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N-terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis

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Concentrations of N-terminal pro brain natriuretic peptide (NT-proBNP) increase in patients with heart failure and other cardiovascular (CV) diseases and are strong prognostic markers. In patients with end-stage renal disease (ESRD) in hemodialysis (HD), levels of NT-proBNP are almost always raised. In ESRD patients undergoing HD, we aimed at (i) identifying the factors that affect levels of NT-proBNP, (ii) determining the effect of HD on NT-proBNP, and (iii) determining the prognostic impact of NT-proBNP. A total of 109 patients underwent physical examination, electrocardiogram, and echocardiography. Serum NT-proBNP was measured before and after HD (Elecsys 2010). NT-proBNP levels were markedly elevated (pre-HD 4079 pg/ml, post-HD 2759 pg/ml, $P<0.001$). There was a strong inverse correlation between NT-proBNP and left ventricular ejection fraction (LVEF) ($P=0.043$), 24-h urine production ($P=0.006$), and K_t/V (efficacy of dialysis) ($P=0.016$) and a positive correlation with left ventricular hypertrophy (LVH) ($P=0.014$). Patients with higher concentrations, both pre- and post-HD had an increased mortality rate compared to those with lower concentrations ($P=0.007$, $P=0.002$). We found age ($P=0.009$) and NT-proBNP (pre-HD $P=0.007$, post-HD $P=0.001$) predictive of death. Our findings demonstrate that CV disease in terms of LVH and reduced LVEF in addition to 24-h urine production and K_t/V determine NT-proBNP levels. Post-HD levels of NT-proBNP were lower than pre-HD levels; both predictive of mortality.

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KEYWORDS: NT-proBNP; cardiac markers; end-stage renal disease; hemodialysis; volume status

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Natriuretic peptides (NPs) for the diagnosis of heart failure have been a diagnostic breakthrough in cardiology, where determination of the concentration in serum can help identify patients with left ventricular systolic dysfunction.[1,2] The main physiological function of NP is homeostasis and protection of among others the cardiovascular (CV) system from the effects of volume overload. They play an important role in regulating blood pressure (BP) and body fluid volume by their natriuretic and diuretic actions, arterial dilatation, and inhibition of the renin–angiotensin system.[3] NPs are synthesized in the cardiac ventricles by cleavage of a precursor protein, pro brain natriuretic peptide (proBNP), into a 32-amino-acid active hormone B-type natriuretic peptide (BNP; MW 3.5 kDa) and a biologically inactive 76-amino-acid peptide N-terminal pro-BNP (NT-proBNP; MW 8.5 kDa), and released into the circulation on an equimolar basis upon ventricular myocyte stretch.[4] Concentrations of NP increase in patients with congestive heart failure (CHF) and other CV diseases owing to pressure and volume overload, whereas levels below cutoff are a strong negative predictor for CHF.[1,5,6] In patients with end-stage renal disease (ESRD) treated with hemodialysis (HD), plasma levels of NP are almost always markedly raised for reasons largely unknown.[7] As a consequence of diminutive residual renal function, volume overload facilitating left ventricular strain could be responsible for the elevated concentrations, but it is also widely recognized that cardiac function is a major confounder for the interpretation of plasma concentrations of NP in chronic renal failure. Furthermore, as the kidneys are generally thought to be partly responsible for the elimination of NP, decreased clearance by the clearance receptors in renal tissue could contribute to the elevated concentrations. Earlier research has hypothesized that the measurement of NP could be of use in monitoring excess fluid volume and dry weight in dialysis patients,[8,9] but this remains to be proven and the significance of plasma NP and their clinical role in HD patients is still unclear. Measuring NT-proBNP may have analytical advantages over BNP because of greater stability due to a longer half-life and the present study aimed at (i) identifying the factors that affect

the concentrations of circulating NT-proBNP, (ii) determining the effect of dialysis on NT-proBNP levels, and (iii) investigating the prognostic impact of elevated concentrations of NT-proBNP in patients undergoing regular HD.

RESULTS

Table 1 shows the characteristics of the included 109 patients on chronic HD. No patients were lost to follow-up. The median duration of HD treatment before entry into the study was 20 months (range 1–216 months). At initiation of HD treatment, 87 patients (79.8%) were classified as hypertensive, but only 22 patients (20.2%) were hypertensive on 24-h ambulatory BP monitoring at entry into the study owing to antihypertensive treatment. Of the total population, 18.3% were on no antihypertensive treatment, 23.9% were treated with one antihypertensive drug (50% of these with diuretics), 23.9% were treated with two antihypertensives, 26.6% were treated with three antihypertensives, and 7.3% were treated with four or more drugs. On treatment average, systolic BP was 143.6 ± 22.1 mm Hg and average diastolic BP 78.5 ± 13.5 mm Hg. LVMI was 46.8 ± 13.6 g/m$^{2.7}$ in male subjects and 46.3 ± 18.5 g/m$^{2.7}$ in female subjects with LVH detected in 40 patients (36.7%). LVEF was 50.7 ± 13.0%. The mean value of K_t/V per week was 4.5 ± 0.9.

Serum NT-proBNP levels were markedly elevated in these patients (predialysis 4079 pg/ml (median; interquartile range 1893–15076) and post-dialysis 2759 pg/ml (1078–11070), $P < 0.001$; Figure 1) compared to population-based normal values.[10] Compared to the cutoff level of 125 pg/ml when screening symptomatic patients for left ventricular systolic dysfunction, all patients had predialysis levels above the cutoff with only one patient below this level post-dialysis. The mean reduction in concentration during dialysis was 38.8 ± 13.6%. There was no correlation between the amount of fluid drawn during HD and the difference in pre- and post-dialysis levels of NT-proBNP or the decrease in concentration in percent. The mean reduction in pre- to post-cartridge levels of NT-proBNP was 7.0%.

Patients with CHF had a significantly higher median predialysis concentration of NT-proBNP than patients not having been diagnosed with CHF (15 323 (3576–35 473) pg/ml, $n = 26$ vs 3355 (1603–9654) pg/ml, $n = 83$, $P = 0.001$). This is also seen in patients with chronic obstructive pulmonary disease (24 523 (3744–51 495) pg/ml, $n = 15$ vs 3536 (1789–10 792) pg/ml, $n = 94$, $P = 0.007$). This remains significant when excluding chronic obstructive pulmonary disease patients also diagnosed with CHF ($P = 0.042$). We found no difference in predialysis levels of NT-proBNP between male and female patients, previously transplanted vs non-transplanted patients, or in patients with or without documented coronary artery disease, atrial fibrillation, or diabetes mellitus. Also, no significant correlation was found between predialysis levels of NT-proBNP and duration of renal disease or period of time undergoing HD treatment.

Multivariate linear regression analysis demonstrated a strong inverse correlation between predialysis ln NT-proBNP and LVEF ($P = 0.043$), 24-h urine production ($P = 0.006$) and K_t/V ($P = 0.016$) and a positive correlation with LVH ($P = 0.014$) (Table 2). The result was almost identical when investigating post-dialysis ln NT-proBNP. Tertiles of variables

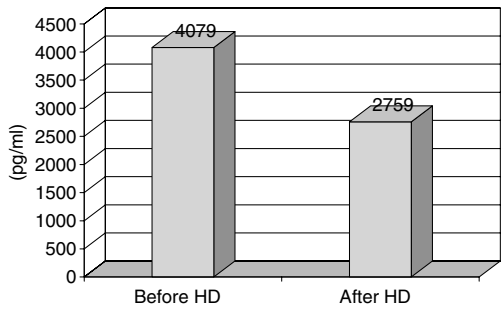


Figure 1 | Pre- and post-dialysis concentrations of NT-proBNP (4079 vs 2759 pg/ml; median values, $P < 0.001$).

Table 1 | Baseline characteristics of 109 study participants

Age (years)[a]	61.8 (19–88)
Sex (m/f), *N*	82/27
Duration of renal disease (years)[b]	3.0 (1–42)
Duration of dialysis treatment (months)[b]	20.0 (1–216)
History of IHD (%)	26.6
History of CHF (%)	23.9
History of diabetes (%)	34.9
Atrial fibrillation (%)	11.0
History of hypertension (%)	79.8
Hypertension on treatment (%)	20.2
Former transplantation (%)	9.2
Systolic BP (mm Hg)[c]	143.6 ± 22.1
Diastolic BP (mm Hg)[c]	78.5 ± 13.5
LVEF (%)[b]	50.7 ± 13.3
LVH (%)	36.7
LVMI (M/F, g/m$^{2.7}$)[c]	$46.8 \pm 13.6/46.3 \pm 18.5$

BP, blood pressure; CHF, chronic heart failure; F, female; IHD, ischemic heart disease; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; M, male.
[a]Expressed as mean, range in parenthesis.
[b]Expressed as median, range in parenthesis.
[c]Expressed as mean with s.d.

Table 2 | Factors affecting the concentration of circulating NT-proBNP in patients undergoing chronic HD (predialysis)

Independent variable	*P*-value	*β*
Age (years)	NS	
Gender	NS	
IHD	NS	
LVH	0.014	0.241
LVEF	0.043	−0.202
24-h urine production	0.006	−0.273
Volume overload	NS	
K_t/V	0.016	−0.246

IHD, ischemic heart disease; HD, hemodialysis; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; NT-proBNP, N-terminal pro brain natriuretic peptide.
Volume overload: predialysis weight above estimated dry weight; K_t/V: K=dialyzer clearance, t=time, V=volume of water a patient's body contains.

significantly affecting NT-proBNP levels are shown in Figure 2. There was no correlation between ln NT-proBNP and age or gender. As a surrogate marker for volume overload, we used the increase in body weight in relation to clinically estimated dry weight, but found no correlation between this and levels of NT-proBNP (correlation coefficient 0.090, $P=0.65$). Quartiles of volume overload in relation to levels of NT-proBNP are shown in Figure 3.

During the mean follow-up of approximately 2 years (712 ± 258 days), 34 patients died (nine from cardiac events). Both pre- and post-dialysis levels of NT-proBNP were significantly higher in patients who died during follow-up than in surviving patients (predialysis 6393 (3272–31 381) vs 3352 (1626–10 425) pg/ml, $n=34$, $P=0.002$; post-dialysis 6130 (2633–23 084) vs 1837 (948–4707) pg/ml, $n=75$, $P<0.001$). Kaplan–Meier estimates revealed an increased mortality rate in patients with concentrations above median values compared to those with lower concentrations, both pre- and post-dialysis ($P=0.007$ vs $P=0.002$) (Figure 4) with a hazard ratio (HR) of 2.665 (1.262–5.586; predialysis values). Excluding patients who have undergone renal transplantation during follow-up did not change the result. Univariate Cox regression analysis (Table 3) found the following factors to be predictive of death in this population: ln predialysis NT-proBNP ($P=0.002$), ln post-dialysis NT-proBNP ($P<0.001$), high sensitivity C-reactive protein ($P=0.002$), age ($P=0.003$), reduced ejection fraction (EF) ($P=0.011$), and diabetes mellitus ($P=0.034$). Other traditional markers in serum predicting mortality in patients in

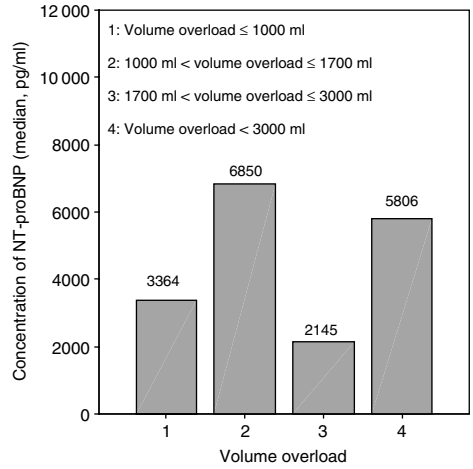


Figure 3 | Serum concentrations of NT-proBNP graphically shown for volume overload (quartiles).

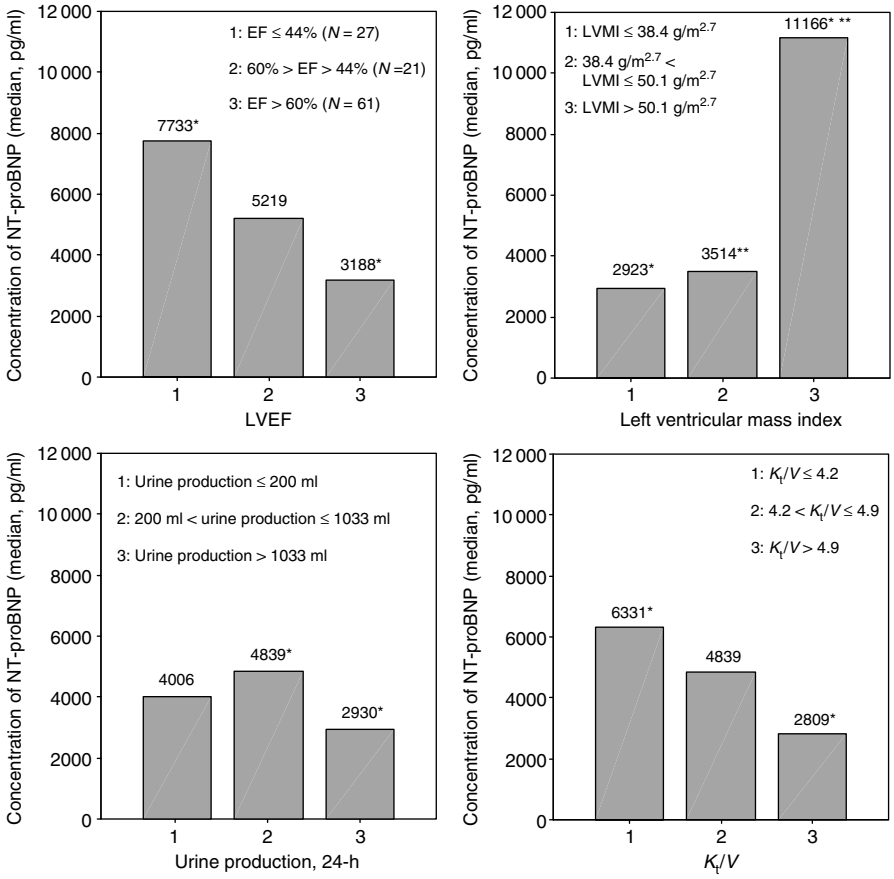


Figure 2 | Serum concentrations of NT-proBNP graphically shown for variables (tertiles) significantly affecting levels in a multivariate linear regression analysis (LVEF in modified tertiles). Significance between groups is indicated by * and **$P<0.05$.

HD such as cholesterol, phosphate, and homocysteine were not significant in our population just as left ventricular end diastolic diameter, LVMI, and body mass index did not predict mortality. Apart from NT-proBNP, only age revealed significance in a multivariate Cox regression analysis; predialysis: age ($P = 0.009$; HR 1.04 (1.01–1.06)), and ln NT-proBNP ($P = 0.007$; HR 1.42 (1.10–1.82)), post-dialysis: age ($P = 0.009$; HR 1.04 (1.01–1.07)) and ln NT-proBNP ($P = 0.001$; HR 1.52 (1.18–1.96)). Receiver operator characteristics curves for NT-proBNP as predictor of subsequent death demonstrated the following areas under the curve: predialysis 0.718 and post-dialysis 0.729 (Figure 5). Serum albumin is still the most powerful predictor of death (HR 5.959 (2.519–14.096; median) and is not included in these calculations.

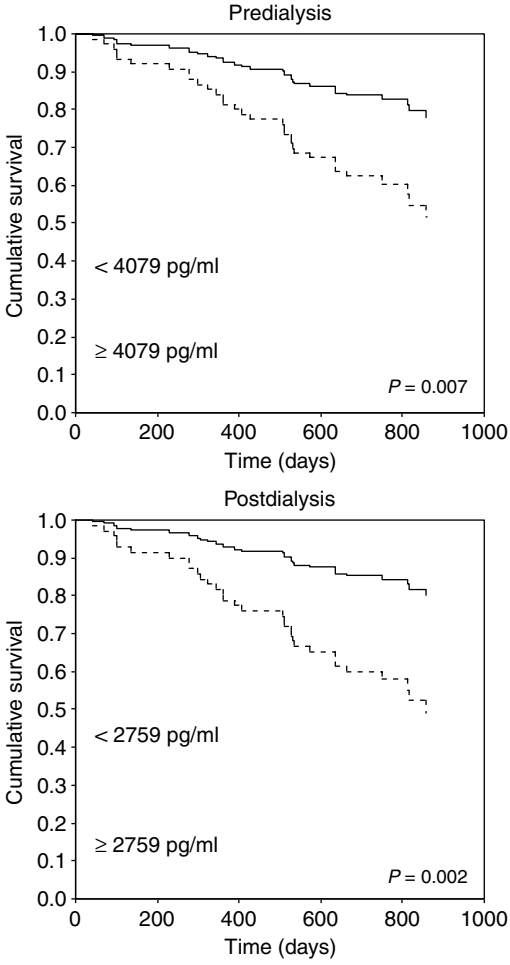
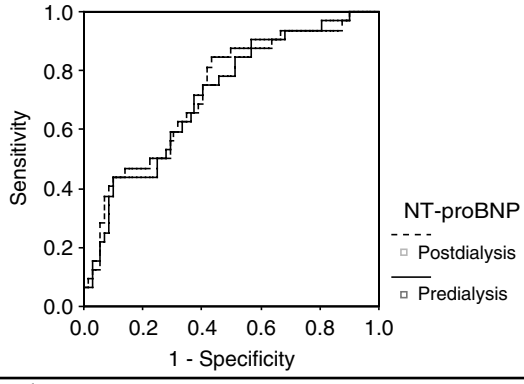


Figure 4 | Kaplan–Meier survival curves according to concentrations of NT-proBNP above and below median values (pre- and post-dialysis).



Concentration (mg/ml)	Sensitivity	Specificity	Hazard ratio
2515	0.875	0.431	3.473 (1.338 – 9.015)
3722	0.750	0.597	2.993 (1.362 – 6.317)
4079	0.718	0.611	2.665 (1.262 – 5.586)
7557	0.500	0.750	2.179 (1.099 – 4.323)

Figure 5 | Receiver operator characteristics curve for pre- and post-dialysis concentrations of NT-proBNP with sensitivity, specificity, and hazard ratio at different cutoff levels of predialysis concentrations of NT-proBNP. Areas under the receiver operator characteristics curve for pre- and post-dialysis values are 0.718 and 0.729, respectively. *Median value.

Table 3 | Uni- and multivariate Cox regression analysis for mortality

	Univariate		Multivariate predialysis		Multivariate post-dialysis	
Independent variable	*P*-value	*β*	*P*-value	HR	*P*-value	HR
Age (years)	0.003	0.038	0.009	1.04 (1.01–1.06)	0.009	1.04 (1.01–1.07)
Gender	NS		—	—	—	—
IHD	NS		—	—	—	—
DM	0.031	0.742	NS		NS	
LVMI	NS		—	—	—	—
LVEF	0.009	−0.030	NS		NS	
LVEDD	NS		—	—	—	—
ln NT-proBNP (pre-HD)	0.002	0.403	0.007	1.42 (1.10–1.82)	—	—
ln γNT-proBNP (post-HD)	<0.001	0.502	—	—	0.001	1.52 (1.18–1.96)
BMI	NS	—	—	—	—	—
hsCRP	0.002	0.190	NS		NS	
S-cholesterol	NS	—	—	—	—	—
S-phosphate	NS	—	—	—	—	—
S-homocysteine	NS	—	—	—	—	—

BMI, body mass index; DM, diabetes mellitus; HD, hemodialysis; hsCRP, high sensitivity C-reactive protein; IHD, ischemic heart disease; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NS, nonsignificant; NT-proBNP, N-terminal pro brain natriuretic peptide.

The number of CV events was too small to reveal any statistical differences regarding NT-proBNP.

DISCUSSION

This study shows that serum concentrations of NT-proBNP are greatly elevated in patients with ESRD undergoing HD and hence they cannot be used as a marker of heart failure in this population. HD induces a significant decrease of NT-proBNP levels, which is consistent with one previous report, whereas another study has shown increasing values.[11,12] Wahl *et al.*,[12] reported that pre- and post-dialysis levels of NT-proBNP are dependent on the type of membrane used during HD. High-flux membranes have a higher ultrafiltration rate than low-flux membranes. They tend to have larger pores, which means that the clearance of NT-proBNP with a molecular weight of 8.5 kDa is higher than when using low-flux membranes, where the post-dialysis concentration increased. In our substudy, the mean reduction in pre- to post-cartridge levels was 7.0%, which can be attributed to adhesion to or elimination over the cartridge. Supportive of the eliminating effect of HD on concentrations is also the fact that our levels were dependent of the effectiveness of dialysis as assessed by K_t/V. However, reduced synthesis may also contribute considering the abrupt fall in intra-vascular volume occurring during dialysis in combination with the half-life of NT-proBNP of 120 min.[4] There was no correlation between ln NT-proBNP and volume overload being an indicator of left ventricular strain, suggesting that other factors than fluid retention predominate in regulating concentrations.

Most studies regarding NP and dialysis have been investigating BNP and have demonstrated a positive correlation between levels of BNP and, for example, LVMI, LV diameters, arterial pressure and pulmonary artery pressure, and the inverse correlation with LVEF.[13–17] This is in agreement with existing evidence on NP in the general population,[2,18–22] but conclusions of the same consistence of other NP in HD patients cannot be drawn. In our study, despite the massive elevation of NT-proBNP concentrations, they nevertheless correlated with LVH and reduced LVEF, when performing a multivariate linear regression analysis. Levels of NT-proBNP in the general population are also age- and sex related as established, for example, by Raymond *et al.*,[23] but these differences cannot be detected in this population of patients in HD. These results suggest that the massively elevated concentrations in part are based on the same parameters as in patients without renal insufficiency, but with different impact: age and gender seem to be of minor importance as opposed to factors affecting left ventricular dimensions and function.

Another debatable issue in explaining the elevated concentrations is the elimination of NT-proBNP. NPs are cleared from the organism by NP receptors in the renal vascular endothelium, but also by proteolysis primarily by neutral endopeptidase located in the glomeruli.[24,25] There is no evidence regarding affinity of the different clearance mechanisms for the various NP, but the clearance mechanism for NT-proBNP vs BNP is thought to be primarily renal because of a stronger correlation with estimated glomerular filtration rate (-0.60 vs -0.20).[1] Also, NT-proBNP has been detected in urine in both healthy subjects and patients with heart failure,[26,27] but experience in this area is limited. Our results sustain this hypothesis given the correlation between increased 24-h urine production and lower concentrations of NT-proBNP levels. If a substantial part of the elimination of NT-proBNP were assumed to take place in the kidneys, an increase in the circulating concentrations would be anticipated if not compensated for by other ways of elimination. The clearance of peptides in the kidney is load dependent[28] and conditioned on extraction ratio and renal blood flow (clearance = extraction ratio × renal blood flow). If one or more of these parameters are affected, as seen in end-stage renal failure, it can cause a substantial rise in measured concentration owing to recirculating substances, but the importance of reduced elimination by the kidneys and its contribution to the elevated concentrations is still unknown.

Patients with CHF or chronic obstructive pulmonary disease had significantly elevated concentrations compared to HD patients not having been diagnosed with any of these disorders, which can be ascribed to the additional strain of the ventricles. We did not find an increased mortality rate among patients with diabetes mellitus compared to patients without diagnosed diabetes mellitus, just as we did not find any significant difference in the concentration of NT-proBNP in the two groups as earlier described by Naganuma *et al.*[16]

As previously established by several studies, the level of NT-proBNP is a strong prognostic marker in both the general population[29] and in various disease states, for example, acute and CHF,[30,31] coronary heart disease,[32,33] and hypertension.[34] The information on the prognostic role in patients with ESRD on HD is sparse with only one study demonstrating elevated levels of NT-proBNP predictive of all-cause death.[35] In our study, concentrations of NT-proBNP revealed increasing mortality with concomitant increasing concentrations, which was shown for both pre- and post-dialysis values. This complements the findings of Apple *et al.*,[35] who demonstrated that NT-proBNP was a marker for mortality prediction in ESRD if tertile analysis was used.

Multivariate Cox regression analysis revealed only age and NT-proBNP to be predictive of all-cause death. Therefore, although large variation and poor sensitivity and specificity, substantially increased levels of NT-proBNP in HD patients could be interpreted as an individual risk marker with the aim of identifying high-risk patients. Even though the synthesis and by that the level of NT-proBNP may decrease further initially after dialysis because of minor myocyte stretch, either pre- or post-dialysis NT-proBNP may function as a baseline marker for each individual patient. A putative therapeutic aim could be to identify patients who would benefit from a more aggressive clinical evaluation and therapeutic intervention in order to diminish concentrations as much as possible and to presumably reduce mortality risk.

We defined CV death as sudden death or death caused by arrhythmias, acute myocardial infarction or CHF. Of the 34 patients who died during follow-up, 25 patients died from non-CV causes with elevated levels of NT-proBNP being predictive of total mortality. In our study, 26 patients suffered from CHF with a mortality rate of 50% during follow-up; only three patients classified as CV deaths. The most likely explanation for the predictive value of mortality of NT-proBNP despite the low incidence of CV death may be based on misclassification of cause of death owing to other concomitant disease or the clinical impact of CHF on the general condition of the patients resulting in deterioration with subsequent death.

We did not find any correlation between volume overload and NT-proBNP, which is consistent with a study performed by Clerico *et al.*,[11] who was unable to detect any correlation between NT-proBNP and acute changes in preload during dialysis. This might lessen the possible use of NT-proBNP as a more accurate marker for estimated dry weight for sufficient HD, but further research within this area is needed.

Limitations to this study are that the sample size and number of adverse events are relatively small and therefore Cox regression analysis is restricted to a limited number of potential confounders.

Conclusion

Our findings demonstrate that CV disease in terms of LVH and reduced LVEF in addition to residual urine production and K_t/V determine NT-proBNP levels. Post-HD concentrations of NT-proBNP were lower than pre-HD levels; both values were predictive of mortality. Levels could be interpreted as an individual risk marker, which makes NT-proBNP monitoring an intriguing perspective.

MATERIALS AND METHODS

Patients

The study was designed as a single center study with prevalent patients recruited from the hemodialysis department, State University Hospital of Copenhagen. One hundred and nine patients were included. All patients underwent physical examination, electrocardiogram, and echocardiography assessing left ventricular ejection fraction (LVEF) and left ventricular mass index (LVMI), measurement of 24-h ambulatory BP and ankle-brachial index. All patients received regular bicarbonate HD using high-flux filters 3–4 times a week in sessions lasting 3–4.5 h. K_t/V (K = dialyzer clearance, t = time, V = volume of water a patient's body contains) is a surrogate marker for the elimination of low molecular weight toxins and represents a standard for effectiveness of HD with a desired value of 3.6 per week. Volume overload is defined as the increase in bodyweight measured just before HD in relation to clinically estimated dry weight.

Ischemic heart disease was defined as present in patients with previous acute myocardial infarction, coronary artery bypass grafting, or percutaneous coronary intervention or in patients with symptoms suggesting stable angina pectoris and responding to anti-ischemic treatment. Systolic CHF was defined in accordance with the guidelines from the European Society of Cardiology as symptoms (shortness of breath or ankle swelling) and objective evidence suggesting cardiac dysfunction, in this study also including patients with estimated LVEF $\leqslant 40\%$.[36] The duration of chronic renal failure is estimated as time since the first discovery of reduced renal function. During follow-up, all hospitalizations and causes of death were registered by continuous observation of medical records. Primary end points were acute coronary syndromes, ischemic stroke, sudden death, CV death, and total mortality.

According to the Declaration of Helsinki Principles, the local ethical committee approved the protocol, and written informed consent was obtained from each participant.

Measurement of clinical parameters

Echocardiography (HP/Agilent Sonos 4500) was performed on a non-dialysis day. Left ventricular dimensions and LVEF were measured by standard techniques; LVEF was estimated by nine-segment wall motion score index. Motion score index multiplied by 0.3 gives an estimate of LVEF.[37] Left ventricular mass (LVM) was calculated according to the regression equation described by Devereux:
$\mathrm{LVM} = 0.832(\mathrm{IVSD} + \mathrm{LVEDD} + \mathrm{PWTD})^3 - (\mathrm{LVEDD})^3 + 0.6$ and indexed to height$^{2.7}$ to determine LVMI, presented in g/m$^{2.7}$. Left ventricular hypertrophy (LVH) was defined as LVMI > 50 g/m$^{2.7}$ in male subjects and > 47 g/m$^{2.7}$ in female subjects.

Standard measurements of 24-h ambulatory BP were performed by oscillometry (A&D TM2420/TM2421, Spacelab 90217). In patients who refused to undergo ambulatory BP monitoring hypertension was estimated by comparison of BP before and after HD for a period of 1 month before study inclusion. HD BP was determined by standard techniques just before the HD session with an autoinflation BP monitor (A&D UA-779) and examination BP was determined by a non-invasive volume-oscillometric method (Artcomp, Critikon, GE Healthcare, Tampa, FL, USA) after 15 min of rest, in both using cuffs adapted to arm circumference. Hypertension was defined as systolic BP > 135 mm Hg and/or diastolic BP > 85 mm Hg independent of method of measurement.[38]

Laboratory measurements

Blood samples were drawn from the arteriovenous fistula just before and at the end of HD before discontinuation of the extra corporal circulation. Samples were centrifuged and serum was stored at -70°C before thawed and analyzed within 1 year of sampling. Serum NT-proBNP was measured using Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Cutoff used was 125 pg/ml taken from the manufacturer's package insert. The lower detection limit is 5 pg/ml and the coefficient of variation (20%) is < 50 pg/ml.

A small substudy on 10 randomly assigned patients was performed in order to evaluate the change in concentration over the cartridge. Pre- and post-cartridge blood samples were collected 30 min after initiation of HD with immediate determination of concentrations of NT-proBNP. No further clinical data were obtained on these patients.

Statistical analysis

All data with a normal distribution are presented as mean $\pm$ s.d., otherwise as median with range or quartiles in parenthesis. NT-proBNP values were skewed and consequently data underwent normalization by natural logarithmic transformation (ln) if required for statistical analysis. Differences between groups were examined with Student's t-tests or Mann–Whitney. Categorical data were compared using χ^2 analysis. Relationships between two variables

were determined by linear regression analysis, and multiple regression analysis was used to assess the combined influence of variables on serum NT-proBNP concentrations. The risk of death among patients with increasing concentrations of serum NT-proBNP was compared using the Kaplan–Meier method. The cutoff values were based on quartiles, and differences between the groups were analyzed with the log-rank test. The independent power of different variables to predict overall mortality was assessed using Cox proportional hazards regression analysis. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS 11.5 PC software.

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