Original Paper

Superior Predictive Value for NTproBNP Compared with High Sensitivity cTnT in Dialysis Patients: A Pilot Prospective Observational Study

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Key Words

Biomarkers • Prognosis • Survival • Cardiovascular events • Dialysis • Bioimpedance

Abstract

Background/Aims: The clinical utility of the new biomarker, high sensitivity cardiac T troponin (hs-cTnT) is still unclear in dialysis patients. Furthermore, the prognostic value of combining N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) and hs-cTnT has not been explored so far. The objective of this pilot study was to determine the utility of hs-cTnT alone versus hs-cTnT in combination with NT-proBNP for predicting death in a stable hemodialysis cohort. **Methods:** A prospective observational pilot study including 98 chronic asymptomatic hemodialysis patients with a follow up period of 24 months was designed. The cut-off values for NT-proBNP and hs-cTnT were calculated using receiver operating characteristic (ROC) analysis, using mortality as an end-point. Based on the cut-off values, the cohort was divided into four groups. Group 1 – NT-proBNP < 14275 pg/ml and hs-cTnT < 69.48 ng/l; group 2 – NT-proBNP < 14275 pg/ml and hs-cTnT > 69.48 ng/l; group 3 – NT-proBNP > 14275 pg/ml and hs-cTnT < 69.48 ng/l; group 4 – NT-proBNP > 14275 pg/ml and hs-cTnT > 69.48 ng/l. Survival for each group was determined using the Kaplan–Meier method and Cox regression analysis. **Results:** During the follow-up period 16 patients died. According to the ROC curves analysis, the cut-off point for hs-cTnT and for NT-proBNP were 69.43 ng/l (AUC = 0.618; p = 0.04) and 14275 pg/ml (AUC = 0.722; p = 0.003), respectively. In univariate Cox analysis, both hs-cTnT (HR = 3.34; p = 0.016) and NT-proBNP (HR = 5.94; p = 0.01) were predictors of death. In the multivariable Cox proportional hazards model, only NT-pro-BNP levels above the cut-off value remained an independent predictor of all-cause mortality. The combined elevation of both biomarkers did not improve significantly the prognostic value compared with NT-

proBNP alone (HR = 6.15 versus HR = 4.78; $p = 0.338$). **Conclusion:** NT-pro-BNP is a strong predictor of overall mortality in asymptomatic hemodialysis patients. The addition of hs-cTnT did not improve the prognostic accuracy compared with NT proBNP alone.

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Introduction

End-stage renal disease (ESRD) patients treated by chronic dialysis have a worryingly high mortality, only comparable with aggressive forms of cancer. Although in the last decade numerous progresses were made in the management of dialysis patients, morbidity and mortality rates remain high, approaching 15-20% annually [1]. Almost 40% of this mortality is attributable to cardiovascular (CV) disease [2]. Unfortunately, there seems to be little hope in sight for improvements since almost all large, randomized controlled trials have persistently shown no survival benefit from several “new” treatment approaches: use of statins [3], ESA’s [4], vitamin D or cinacalcet [5, 6], antioxidant or homocysteine lowering therapies [7], prolonged or more frequent dialysis sessions [8] or convective therapies [9].

Numerous explanations were found for these disappointing results: underpowered studies, selection of ‘healthier’ patients for trials, better survival due to higher intensity of care during trial, high drop-out rates, and competing risks for mortality. Therefore, recognizing which patients are at the greater risk of cardiac mortality and could benefit from targeted management remains the best available approach for reducing the impressive mortality.

Cardiac biomarkers are a useful target for risk stratification. Although biomarkers such as albumin [10] or C reactive protein or natriuretic peptides [11, 12] have already been validated for predicting overall and cardiovascular mortality in CKD patients, the optimal biomarker and/or combination of biomarkers for risk stratification remains uncertain.

Cardiac troponins are frequently elevated even in the absence of acute coronary syndrome in chronic kidney disease (CKD) and in dialysis populations [13]. Classical cardiac troponins T (cTnT) and I (cTnI) have been corroborated with all-cause mortality or cardiac ischemia in stable dialysis cohorts [14]. The fifth-generation high sensitivity cardiac troponin T (hs-cTnT) test provides a high assay precision at the 99th percentile of the normal reference population. In the general population it has improved the detection, especially in the early phase of myocardial infarction; additionally, it is able to predict more accurately the risk for future cardiovascular events and death compared with conventional assays [15].

The clinical utility of hs-cTnT in a stable dialysis cohort is still unclear. Hs-cTnT levels are strongly associated with left ventricular hypertrophy in a large dialysis cohort [16], and a predictor of death in several recent reports [17-19]. However, the utility of a combination of hs-cTnT with natriuretic peptides for recognizing patients at high risk for major CV events or death has still not been explored.

In this context we performed a prospective observational study in a stable dialysis cohort to determine the utility of hs-cTnT alone versus hs-cTnT in combination with NT-proBNP for predicting death and fatal or non-fatal major CV events.

Material and Methods

Study population

All prevalent patients undergoing chronic HD treatment for at least 3 months in a single unit were assessed for eligibility for inclusion (prevalent cross-sectional cohort approach). Patients fitted with a cardiac pacemaker (N=5), chronic atrial fibrillation (N=20), lower limb amputation (N=6), poor echocardiographic window (N=3), non-functioning arterio-venous fistula (AVF) (N=5), less than 3 months dialysis vintage (N=10) or refusal to participate (N=12) were excluded from the study. All remaining patients (N=98) were treated uniformly with 12 hours of dialysis/week, using high-flux polysulphone membranes (FX60), a mean

blood flow of 400 ml/min and conductivity 135 ms. All patients signed an informed consent, and ethical approval was obtained from the Hospital and University ethical committee, as per country protocol.

Demographic, clinical and biochemical parameters

The following parameters were recorded: age, gender, body surface area, diabetes, residual diuresis, interdialytic weight gain (mean of the last 6 HD sessions). Additionally, eKt/V was calculated using the Daugirdas formula [19]. Time spent on HD (HD vintage), and other dialysis modalities preceding HD (PD, transplant) were noted in months.

The following biochemical parameters were recorded: serum hemoglobin, albumin, urea, creatinine, parathormone level, and calcium and serum inorganic phosphates. Blood samples for hs-cTnT assay were collected before the midweek HD session; the determination was made using a fifth-generation electrochemiluminescence assay (Elecsys, Cobas e411 analyzer, Roche Diagnostics). According to the manufacturer of the assay, for hs-cTnT the stated limit of detection is 5 ng/L, the analytical range is 3–10 000 ng/L, and the upper reference limit (99th percentile) in the normal population is 14 ng/L.

NT-proBNP in serum samples were collected before the midweek HD session and were analyzed centrally using the Roche Elecsys® kit, an electrochemiluminescence 'sandwich' immunoassay based on polyclonal antibodies against NT-proBNP.

Congestive heart failure was defined according to the New York Heart Association (NYHA) classification. Ischemic heart disease was defined based on clinical and EKG characteristic alterations or angiography. Blood pressure assessment was performed pre-HD, after 30 min. of recumbency; the last BP value of a three measurements series was used throughout the study. During the HD session, BP was recorded every 30 minutes.

Hydration status and body composition bioimpedance-derived measurements.

Measurements of hydration and body composition were performed using a multi-frequency bioimpedance device (BCM®). This device measures the impedance spectroscopy at 50 frequencies. Measurements were performed before the start and 30 min after the end of the HD treatment. Based on a fluid model using these resistances, the extracellular water (ECW), the intracellular water (ICW), and the total body water (TBW) are calculated [20]. Absolute fluid overload (AFO) is the difference between the expected patient's ECW under normal physiological conditions and the actual ECW, whereas the relative fluid overload (RTH) is defined as the AFO to ECW ratio.

Echocardiography and arterial stiffness assessment.

All patients had a baseline echocardiography. Echocardiographic evaluations were made in each patient before the midweek HD session. All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography [21] by an independent observer. Applanation tonometry was done with a SphygmoCor device (AtCor Medical, Westmead, Sydney, Australia). The PWV was computed from carotid and femoral artery waveforms recorded consecutively, using an electrocardiogram-gated signal and anthropometric distances. All measurements were done before the midweek HD session.

Follow-up and outcomes

All patients were enrolled in study in June–September 2011. All patients were followed at regular dialysis sessions for a pre-specified period of 24 months (until September 2013) or until death; the main outcome was death from all cause. Fatal or non-fatal major cardiovascular events were recorded in the EUCLID® database.

Statistics

Statistical significance of differences was tested with the ANOVA test for continuous and normally distributed parameters, the Mann Whitney U test for non-normally distributed parameters or Fisher's exact test for categorical parameters. The cut-off values for NT proBNP and hs-cTnT for overall mortality was calculated using receiver operating characteristic (ROC) analysis ROCs and the area under the curve (AUC). Kaplan–Meier and Cox regression analysis were used to investigate the prognostic value of these two biomarkers for predicting mortality, analyzing hs-cTnT and NT-proBNP as a categorical variable.

Table 1. Comparison (at 24 months follow-up) of various parameters between patients who survived and those who died

	Total (N=98)	Survivors (N=82)	Deceased (N=16)	P value*
Age (years)	55.4 ± 13.2	53.4 ± 12.3	65.5 ± 13.1	0.002
Dialysis vintage (mo)	112.5 (78.9-157.9)	112.5 (78.8-156.4)	111.5 (85.6-188.4)	0.75
Male, n (%)	49 (50.0)	40 (48.8)	9 (56.3)	0.59
Diabetes, n (%)	6 (6.1)	6 (7.3)	0 (0)	0.26
Hypertension, n (%)	66 (67.3)	54 (65.9)	12 (75.0)	0.48
SBP, mmHg	140.0 (130.0-148.2)	140.0 (132.0-150.0)	139.4 (130.0-140.2)	0.33
DBP, mmHg	75.8 (70.0-80.0)	68.9 (60.0-75.4)	80.0 (70.0-86.0)	0.59
Ischaemic heart disease, (%)	24 (24.5)	15 (18.3)	9 (56.3)	0.001
NYHA class, n (%)	-	-	-	0.007
II	26 (26.5)	20 (24.4)	6 (37.5)	-
III	8 (8.2)	5 (6.1)	3 (18.8)	-
IV	3 (3.1)	1 (1.2)	2 (12.5)	-
Peripheral vascular disease (%)	9 (9.2)	4 (4.9)	5 (31.3)	0.001
Stroke, n (%)	5 (5.1)	4 (4.9)	1 (6.3)	0.82
Hemoglobin, g/dl	12.3 ± 1.5	12.4 ± 1.5	11.8 ± 1.5	0.20
Calcium, mg/dl	8.6 ± 0.6	8.6 ± 0.6	8.5 ± 0.4	0.25
Phosphate, mg/dl	5.1 (4.2-6.5)	5.2 (4.3-6.6)	4.8 (3.4-5.9)	0.30
Albumin, g/dl	4.1 (3.8-4.2)	4.1 (3.9-4.2)	3.8 (3.3-4.2)	0.07
C reactive protein, mg/dl	12.8 (4.6-24.9)	13.2 (4.4-23.9)	8.9 (4.7-32.8)	0.84
PWV, m/s	11.1 (8.9-14.6)	10.7 (8.6-14.3)	14.5 (10.1-18.9)	0.01
EF, %	64.5 ± 7.5	64.2 ± 7.5	66.1 ± 7.6	0.37
LVMI, g/m ^{2.7}	142.9 (118.5-169.9)	142.4 (118.4-161.2)	148.9 (124.9-180.8)	0.61
NT proBNP, pg/ml	6662.0 (2960.8-25755.3)	5896.0 (2692.3-17240.0)	26354.5 (13160.0-44550.3)	0.005
hs-cTnT, pg/ml	44.7 (22.6-71.6)	37.4 (21.6-64.9)	71.5 (29.1-117.9)	0.07

SBP – systolic blood pressure; DBP – diastolic blood pressure; PTH – parathormone; PWV – pulse wave velocity; EF – ejection fraction; LVMI – left ventricular mass index; * survival vs non-survival

In a Cox proportional hazards model, NT-proBNP, hs-cTnT, and baseline parameters (age, gender, presence of diabetes mellitus, cardiovascular diseases, hepatitis), together with laboratory parameters, blood pressure, arterial stiffness, hydration status were included to assess the relative risk of mortality. These factors were used first in a univariate model. All parameters showing an association with mortality at a p value <0.05 were included in a multivariate model; To avoid the problem of over fitting due to the low number of incident outcomes, we performed bootstrapping validation, in order to determine the confidence intervals for estimating β in the Cox proportional hazard regression.

To determine the potential utility of simultaneous hs-cTnT and NTproBNP assessment, we divided the sample into four groups based on hs-cTnT and NTproBNP cut-off points: group 1 - NT-proBNP < 14275 pg/ml; hs-cTnT < 69.48 ng/l; group2 - NT proBNP < 14275 pg/ml; hs-cTnT > 69.48 ng/l; group 3 - NT proBNP > 14275 pg/ml; hs-cTnT < 69.48 ng/l and group 4 NT proBNP > 14275 pg/ml, hs-cTnT > 69.48 ng/l.

All statistical analyses were performed using SPSS version 19.0. A p value of 0.05 was considered significant.

Results

Demographic characteristics are presented in Table 1: mean age of participants was 61.4 ± 13.9 years, 50% were women; the dialysis vintage was 112.2 ± 13.5 months; almost 68% of the patients were hypertensives and 38% had a previous heart failure hospitalization.

The median hs-cTnT level was 44.7 ng/l (table 1). Using this high sensitivity assay, 95 participants (94%) had hs-cTnT above the 99th percentile of the normal population (14 ng/l, see methods). Levels of hs-cTnT were correlated with patients' age (r = 0.207; p = 0.041), dialysis vintage (r = 0.200; p = 0.05), and NT-proBNP levels (r = 0.339, p = 0.0001). Men tended to have higher values than women (73.2 versus 45.8 ng/l; p = 0.04). Hypertensive patients also had significantly higher levels of hs-cTnT (74.1 versus 48.7 ng/l in normotensives;

Table 2. Hydration status in survival vs non-survival patients

	All cohort			Patients with a previous history of heart failure		
	Survival	Non survival	p	survival	Non-survival	P
Overhydration predialysis	1.73 ±1.23	1.55±0.9	0.580	1.94 ± 1.14	1.72±0.9	0.564
Overhydration post dialysis	-0.6±1.9	-0.25±1.7	0.508	-0.6 ± 1.9	-0.25±.77	0.535
Ultrafiltration volume	2.32±1.49	1.81±1.08	0.199	2.56±1.5	1.90±1.19	0.219
Total body water	34.11±5.5	34.26±6.8	0.925	36.12±6.20	34.77±8.13	0.583
Extracellular water	16.18±2.48	16.28±3.08	0.893	17.10±2.42	16.50±3.59	0.556
Intracellular water	17.89±3.40	17.98±3.99	0.925	18.9±4.06	4.06±4.7	0.645
LEAN	21.44±11.7	29.59±10.8	0.012	26.24±14.0	32.25±8.86	0.705
FAT	13.73±8.28	16.10±8.04	0.297	14.12±10.1	16.9±5.8	0.199
RTH	10.08±7.4	10.53±5.4	0.891	10.99 ±6.6	10.51±6.0	0.827
RTH – relative tissue hydration						

p = 0.01). With the exception of a higher systolic BP, hs-cTnT levels were not consistently associated with most traditional CV risk factors, including smoking, diabetes, obesity, and high cholesterol levels.

The median NT-proBNP level was 6662 pg/ml (table 1). Baseline NT-proBNP values correlated positively with age (r = 0.295, p = 0.03), left ventricular mass index (r = 0.260; p = 0.015), inter-ventricular septum thickness (r = 0.288; p = 0.05) and posterior wall thickness (r = 0.333; p = 0.009). There were no correlations between baseline NT-proBNP values and other known confounders.

Survival analysis

Patients in the study were monitored for 24 months; during this period 16 deaths (16.3%) were recorded. Cardiac death was recorded in 9 patients; 6 patients developed sudden cardiac death, while 3 patients had an acute coronary syndrome. The main cause of non-cardiovascular death was infection-related (7 patients).

There was a higher baseline level of NT-proBNP (26354.5 vs 5896.0 pg/ml; p = 0.005) and of hs-cTnT (71.5 vs 37.4 ng/l; p = 0.007) in patients who died compared with survivors. Additionally, deceased patients were older (65.5 vs 53.4 yrs; p = 0.002) and had higher arterial stiffness in comparison with patients who survived (table 1). There was no difference in hydration status between survivors and non-survivors (see table nr. 2). Additionally, there was no difference in hydration status between survivors and non-survivors, in patients with previous history of heart failure hospitalization (see table nr.2).

N- proBNP and hs-cTnT as predictors of mortality

According to the ROC curves, the cut-off point for NT-proBNP as a predictor of mortality was 14275 pg/ml, with a sensitivity of 68.7% and a specificity of 79.3%. The area under the curve was 0.722 (p = 0.003). The cut-off point for hs-cTnT as a predictor of mortality, according to the ROC curves, was 69.43 ng/l, with a sensitivity of 68% and a specificity of 79%. The area under the curve was 0.618 (p = 0.04). The area under the curve for both NT-proBNP and hs-cTnT was 0.756 (p = 0.001). Both NT-proBNP and the combination (NT-proBNP and hs-cTnT) exhibited good prognostic accuracy for survival, with AUC values of 0.722 and 0.756, respectively.

Kaplan Meier analysis showed that all cause mortality was significantly higher in patients with NT-proBNP >14275 pg/ml (p = 0.002) and in patients with an hs-cTnT >69.43 mg/dl (see figure nr 1a and 1b).

Fig. 1. Kaplan Meyer analysis for NTproBNP and hs - cTnT as a predictor of mortality.

Univariate and multivariate survival analysis

Univariate Cox regression analysis, including traditional and non-traditional risk factors, showed the following items to be predictive for death, in this HD population: NT-proBNP >14275 pg/ml, hs-cTnT >69.43 ng/l, age, ischemic heart disease and arterial stiffness (table 3). In multivariate analysis, only NT-proBNP, age and ischemic heart disease remained independent predictor of mortality, whereas hs-cTnT lost its predictive significance - (Table 3).

As a continuous variable, NT proBNP but not hs-cTnT predicted death from all cause mortality in univariate Cox analysis (HR = 2.16; 95% CI = 1.27-3.67). In multivariable analysis, this biomarker remains an independent predictor of mortality (see table 4).

Nine deaths were attributing to CV events. Univariate analysis showed that NT-proBNP >14275 pg/ml, hs-cTnT >69.43 ng/l, age, ischemic heart disease were predictors for CV death. In multivariate analysis, only NT-proBNP and ischemic heart disease remained independent predictor of mortality, whereas hs-cTnT lost its predictive significance (see table nr.5).

Combined analysis of the biomarkers for mortality

To determine the potential utility of simultaneous hs-cTnT and NT-proBNP assessment, we divided the samples into four groups based on hs - cTnT and NT-proBNP cut-off points (see

Fig. 2. Kaplan Meyer analysis for NTproBNP and hs - cTnT as a predictor of mortality.

Table 3. Univariate and multivariate COX regression analysis

	Univariate analysis				Multivariate analysis			
	Sig.	Exp(B)	95.0% CI for Exp(B)		Sig.	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper			Lower	Upper
<i>Nt proBNP (14275 pg/ml)</i>	0.001	5.938	2.061	17.104	0.009	5.782	1.558	21.461
<i>Age (yrs)</i>	0.001	1.072	1.031	1.115	0.011	1.072	1.016	1.132
<i>Ischemic heart disease</i>	0.002	4.684	1.742	1.843	0.038	2.983	1.061	8.392
<i>hs – cTnT (69.43 pg/ml)</i>	0.016	3.344	1.255	8.912		NS		
<i>PWV (m/sec)</i>	0.009	1.149	1.035	1.277		NS		
Gender	0.672	0.808	0.301	4.825		NS		
Dialysis vintage (mo)	0.667	1.002	0.994	0.993		NS		
Diabetes	0.485	0.045	0.000	1.736		NS		
RTH (l)	0.532	0.893	0.627	1.273		NS		
LVMI (g/m ²)	0.625	1.003	0.992	1.014		NS		
Hemoglobin (g/dl)	0.255	0.815	0.572	1.160		NS		
Calcium(mg/dl)	0.631	0.802	0.327	1.971		NS		
Phosphate (mg/dl)	0.586	0.916	0.668	1.256		NS		
Cholesterol (mg/dl)	0.806	1.002	0.986	1.019		NS		
Creatinine (mg/dl)	0.358	0.911	0.748	1.111		NS		
Albumin (g/dl)	0.078	0.064	0.017	0.249		NS		

PTH – parathormone level; PWV – pulse wave velocity; LVMI – left ventricular mass index;
RTH – relative tissue hydration

Table 4. Univariate and multivariate COX regression analysis using continous variable

	Univariate analysis				Multivariate analysis			
	Sig.	Exp(B)	95.0% CI for Exp(B)		Sig.	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper			Lower	Upper
<i>log_NTprobnp_SD</i>	0.004	2.167	1.278	3.674	0.023	1.831	1.085	3.089
<i>Log PWV SD</i>	0.013	1.069	1.014	1.127		NS		
<i>log_Creatinine_SD</i>	0.336	0.782	0.475	1.290		NS		
<i>log_CRP_SD</i>	0.783	0.936	0.582	1.503		NS		
<i>log_Cholesterol_SD</i>	0.629	1.134	0.682	1.886		NS		
<i>Log-Triglycerides SD</i>	0.143	0.696	0.428	1.130		NS		
<i>log_Phosphate_SD</i>	0.439	0.832	0.523	1.324		NS		
<i>log_LVMI_SD</i>	0.706	1.098	0.677	1.781		NS		
<i>log_hs-cTnT_SD</i>	0.128	1.470	0.895	2.412		NS		
<i>log_PTH_SD</i>	0.006	0.568	0.380	0.848		NS		
<i>Log PWV SD</i>	0.013	1.069	1.014	1.127		NS		

PTH – parathormone level; PWV – pulse wave velocity; LVMI – left ventricular mass index;
RTH – relative tissue hydration; CRP – C reactive protein

methods). The group 1 – NT-proBNP <14275 pg/ml and hs-cTnT < 69.48 ng/l included 58 patients, group 2 – NT-proBNP <14275 pg/ml and hs-cTnT >69.48 ng/l included 10 patients, group 3 – NT-proBNP >14275 pg/ml and hs-cTnT <69.48 ng/l included 16 patients, while group 4 – NT-proBNP >14275 pg/ml and hs-cTnT >69.48 ng/l included 14 patients.

We considered the first group - defined as both NT-proBNP and hs-cTnT below the cut-off) to be the reference group. Compared with the reference group, patients with high hs-

Table 5. Univariate and multivariate COX regression analysis (CV mortality)

	Univariate analysis				Multivariate analysis			
	Sig.	Exp(B)	95.0% CI for Exp(B)		Sig.	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper			Lower	Upper
<i>Nt proBNP (14275 pg/ml))</i>	0.016	5.468	1.366	21.894	0.030	4.704	1.163	19.029
<i>Ischemic heart disease</i>	0.023	4.605	1.234	17.188	0.047	3.840	1.018	14.480
<i>hs – cTnT (69.43 pg/ml)</i>	0.032	4.226	1.134	15.744		NS		
Age (yrs)	0.302	1.028	0.976	1.082		NS		
hs – cTnT (69.43 pg/ml)	0.032	4.226	1.134	15.744		NS		
Gender	0.353	1.928	0.482	7.709		NS		
Dialysis vintage (mo)	0.162	1.007	0.997	1.017		NS		
Diabetes	0.485	0.045	0.000	1.736		NS		
PWV (m/sec)	0.074	1.137	0.988	1.309		NS		
RTH (l)	0.532	0.893	0.627	1.273		NS		
LVMi (g/m ²)	0.838	1.002	0.987	1.017		NS		
Hemoglobin (g/dl)	0.284	0.791	0.515	1.214		NS		
Calcium(mg/dl)	0.414	0.644	0.224	1.853		NS		
Phosphate (mg/dl)	0.603	1.093	0.782	1.528		NS		
PTH (pg/dl)	0.217	0.999	0.998	1.000		NS		
Creatinine (mg/dl)	0.463	1.089	0.867	1.368		NS		
Cholesterol (mg/dl)	0.246	1.009	0.994	1.026		NS		
Albumin (g/dl)	0.766	0.743	0.106	5.227		NS		

PTH – parathormone level; PWV – pulse wave velocity; LVMi – left ventricular mass index;
RTH – relative tissue hydration;

Table 6. COX regression analysis based on cutt-off levels of hs-cTnT and NTproBNP

	Sig.	Exp(B)	95.0% CI for Exp(B)	
			Lower	Upper
NT proBNP <14275; hs-cTnT < 69.48 (<i>reference group</i>)				
NT proBNP <14275; hs-cTnT > 69.48	0.977	0.968	0.104	8.989
NT proBNP >14275; hs-cTnT < 69.48	0.033	4.718	1.132	19.662
NT proBNP >14275; hs-cTnT > 69.48	0.005	6.159	1.737	21.843

cTnT levels but normal NT-proBNP did not have a worse outcome (HR = 0.98; p = 0.968) - see table 3. In contrast, both groups with high NT-proBNP levels (with normal hs-cTnT or with high hs-cTnT) had a significant higher risk of overall mortality (HR = 4.78; p = 0.033 and HR = 6.15; p = 0.005, respectively) compared with the reference group (see table 6 and figure 2).

However, the addition of hs-c TnT to NTproBNP did not improve significantly the predictive power of NT-proBNP alone (HR = 6.15 versus HR = 4.78; p = 0.338).

Discussion

This observational prospective pilot study provides a comprehensive analysis of the prognostic value of high-sensitivity cTnT alone or in combination with NT-proBNP, as a predictor of mortality, over a 2-year period, in a stable cohort of chronic HD patients. We showed that hs-CTnT, in contrast to NT proBNP is not a valuable biomarker for predicting

mortality; additionally, we found that the combination of these two biomarkers did not improve risk stratification for death above and beyond a model including NT-proBNP alone.

Plasmatic levels of natriuretic peptides in both general and renal populations are tightly correlated with all-cause mortality, and especially with cardiovascular mortality [12]. Our data reconfirm the prognostic value of NT-proBNP for subsequent mortality risk in HD patients.

Similar to previous studies conducted in CKD and dialysis patients [17-19], we found a high proportion of patients in our cohort (95%) with a hs-cTnT concentration above the 99th percentile of the normal population (14 ng/l). In contrast, the older studies, using the classical cTnT assay, reported only 15-45% of patients having increased concentration [13, 22]. This higher sensitivity is associated with a better predictive value of mortality in general population [15, 23].

The prognostic value of classical troponins, in dialysis patients, was demonstrated by numerous studies including a recent meta-analysis [14]. In almost 4000 patients, elevated classical TnT (>0.1 ng/mL) was significantly associated with increased all-cause mortality (RR = 2.64; 95%CI = 2.17 to 3.20). The ability of hs-cTnT to predict all cause and CV mortality in hemodialysis patients has been examined in few studies. Hassan et al. [19] demonstrated an increased risk for myocardial infarction and for general mortality with increasing hs troponin quartiles in 393 dialysis patients (including 275 on hemodialysis). Similarly, Artunc et al. [18] reported that hs-cTnT concentrations above 38 pg/mL were associated with a 5-fold risk of death, during a follow-up period of almost two years, in a 239 hemodialysis cohort. We showed, in our observational prospective study, that hs-cTnT is an effective marker for survival in univariate Cox analysis. Patients with hs-cTnT above 69.43 pg/ml had a 3.3-fold increased risk of death; the predictive value was lost in multivariate analysis, when adjusting for confounders. Similar data are reported by McGill et al. [24]: in a stable dialysis cohort, after a median 30 months of follow-up, NT-proBNP was the only biomarker predictive for all cause mortality and not hs-cTnT. Moreover, in the same study, they determined both classical and hs-cTnT in 143 patients and followed these patients during 3.9 years. The AUC showed that hs-TnT improves the prediction for all-cause mortality as compared to the fourth generation TnT (0.760 vs. 0.746).

Comparison of the diagnostic performance of NT-proBNP with classical or with hs-cTnT, in dialysis patients

There are several studies that compared directly the prognostic value of NT-proBNP and classical troponins in HD populations, with strikingly different results. Sommerer et al. [25] found in 134 stable asymptomatic patients that both NT-proBNP and cTnT are independent predictors of the composite end-point of death and CV events. Fernandes Reyes et al [26], in a small dialysis cohort (58 pts) found that NT-proBNP level was strongly correlated with cTnT and cTnT was the best predictor of death. Apple et al. [27] found in 399 hemodialysis patients that both cTnT and cTnI are independent predictors of all cause mortality, but NT-proBNP, despite being elevated in nearly 99% of the patients. In contrast, Satyan et al. [28] showed in 150 asymptomatic dialysis patients that NT-proBNP is strongly related with all cause and CV mortality; adding troponin did not improve the prognostic value compared with NT-proBNP alone.

The diagnostic performance of NT-proBNP compared to the new hs-cTnT was described until now in only one previous study. McGill et al. [24], found in 143 dialysis patients that NT-proBNP and albumin had a strong predictive value for all-cause mortality at 30 months of follow-up; however, after a median follow-up of 46.7 months the new hs-cTnT assay was the only cardiac biomarker predictive of all-cause mortality, suggesting that NT-proBNP is a better predictor for early mortality and troponin for long-term mortality. Similar data were found in our study: at 24 months of follow-up, NT-proBNP and not hs-cTnT was the only predictor of overall mortality risk.

The diagnostic performance of the combination of hs-cTnT and NT proBNP for risk stratification in dialysis

In general population combination of these two biomarkers improves substantially the predictive accuracy. In a large heart failure cohort, de Antonio et al. [29] found that both NT-proBNP and hs-cTnT were predictors of mortality, but the combination of both biomarkers was associated with substantially higher accuracy, compared with either biomarker alone, reaching a very significant HR of 7.42. In another large (3800 pts) diabetes cohort, the combination of NT-proBNP and hs-cTnT also greatly improved the accuracy with which the risk of cardiovascular events or death could be estimated [30]. The addition of either marker improved 5-year risk classification for cardiovascular events (net reclassification index in continuous model, 39% for NT-proBNP and 46% for hs-cTnT).

For the first time, we used the combination of NT-proBNP and hs-cTnT for predicting mortality risk in HD patients and found that hs-cTnT is not an independent contributor to the survival prediction. The addition of hs-cTnT to a NT proBNP prediction model failed to be associated with a significantly better risk discrimination, as compared to NT-proBNP alone.

Limitations

The limitations of our study were the relatively small sample size but with 2-years period of follow-up. Our study population had a long-lasting HD treatment; this does not represent a common situation in daily dialysis practice and results cannot be generalized for all dialysis patients. Although albumin and diabetes have been considered as survival predictors, our study included a small number of patients with diabetes and a small number of patients with low albumin level, thus precluding a possible statistical effect of these parameters. Levels of hs-cTnT have been measured from frozen rather than fresh samples. There is little information about long-term stability of frozen hs-cTnT. The deceased groups of our patients were older and with higher percentage of ischemic heart disease compared with the survivor group. However, the prognostic power of NT proBNP remains statistically significant after adjusting for ischemic heart disease.

Conclusions

NT-proBNP and not hs-cTnT is a predictor of all-cause mortality in stable, long-term dialysis patients, at 24 months of follow-up. The combination of NT-proBNP and hs-cTnT did not improve the prognostic accuracy compared to NT-proBNP alone.

Whether or not these biomarkers should be routinely measured in ESRD patients still remains to be answered. Future studies will hopefully address the significance of sequential measurement of these biomarkers in ESRD patients and the effects of therapeutic interventions based on biomarker levels.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

Acknowledgements

Part of this study was funded by the University of Medicine and Pharmacy, Iasi (grants Nos. 1640/01.02.2013, 1641/01.02.2013, 1643/01.02.2013 and IDEI-PCE 2011, PN-II-ID-PCE-2011-3-0637).

References

- Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Johansen K, Kasiske B, Kutner N, Liu J, St Peter W, Guo H, Gustafson S, Heubner B, Lamb K, Li S, Li S, Peng Y, Qiu Y, Roberts T, Skeans M, Snyder J, Solid C, Thompson B, Wang C, Weinhandl E, Zaun D, Arko C, Chen SC, Daniels F, Ebben J, Frazier E, Hanzlik C, Johnson R, Sheets D, Wang X, Forrest B, Constantini E, Everson S, Eggers P, Agodoa L: United States renal data system 2011 annual data report: atlas of chronic kidney disease & end-stage renal disease in the United States. *Am J Kidney Dis* 2012;59:A7:e1-420.
- de Jager DJ, Grootendorst DC, Jager KJ, van Dijk PC, Tomas LM, Ansell D, Collart F, Finne P, Heaf JG, De Meester J, Wetzels JF, Rosendaal FR, Dekker FW: Cardiovascular and noncardiovascular mortality among patients starting dialysis. *JAMA* 2009;302:1782-1789.
- Palmer SC, Craig JC, Navaneethan SD, Tonelli M, Pellegrini F, Strippoli GF: Benefits and harms of statin therapy for persons with chronic kidney disease: a systematic review and meta-analysis. *Ann Int Med* 2012;157:263-275.
- Jing Z, Wei-jie Y, Nan Z, Yi Z, Ling W: Hemoglobin targets for chronic kidney disease patients with anemia: a systematic review and meta-analysis. *PloS one* 2012;7:e43655.
- Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, Bhan I, Agarwal R, Zoccali C, Wanner C, Lloyd-Jones D, Cannata J, Thompson BT, Andress D, Zhang W, Packham D, Singh B, Zehnder D, Shah A, Pachika A, Manning WJ, Solomon SD: Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. *JAMA* 2012;307:674-684.
- EVOLVE Trial Investigators, Chertow GM, Block GA, Correa-Rotter R, Drüeke TB, Floege J, Goodman WG, Herzog CA, Kubo Y, London GM, Mahaffey KW, Mix TC, Moe SM, Trotman ML, Wheeler DC, Parfrey PS: Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis. *New Engl J Med* 2012;367:2482-2494.
- Pan Y, Guo LL, Cai LL, Zhu XJ, Shu JL, Liu XL, Jin HM: Homocysteine-lowering therapy does not lead to reduction in cardiovascular outcomes in chronic kidney disease patients: a meta-analysis of randomised, controlled trials. *Br J Nutr* 2012;108:400-407.
- Cheung AK, Levin NW, Greene T, Agodoa L, Bailey J, Beck G, Clark W, Levey AS, Leypoldt JK, Ornt DB, Rocco MV, Schulman G, Schwab S, Teehan B, Eknayan G: Effects of high-flux hemodialysis on clinical outcomes: results of the HEMO study. *J Am Soc Nephrol* 2003;14:3251-3263.
- Grooteman MP, van den Dorpel MA, Bots ML, Penne EL, van der Weerd NC, Mazairac AH, den Hoedt CH, van der Tweel I, Lévesque R, Nubé MJ, ter Wee PM, Blankestijn PJ; CONTRAST Investigators: Effect of online hemodiafiltration on all-cause mortality and cardiovascular outcomes. *J Am Soc Nephrol* 2012;23:1087-1096.
- Kalantar-Zadeh K, Kilpatrick RD, Kuwae N, McAllister CJ, Alcorn H Jr, Kopple JD, Greenland S: Revisiting mortality predictability of serum albumin in the dialysis population: timedependency, longitudinal changes and population-attributable fraction. *Nephrol Dial Transplant* 2005;20:1880-1888.
- Bazeley J, Bieber B, Li Y, Morgenstern H, de Sequera P, Combe C, Yamamoto H, Gallagher M, Port FK, Robinson BM: C-reactive protein and prediction of 1-year mortality in prevalent hemodialysis patients. *Clin J Am Soc Nephrol* 2011;6:2452-2461.
- Paniagua R, Ventura MD, Avila-Díaz M, Hinojosa-Heredia H, Méndez-Durán A, Cueto-Manzano A, Cisneros A, Ramos A, Madonia-Juseino C, Belio-Caro F, García-Contreras F, Trinidad-Ramos P, Vázquez R, Ilabaca B, Alcántara G, Amato D: NT-proBNP, fluid volume overload and dialysis modality are independent predictors of mortality in ESRD patients. *Nephrol Dial Transplant*.2010;25:551-557.
- Havekes B, van Manen JG, Krediet RT, Boeschoten EW, Vandenbroucke JP, Dekker FW; NECOSAD Study Group: Serum troponin T concentration as a predictor of mortality in hemodialysis and peritoneal dialysis patients. *Am J Kidney Dis* 2006;47:823-829.
- Khan NA, Hemmelgarn BR, Tonelli M, Thompson CR, Levin A: Prognostic value of troponin T and I among asymptomatic patients with end-stage renal disease: a meta-analysis. *Circulation* 2005;112:3088-3096.
- Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, Biedert S, Schaub N, Buerge C, Potocki M, Noveanu M, Breidthardt T, Twerenbold R, Winkler K, Bingisser R, Mueller C: Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med* 2009;361:858-867.

- 16 Smith K, deFilippi C, Isakova T, Gutiérrez OM, Laliberte K, Seliger S, Kelley W, Duh SH, Hise M, Christenson R, Wolf M, Januzzi J: Fibroblast growth factor 23, high-sensitivity cardiac troponin, and left ventricular hypertrophy in CKD. *Am J Kidney Dis* 2013;61:67-73.
- 17 Kumar N, Michelis MF, DeVita MV, Panagopoulos G, Rosenstock JL: Troponin I levels in asymptomatic patients on haemodialysis using a high-sensitivity assay. *Nephrol Dial Transplant* 2011;26:665-670.
- 18 Artunc F, Mueller C, Bredthardt T, Twerenbold R, Peter A, Thamer C, Weyrich P, Haering HU, Friedrich B: Sensitive troponins--which suits better for hemodialysis patients? Associated factors and prediction of mortality. *PLoS One* 2012;7:e47610.
- 19 Hassan HC, Howlin K, Jefferys A, Spicer ST, Aravindan AN, Suryanarayanan G, Hall BM, Cleland BD, Wong JK, Suranyi MG, Makris A: High-sensitivity troponin as a predictor of cardiac events and mortality in the stable dialysis population. *Clin Chem* 2014;60:389-398.
- 20 Wabel P, Moissl U, Chamney P, Jirka T, Machek P, Ponce P, Taborsky P, Tetta C, Velasco N, Vlasak J, Zaluska W, Wizemann V: Towards improved cardiovascular management: the necessity of combining blood pressure and fluid overload. *Nephrol Dial Transplant* 2008;23:2965-2971.
- 21 Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard H, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography: Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005;18:1440-1463.
- 22 Mongeon FP, Dorais M, Lorier JL, Froment D, Letendre E, Rinfret S: Effect of hemodialysis, coronary artery disease and diabetes on cardiac troponin T: a prospective survey over one year. *Open Cardiovasc Med J* 2009;3:69-77.
- 23 Kavsak PA, Wang X, Ko DT, MacRae AR, Jaffe AS: Short- and long-term risk stratification using a next-generation, high-sensitivity research cardiac troponin I (hs-cTnI) assay in an emergency department chest pain population. *Clin Chem* 2009;55:1809-1815.
- 24 McGill D, Talaulikar G, Potter JM, Koerbin G, Hickman PE: Over time, high-sensitivity TnT replaces NT-proBNP as the most powerful predictor of death in patients with dialysis-dependent chronic renal failure. *Clin Chim Acta* 2010;411:936-939.
- 25 Sommerer C, Beimler J, Schwenger V, Hecke N, Katus HA, Giannitsis E, Zeier M: Cardiac biomarkers and survival in haemodialysis patients. *Eur J Clin Invest* 2007;37:350-356.
- 26 Fernández-Reyes MJ, Mon C, Heras M, Guevara P, Garcia MC, Sanchez R, Velasco S, Alvarez-Ude F: Predictive value of troponin T levels for ischemic heart disease and mortality in patients on hemodialysis. *J Nephrol* 2004;17:721-727.
- 27 Apple FS, Murakami MM, Pearce LA, Herzog CA: Multi-biomarker risk stratification of N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and cardiac troponin T and I in end-stage renal disease for all-cause death. *Clin Chem* 2004;50:2279-2285.
- 28 Satyan S, Light RP, Agarwal R: Relationships of N-terminal pro-B-natriuretic peptide and cardiac troponin T to left ventricular mass and function and mortality in asymptomatic hemodialysis patients. *Am J Kidney Dis* 2007;50:1009-1019.
- 29 de Antonio M, Lupón J, Galan A, Vila J, Urrutia A, Bayes-Genis A: Combined use of high-sensitivity cardiac troponin T and N-terminal pro-B type natriuretic peptide improves measurements of performance over established mortality risk factors in chronic heart failure. *Am Heart J* 2012;163:821-828.
- 30 Hillis GS, Welsh P, Chalmers J, Perkovic V, Chow CK, Li Q, Jun M, Neal B, Zoungas S, Poulter N, Mancina G, Williams B, Sattar N, Woodward M: The relative and combined ability of high-sensitivity cardiac troponin T and N-terminal pro-B-type natriuretic Peptide to predict cardiovascular events and death in patients with type 2 diabetes. *Diabetes Care* 2014;37:295-303.