dysfunction, time on dialysis and blood pump speed and they were all negatively correlated to residual diuresis. MR-pro-ANP and NT-pro-BNP were further positively correlated to interdialytic weight gain and plasma β2-microglobulin concentration.

Table 2: Univariate correlations (Pearson's r) of the plasma natriuretic peptide concentration with general and dialysis-specific parameters ($n = 210 - 239$)

	MR-pro-ANP, pmol/L	NT-pro-BNP, pg/ml	BNP, pg/ml
age, y	0.25 **	0.20 **	0.32 ***
systolic LVF, classes	0.40 ***	0.26 **	0.46 ***
time on dialysis, months	0.25 **	0.12 #	0.13 *
residual diuresis, ml/day	- 0.26 ***	- 0.17 **	- 0.16 *
interdialytic weight gain, kg	0.17 **	0.11 #	n.s.
shunt flow, ml/min	n.s.	n.s.	n.s.
systolic blood pressure, mm Hg	n.s.	n.s.	n.s.
diastolic blood pressure, mm Hg	n.s.	n.s.	n.s.
pulse pressure, mm Hg	n.s.	n.s.	0.11 #
duration of a HD session	n.s.	n.s.	n.s.
blood pump speed, ml/min	0.13 #	0.14 #	0.12 #
Kt/V	n.s.	n.s.	n.s.
hemoglobin, g/dL	n.s.	n.s.	n.s.
plasma albumin, g/L	n.s.	n.s.	n.s.
C-reactive protein, mg/L	0.21 **	n.s.	0.17 **
parathormone, pg/mL	0.11 #	n.s.	0.15 *
β 2-microglobulin, mg/dL	0.32 ***	0.27 ***	n.s.

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = not significant ($p > 0.10$)

Fig. 1. A: Box-and-Whiskers-Plot of the plasma natriuretic peptide concentration indicating median, interquartile range and the range between the 2.5th and 97.5th percentile. Solid lines represent the upper reference limit used to diagnose heart failure (<100 pg/mL for BNP [9], 300 pg/mL for NT-pro-BNP [21], 130 pmol/L for MR-pro-ANP [20]). B: Box-and-Whiskers-Plot of the plasma natriuretic peptide concentration expressed as multiples of the cut-off used for diagnosing heart failure, respectively. *** indicates significant difference ($p < 0.0001$) between the natriuretic peptide concentration.

Multivariate analyses

To analyze independent determinants of the MR-pro-ANP plasma concentrations, a multivariate linear regression model was performed with a stepwise forward approach. Table 3 lists independent determinants with a p -value of < 0.05 and their contribution to the

Table 3: Independent factors determining plasma natriuretic peptide concentration by stepwise multivariate linear modelling (n = 155 - 201)

	covariate	estimate ± SD	standardized estimate ± SD	p-value
MR-pro-ANP	y-intercept	-0.06 ± 0.65		
	age, y	0.68 ± 0.14	0.19 ± 0.04	<0.0001
	systolic LVF, classes [3 vs. 2]	0.15 ± 0.05	0.15 ± 0.05	0.0050
	diastolic blood pressure, mm Hg	0.80 ± 0.27	0.13 ± 0.04	0.0040
	residual diuresis, ml/24 h	$-8 \cdot 10^{-5} \pm 2 \cdot 10^{-5}$	-0.11 ± 0.02	<0.0001
	systolic LVF, classes [2 vs. 1]	0.10 ± 0.04	0.10 ± 0.03	0.0115
NT-pro-BNP	shunt flow, ml/min	0.11 ± 0.05	0.07 ± 0.03	0.0380
	y-intercept	0.36 ± 0.92		
	age, y	1.37 ± 0.38	0.37 ± 0.10	0.0005
	systolic LVF, classes [2 vs. 1]	0.36 ± 0.12	0.36 ± 0.12	0.0030
	residual diuresis, ml/24 h	$-8 \cdot 10^{-4} \pm 5 \cdot 10^{-5}$	-0.31 ± 0.07	<0.0001
	systolic LVF, classes [3 vs. 2]	0.30 ± 0.16	0.30 ± 0.16	0.0594
BNP	shunt flow, ml/min	0.28 ± 0.16	0.18 ± 0.10	0.0838
	y-intercept	-2.07 ± 1.37		
	systolic LVF, classes [3 vs. 2]	0.29 ± 0.09	0.29 ± 0.09	0.0016
	systolic LVF, classes [2 vs. 1]	0.40 ± 0.12	0.30 ± 0.11	0.0124
	residual diuresis, ml/24 h	$-1 \cdot 10^{-4} \pm 4 \cdot 10^{-5}$	-0.18 ± 0.06	0.0065
	age, y	1.44 ± 0.33	0.47 ± 0.11	<0.0001
	diastolic blood pressure, mm Hg	1.04 ± 0.59	0.21 ± 0.12	0.0762

Fig. 2. Survival curves of the cohort stratified according to tertiles (T1, T2 and T3) of MR-pro-ANP (< 724; 724 - 1159; > 1159 pmol/l), NT-pro-BNP (< 2384; 2384 - 8412; > 8412 pg/ml) and BNP (< 195; 195 - 528; > 528 pg/ml) plasma concentration.

model indicated by the standardized estimate. Independent determinants of the MR-pro-ANP, NT-pro-BNP and BNP plasma concentration were age, systolic LV function and residual diuresis. Diastolic blood pressure and shunt flow were also determinants of MR-pro-ANP. MR-pro-ANP had the highest r^2 with 0.4185 indicating that 42% of the variability in plasma MR-pro-ANP concentration could be explained by these factors (Table 3). In contrast, the best model found for BNP and NT-pro-BNP had an r^2 of 0.3283 and 0.3190 respectively.

Prognostic value

During a median follow-up time of 710 days (679; 761), 44 patients died corresponding to an annual mortality rate of 9.46%. Compared to surviving patients, deceased patients were

Table 4: Prognostic performance of the plasma natriuretic peptide concentration in predicting death during follow-up

	cut-off	AUC (c-index)	Youden index	sensitivity, %	specificity, %	positive predictive value, %	negative predictive value, %
MR-pro-ANP	1684 pmol/l	0.62	0.25	32	93	50	86
NT-pro-BNP	18611 pg/ml	0.64	0.27	43	84	13	82
BNP	958 pg/ml	0.66	0.31	45	85	41	87

Fig. 3. Hazard ratio for mortality according to tertiles (T1, T2 and T3) of MR-pro-ANP (< 724; 724 - 1159; > 1159 pmol/l), NT-pro-BNP (< 2384; 2384 - 8412; > 8412 pg/ml) and BNP (< 195; 195 - 528; > 528 pg/ml) plasma concentration. The hazard ratio of the first tertile was set to 1. * $p < 0.05$, ** $p < 0.01$.

significantly older (77 years [68;82] vs. 69 [59; 76]; $p < 0.0001$), were more likely to have LV dysfunction (68% vs. 24%; $p < 0.0001$) and had lower diastolic blood pressure (65 mm Hg [59;72] vs. 69 [63; 74]; $p = 0.0087$). The concentration of MR-pro-ANP was significantly ($p < 0.01$) higher in deceased patients compared to surviving patients 1106 [746;1806] pmol/L vs. 865 [634;1235] pmol/L, which was similar for NT-pro-BNP 12993 [4360; 29416] pg/mL vs. 3760 [1485; 11571] and BNP 577 [257;1772] pg/mL vs. 299 [127;573] pg/mL.

The survival curves stratified for tertiles of plasma natriuretic peptide concentrations are shown in Figure 2, the relative risk compared to the first tertile is shown in Figure 3. When analyzing the diagnostic performance of plasma natriuretic peptides concentrations using contingency tables and receiver-operator curves (Table 4), all natriuretic peptides showed similar AUC-values and had a high negative predictive value (82 - 87%). For each increase of the plasma natriuretic peptide concentration by 100 pmol/L for MR-pro-ANP, by 100 pg/mL for BNP and 1000 pg/mL for NT-pro-BNP, the risk ratio was increased by 7.8%, 6.1% and 2.4%, respectively. In a multivariate proportional hazards model with age, systolic function and plasma natriuretic peptides concentration, plasma BNP and NT-pro-BNP concentration, but not plasma MR-pro-ANP concentration, were independent predictors ($p < 0.05$) of mortality in addition to systolic function (data not shown).

Discussion

This study demonstrates that MR-pro-ANP has a similar performance in stable hemodialysis patients compared to BNP and NT-pro-BNP. Our data show that the MR-pro-ANP plasma concentrations were to a much greater extent dependent on residual renal clearance compared to BNP and resulted in concentrations above the upper limit used to diagnose heart failure in all patients. This finding was also evident for NT-pro-BNP plasma concentrations suggesting that the clearance of these fragments is highly dependent on residual function in contrast to BNP which can be eliminated after binding to the natriuretic peptide clearance receptor and the action of neutral endopeptidase [22, 23]. The accumulation of the plasma natriuretic peptide concentration limits its clinical use in patients with chronic kidney disease. Müller et al. found that determination of BNP in dyspnoeic patients presenting to an emergency department improved patient management only little as opposed to patients without renal disease due to increased baseline concentrations of BNP which decreases the negative predictive value to rule out cardiac failure [24]. In our study, the negative predictive value for the end point mortality was still high with values >80% indicating a good prognosis in patients with a plasma natriuretic peptide concentration below the calculated cut-off values (Table 4). However, the sensitivity and positive predictive values were low and preclude accurate prediction in an individual patient.

The diagnostic value of plasma natriuretic peptide concentration in hemodialysis patients can be improved by establishing specific cut-off values for this population. McCullough et al. found that a BNP plasma concentration of >225 pg/ml should be applied when a patient has a GFR <30 ml/min/1.73m² [25]. For MR-pro-ANP, the cut-off for diagnosing heart failure was found to be 130 pmol/L in the BACH (Biomarkers in Acute Heart Failure) trial [20]. However, a cut-off value adapted to patients with CKD and, even hemodialysis, has not been established. Our data show, that there is substantial retention of MR-pro-ANP in hemodialysis patients resulting in MR-pro-ANP plasma concentrations greater than this threshold in all patients included in this study.

In the study of Gouya et al. a MR-pro-ANP plasma concentration in the upper tertile (greater than 795 pmol/l) was associated with a 1.76-fold increased mortality risk in 201 hemodialysis patients during a follow-up of seven years (p=0.08) [14]. In our study, we found a significant (p <0.01) 2.4-fold increase in mortality in patients with a MR-pro-ANP plasma concentration in the upper tertile (greater than 1159 pmol/l) and a 1.7 fold increase in mortality in patients in the middle tertile (MR-pro-ANP plasma concentration between 724 and 1159) which did not reach statistical significance (p=0.20). More studies are needed to better define cut-off values of the MR-pro-ANP plasma concentration for diagnostic and prognostic purposes.

The dependence of the plasma concentration of MR-pro-ANP and NT-pro-BNP on residual diuresis and kidney function in addition to heart function makes these fragments useful markers of the cardio-renal syndrome since elevated plasma concentrations reflect both increased cardiac secretion and reduced residual renal function which is an independent predictor of mortality in hemodialysis patients [26]. This could be the reason for the high range of the observed NT-pro-BNP plasma concentration with a 800-fold difference between the lowest and the highest value (Figure 1) which translated to a higher relative mortality risk of the upper tertile compared to that of BNP (4.95-fold vs. 3.17-fold; Figure 3). However, the fragment MR-pro-ANP (98 amino acids) had the smallest range with only a 17-fold difference between the lowest and the highest value (Figure 1). The reason for this remains unclear but could involve differences of the secretion compared to ventricle-derived BNP. Accordingly, the mortality risk of the upper tertile of MR-pro-ANP was the lowest (2.36-fold; Figure 3).

The limited number of cardiac parameters that were collected might have contributed to an unexplained variability during multivariate modeling. Survival analyses might be limited by the low mortality during the follow-up period. This study focused on nephrological parameters that are commonly available and accessible during hemodialysis treatment, such

as residual diuresis or shunt flow. Systolic left ventricular function served as a surrogate for cardiac status and still showed a high correlation to the plasma natriuretic peptide concentration. Other cardiac morbidities, such as diastolic function [16], valvular disease or rhythm disturbances, might also contribute to elevations in natriuretic peptide concentration, but were not entered into the model. Again it should be emphasized that association studies cannot prove causality between the studied parameters and elevated plasma natriuretic peptide concentrations and cannot distinguish between cause and consequence.

In hemodialysis patients, the fragment MR-pro-ANP is largely elevated due to accumulation. The prognostic performance of MR-pro-ANP is not superior to that of NT-pro-BNP or BNP.

Conflict of Interest

none

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