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Natriuretic peptides as predictors of protein energy wasting in hemodialysis population.

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AUTHOR CONTRIBUTIONS

ML and EB performed the study, researched data, analyzed the results, and wrote the manuscript. YD, MP, SG, JCS, ML, WA, AB and DF researched data and analyzed the results. CS and LK conceived the study, analyzed the results, and wrote the manuscript. LK is the guarantor of this work and, as such, take full responsibility for it.

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Natriuretic peptides as predictors of protein energy wasting in hemodialysis population.

Abstract (242 words)

Objective: Imbalance between anabolism and catabolism is linked to cachexia and protein energy wasting (PEW), especially in frail populations such as chronic kidney disease (CKD) patients. PEW is responsible of poor outcomes with increased morbidity and mortality. Several causes are involved in PEW like insulin-resistance, acidosis or hyperparathyroidism. Natriuretic peptides (NPs) have recently been described as activators of resting energy expenditure (REE) through the induction of browning of white adipose tissue in rodent with CKD. The present study was therefore implemented to investigate whether NPs could be associated with PEW criteria and predict clinical outcomes.

Methods: We quantified serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) in a prospective cohort of 231 patients undergoing maintenance hemodialysis and atrial natriuretic peptide (ANP) in subgroup of 35 patients. Body composition parameters were measured with bioimpedance spectroscopy.

Results: NT-proBNP was inversely associated with serum albumin, prealbumin and BMI and, conversely, positively associated with age and CRP. NT-proBNP as well as ANP were significantly higher in patients with PEW criteria. NT-proBNP was negatively associated with body fat mass. In multiple linear regression, NT-proBNP remained associated with BMI. Kaplan-Meier analysis revealed a significant correlation between serum NT-proBNP concentrations and all-cause mortality and cardiovascular events. This association remained significantly after multivariable Cox regression models adjusted for demographic factors and **cardiovascular risk factors**.

Conclusion: NPs accumulation seems to be associated with poor nutritional status and reduced survival among hemodialysis patients. Further studies are needed to confirm this association using REE measurement and adipose tissue biopsy.

Key words: Protein energy wasting, chronic kidney disease, hemodialysis, cardiac natriuretic peptides

INTRODUCTION

Protein energy wasting (PEW) is defined as an increase of resting energy expenditure (REE) associated with an imbalance between anabolism and catabolism. PEW is associated with poor outcomes and impairs survival, especially in patients with chronic kidney disease (CKD). Hemodialysis (HD) patients are suffering from PEW in 20 to 80% of cases.^{1,2} Several causes are involved in the occurrence of PEW in CKD patients but the underlying mechanisms remain unclear. Energy-storing white adipose tissue (WAT) could be activated to become “beige cells”, a recently recognized metabolic process often referred to as WAT browning, which can lead to increased REE and could contribute of cachexia in different hypermetabolic situations like cancers or burning.³

Uremic toxins (UTs) are compounds that accumulate in kidney failure as a result of decreased renal clearance and exert adverse biologic impacts. Some links between accumulation of UTs, “browning” and cachexia have emerged. Luce et al have recently highlighted that uremic serum was able to trigger the expression of markers of brown adipocytes (such as uncoupling protein1 or Ucp1) in primary white adipocytes.⁴ Kir et al further have demonstrated that parathyroid hormone was involved in stimulating a thermogenic gene program in 5/6 nephrectomized mice. Fat-specific knockout of parathyroid hormone-receptor prevented adipose browning and cachexia in CKD mice.³

Atrial- and B-type (or Brain) Natriuretic Peptides (NPs), ANP and BNP respectively, have initially been described as peptide hormones responsible for the natriuretic and diuretic activity. NT-proBNP is an inactive N-terminal fragment generated by the enzyme-mediated cleavage of proBNP. However, the view that NPs is simply a cardiac hormone has recently been challenged. Over the last decade, NPs have emerged as potent metabolic hormones.^{5,6} Sarzani et al demonstrated that some NPs receptor were expressed in adipose tissue from rats and human.⁷ Therefore, NPs were identified as potent lipolytic hormones on human adipocytes⁸. They signal through increases in intracellular cyclic guanosine monophosphate (cGMP)⁹. NPs accumulation during heart failure have subsequently been shown to induce adipokine secretion¹⁰, and the browning of white adipose tissue.¹¹ Recently, Luce et al demonstrated that NPs, at concentrations found in CKD patients, increase Ucp1 expression in primary adipocytes.⁴ Also increasing of NPs levels in mice using a neprilysin inhibitor that prevent the breakdown of NPs or after 5/6 nephrectomy resulted in an increased REE and expression of Ucp1 in WAT.⁴

Several cohorts and community-based studies have reported an inverse relationship between plasma NPs levels and body mass index (BMI) in obese patients.^{12–14} However, very few studies have explored the association between NPs and PEW in CKD patients.^{15,16,17} In HD patients, BNP and NT-proBNP are commonly used to monitor cardiac dysfunction and fluid overload.^{18–22} High levels of BNP and NT-proBNP are associated with poor cardiac prognosis^{23,24,25} and outcomes in the CKD population.^{26,27}

As preclinical data indicate that NPs could play a role in PEW and mortality, we measured the serum concentrations of NT-proBNP in a large cohort of patients on hemodialysis and ANP in a subgroup of patients, sought its functional correlates with PEW criteria, and tested its predictive power for all-cause mortality and cardiovascular (CV) events.

MATERIALS AND METHODS

Research Participants

The study protocol was approved by the local ethics committee. Serum samples were selected from prevalent HD patients followed at a single dialysis center. Inclusion criteria were 4-h HD sessions, 3 times a week, for at least 3 months and over 18 years old, and a signed internal review board consent form. Exclusion criteria were current hospitalization, pregnancy or active or invasive tumor. The medical charts from each patient were reviewed for pertinent histories of comorbid conditions and any concomitant medications. History of CV disease was defined as a history of any of the following events: myocardial infarction, stroke, heart failure, angina pectoris, surgical procedures for angina or coronary/peripheral artery disease (including percutaneous transluminal angioplasty). Body mass index (BMI) was defined as the post dialysis weight (in kilograms) divided by the squared height (in meters squared). According to Fouque et al.,^{2,28} patients with CKD were considered having PEW if they presented at least 3 of the following 4 criteria: BMI < 23 kg/m², albumin level < 38 g/L, prealbumin level < 300 mg/L, and normalized protein catabolic rate < 0.8 g/kg/d. Body composition was assessed using whole-body bioimpedance spectroscopy (BIS) (Fresenius Medical Care, Bad Homburg, Germany) at baseline in a subpopulation of 68 patients.

Laboratory Tests

Routine laboratory tests were performed using standard methods in the routine clinical laboratory at the baseline visit, in a fasting state, during a midweek dialysis session. Serum NT-proBNP was quantified by electrochemiluminescence immunoassay on the Cobas E601

analyser (Roche Diagnostics Corp., Meylan, France). In a sub-population of 35 HD patients, serum ANP was quantified with an enzyme immunoassay (Sigma-Aldrich, RAB00385).

Follow-up

During the study period, all-cause mortality and CV events, were identified in all patients included at least 18 months before the end of the study date. CV events were defined as CV death or any adverse event directly related to a CV system dysfunction namely stroke, angina pectoris/myocardial infarction, congestive cardiac failure, new-onset arrhythmia, peripheral ischemia, or any surgical procedures for angina or coronary/peripheral arterial disease.

Statistical analyses

Data were analyzed using GraphPad Prism 8.0 (GraphPad softwares, La Jolla, USA) software and R (<https://www.r-project.org/>). The data are expressed as mean \pm standard error of the mean (SEM), or as median (interquartile range – IQR) when variables were not normally distributed. Distributions were tested for normality using d'Agostino-Pearson **and Shapiro-Wilk tests**. NT-proBNP and ANP values was classified as nonnormally distributed; therefore, a logarithmic transformation of data was performed. Spearman correlations were used as deemed appropriate for association studies. Continuous measures were compared between two independent groups using Mann & Whitney U test or Student t tests. Chi-squared tests were used to compare categorical measures.

Kaplan-Meier plots for death and levels of NT-proBNP were used for initial analysis of patient survival. The log-rank test was used to compare the Kaplan–Meier curves. Univariate and multivariate analyses of overall mortality, CV mortality and CV events were performed using a Cox proportional hazard model as a function of NT-proBNP as continuous variable. In multivariate analyses, models were adjusted for demographic variables (model 1, age and sex and dialysis vintage) or classical CV risk factors (model 2, age, smoking, presence of diabetes, dyslipidemia and history of cardiovascular disease **and proxy of residual renal function i.e. diuretics**). **For overall mortality and CV events, data were censored at renal transplantation, loss to follow-up, or the end of the study observation period.** A $P < 0.05$ was considered as statistically significant in all analysis.

RESULTS

266 prevalent patients undergoing HD for >3 months were included. Measurements of serum NT-proBNP were available for a total of 231 hemodialysis patients. Baseline characteristics of the cohort are summarized in **Table 1**. The median age was 64.1 [IQR, 49.3-75.5] year, 38% of patients were female (N=89) and median dialysis vintage was 2.21 [IQR, 1.07-4.73] years. The proportion of diabetic patients was 32 %. The median serum albumin and prealbumin levels were 39.0 g/l [IQR, 36.0–41.3 g/l] and 330 mg/l [IQR, 280–390 mg/l], respectively. The median normalized protein catabolic rate was 1.09 g/kg/d [IQR, 0.90–1.29 g/kg/d].

The correlation between serum NT-proBNP concentration and relevant clinical characteristics as well as serum laboratory tests are presented in **Table 2**. NT-proBNP was positively correlated with age ($r_s = 0.260$, $p < 0.01$), dialyse vintage ($r_s = 0.260$, $p < 0.01$), and inflammatory markers (CRP, $r_s = 0.230$, $p < 0.01$). NT-proBNP was negatively correlated with nutritional markers such as albumin ($r_s = -0.130$, $p = 0.02$), prealbumin ($r_s = -0.290$, $p < 0.01$), and body mass index (BMI) ($r_s = -0.200$, $p < 0.01$). There was no significant correlation with nPCR. NT-proBNP concentration remained a significant predictor of BMI ($p < 0.05$) after adjustment for age and other common indexes of PEW (i.e albumin, prealbumin and nPCR) in multivariate analysis. (see **Supplementary Table 1**).

To determine if levels of NT-proBNP were correlated with the severity of PEW, patients were divided in two groups according to their nutritional status (i.e. the number of PEW criteria) (see the characteristics of the 2 sub-populations in **Table 1**). **The treatments and cardio-vascular history were not different between HD patient with and without PEW criteria.** **Figure 1** shows that impairment of nutritional status was associated with higher NT-proBNP levels (+48%, $p < 0.01$). To confirm that NT-proBNP was associated with PEW cachexia in HD patients, we measured in a subgroup of 68 patients body composition with BIS. We found a negative association between NT-proBNP levels and adipose tissue mass (ATM, $r_s = -0.23$, $p < 0.05$) (**Figure 2**). Similar observation was made for fat tissue index (FTI, $r_s = -0.22$, $p < 0.05$) but not with lean mass or total body water (**Supplementary Table 2**).

In our cohort, the mean follow-up was 976 days. Patients were stratified into 2 groups (below and above mean NT-pro-BNP levels) (**Supplementary Table 3**). We confirmed that NT-proBNP accumulation in HD was associated with an increase of overall mortality ($p < 0.05$), CV mortality ($p < 0.01$) and CV events ($p < 0.01$) (**Figure 3A-C**). Serum NT-proBNP concentration was significantly associated with all-cause mortality, CV mortality as well as CV events in univariable analysis using NT-proBNP concentration as a continuous variable. In addition, the

relationship between serum NT-proBNP concentration and mortality or CV events during follow-up remained significant ($p < 0.05$) after multivariable adjustments (**Supplementary Table 4A, B**).

In a subgroup of patients with availability of serum, ANP concentration was measured with an immunoassay. 35 HD patients were included and were classified either as no PEW ($n=19$) or PEW ($n=16$). Baseline characteristics are summarized in **Supplementary Table 5**. As similar as observed with NT-proBNP, we shown that serum ANP levels were significantly increased in PEW patients (+49%, $p < 0.05$) (**Figure 4**).

DISCUSSION

In the present study, we demonstrated that NT-proBNP and ANP accumulation in stable adult HD patients may mediate the PEW that characterize this disease state. NT-proBNP was negatively associated with adipose tissue mass and BMI. As with previous studies, we confirmed that NT-proBNP can predict all-cause mortality, and specifically CV events, in patients on hemodialysis.^{23,24,25,26,27}

The implication of NPs on cachexia have been first demonstrated in heart failure patients. Patients with chronic heart failure tend to exhibit higher NT-proBNP levels; however, the levels are higher in patients with cachexia than in patients without cachexia²⁹. The infusion of BNP or ANP in mice at the levels found in cardiac dysfunction induces a cachexia.¹¹ ANP, at a concentration relevant for uremia, can also induce a decrease of fat mass in mice.⁴ Given that the circulating level of NT-proBNP is as a sensitive marker of cardiac stress and volume expansion, and that elevated levels are indicative of heart failure, we found similar results when all patients with CV history were excluded (*data not shown*). Unfortunately, cardiac function was not estimated in the present study to formally exclude cardiac dysfunction. **We failed to observe in the subpopulation a significant correlation between overhydration and NT-proBNP. The role of NT-proBNP in detecting abnormalities of fluid status is still under debate; the relationship between volume status and BNPs differs greatly among various study. Furthermore, the small size of the cohort and the selection of stable patients without overhydration status could explain these results.**

The association between NPs and PEW in CKD patients have been suggested in previous study. Chazot et al found a significant association between BNP and nutrition markers in HD patients¹⁶. Unfortunately, the body composition in this study was not evaluated and the criteria of PEW were only based on nPCR, albuminemia and prealbuminemia. Ducros and al found in Afro-Caribbean dialysis patients that NT-proBNP was independently associated with

PEW¹⁷. A negative correlation between BMI and NT-proBNP levels have further been found in non-dialysis afro-americans CKD patients.¹⁵ However, the nutritional markers may vary according to ethnic groups and cannot be transposed to Caucasian population. Booth et al found a negative association between NT-proBNP and albumin in a small cohort of 72 HD patients.³⁰ NT-proBNP was also associated with predialysis ratio of extracellular water to total body water and these could be a dilutional effect due to fluid overload. In our study, we did not observe any significant correlation with extracellular water suggesting that in our cohort the correlation between NT-proBNP and nutritional markers could reflect poor nutrition status. Ikeda et al³¹ shown **that** in Asian HD patients that NT-proBNP was an independent predictor for decrease in lean body mass measured by DEXA (dual-energy X-ray absorptiometry). In the present study, we did not find any significant correlation between NPs and lean tissue mass. We used BIS to estimate body composition and this methodology could potentially explain conflicting results. The most appropriate method to estimate body composition, lean and fat mass in HD patients is still under debate. However, BIS appears to be a robust tool to measure and monitor total-body fat and lean in this population as compared to the gold standard DEXA (dual-energy X-ray absorptiometry)⁴⁸⁻⁵¹. Therefore, we can conclude with good confidence that NT-proBNP level is correlated with WAT mass but not with LBM in our Caucasian HD patients. In our study, even if only a small number of patients were included, we observed an association between PEW and ANP concentrations as suggested in by Nakatani et al where ANP was negatively correlated with albumin.³² We were unable to measure BNP in the present study. NT-proBNP is known to be more influenced by alteration of kidney function and dialysis treatment than BNP (half-life 20 min versus 2h for NT-proBNP).³³ However some data clearly demonstrated that NT-proBNP levels are significantly correlated with BNP levels at all stages of kidney dysfunction including HD patients.³⁴ Therefore, we can speculate that NT-proBNP levels can be a proxy of BNP concentrations event if further study needs to confirm this point.

Several hypotheses have been proposed regarding the association between high NT-proBNP and adipose tissue loss. NT-proBNP levels are frequently increased in patients with cardiac dysfunction or end stage renal disease with concomitant inflammatory status.³⁵ Inflammation is considered a main factor for PEW in CKD patients.³⁶ As previously demonstrated^{31,37}, NT-proBNP was significantly correlated with CRP levels in our cohort. If the inflammation, and NT-proBNP are linked, the underline mechanisms of regulation are unclear. Because there are some in vitro studies that showed the opposite effect of NPs. ANP in subcutaneous adipocytes may inhibit the production of inflammatory cytokines involved in obesity-related inflammatory state and insulin resistance.³⁸ The NPs can induce lipid

mobilization and oxidation. Perfusion of ANP in humans induces a lipolytic effect with an increase in serum levels of glycerol and non-esterified fatty-acids regardless of their BMI³⁹ while it also enhances REE.⁴⁰ In rodents, ANP and BNP induce browning of adipocytes along with mitochondrial biogenesis through the same NP receptor A - cGMP- protein kinase G signaling cascade. Similarly ANP, at an uremic relevant concentration, can also induce browning in WAT.⁴ Altogether, NT-proBNP and ANP may not only be indirect biomarkers for PEW, but also trigger the loss of adipose tissue mass in HD patients.

However, the present study results must be considered with the following caveats. First, the number of patients was small, and we could only evaluate the findings for prevalent HD patients. **Secondly, the single-point measurement of NT-proBNP did not permit an accurate evaluation. A longitudinal study, with regular measurement of NT-proBNP and BIS evaluation during follow-up would be useful to confirm this association. We have however carefully selected stable patients. Fahim et al observed that the within-person coefficient of variation of NT-proBNP among stable dialysis patients was smaller, equating to 27% for the weekly measurement intervals.⁴¹ Third, several operational definitions exist for cachexia regarding it as the most severe stage of PEW.⁴² Until today there is limited evidence to suggest the use of one tool over others for identifying those at risk of PEW.⁴³ We selected this classification because a study conducted in patients receiving HD, recorded that PEW score predicts survival. However, we cannot exclude that some patients could have been misclassified.²⁸ Finally, it was unfortunately not possible to monitor REE and realize some adipose tissue biopsy in HD patients. We thus considered body composition and PEW criteria as “proxies” of browning activation and further studies are needed to confirm the direct link between NPs accumulation and thermogenesis activation.**

In conclusion, NT-proBNP could be an independent biomarker for PEW in HD patients with adipose tissue loss. With increasing knowledge of molecular mechanisms, we hope to develop new approaches to prevent PEW in CKD patients.

PRACTICAL APPLICATION

We confirmed the potential clinical utility of NT-proBNP levels for risk stratification in patients on HD. It may help practitioners to monitor nutritional state after therapeutic intervention, along with albumin and prealbumin dosages.

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FIGURES

Figure 1: Elevation of serum NT-proBNP is associated to PEW in hemodialysis patients (HD)

NT-proBNP levels (log-transformed) in HD patients without (n=161) or with PEW criteria (n=70). Data were compared using Mann Whitney U test. Data are expressed as mean \pm SEM. **p < 0.01, compared with patients without PEW

Abbreviation: PEW, protein energy wasting.

Figure 2. NT-proBNP concentration is negatively correlated with adipose tissue mass (ATM) in hemodialysis patients (HD)

Correlation between log NT-proBNP and ATM. Correlation analysis was performed with Spearman rank test. ATM was measured with bioimpedance spectroscopy. Difference was considered significant at the P<0.05 level.

Figure 3. Kaplan-Meier estimates of cumulative survival (A) cumulative cardiovascular mortality (B) or time to first cardiovascular event (C) of all patients according to plasma NT-proBNP levels at the time of inclusion. Quantile cutoff (NT-proBNP level): quantile 1, ≤ 3.27 ng/L; quantile 2, > 3.27 ng/L. For cumulative survival, the number of events was 14, and 32 for quantile 1, and quantile 2, respectively. For cumulative cardiovascular mortality, the number of events was 15, and 39 for quantile 1, and quantile 2, respectively. For time to first cardiovascular event, the number of events was 15, and 39 for quantile 1, and quantile 2, respectively. **The number of patients indicated in the tables is the number of patients still followed at the indicated time.**

Figure 4. Elevation of serum ANP concentration is correlated to PEW in hemodialysis patients

ANP levels (log-transformed) in HD patients without (n=19) or with PEW criteria (n=16). Data were compared using Mann Whitney U test. Data are expressed as mean \pm SEM. *p < 0.05.

Abbreviation: PEW, protein energy wasting.

Figure 1

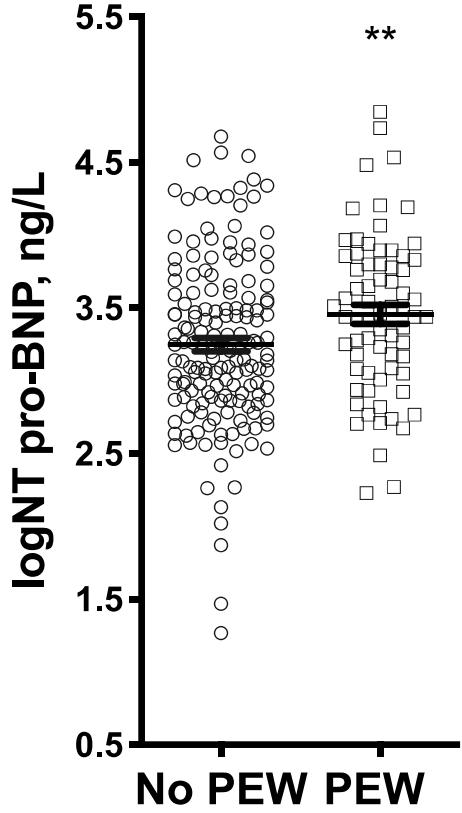


Figure 2

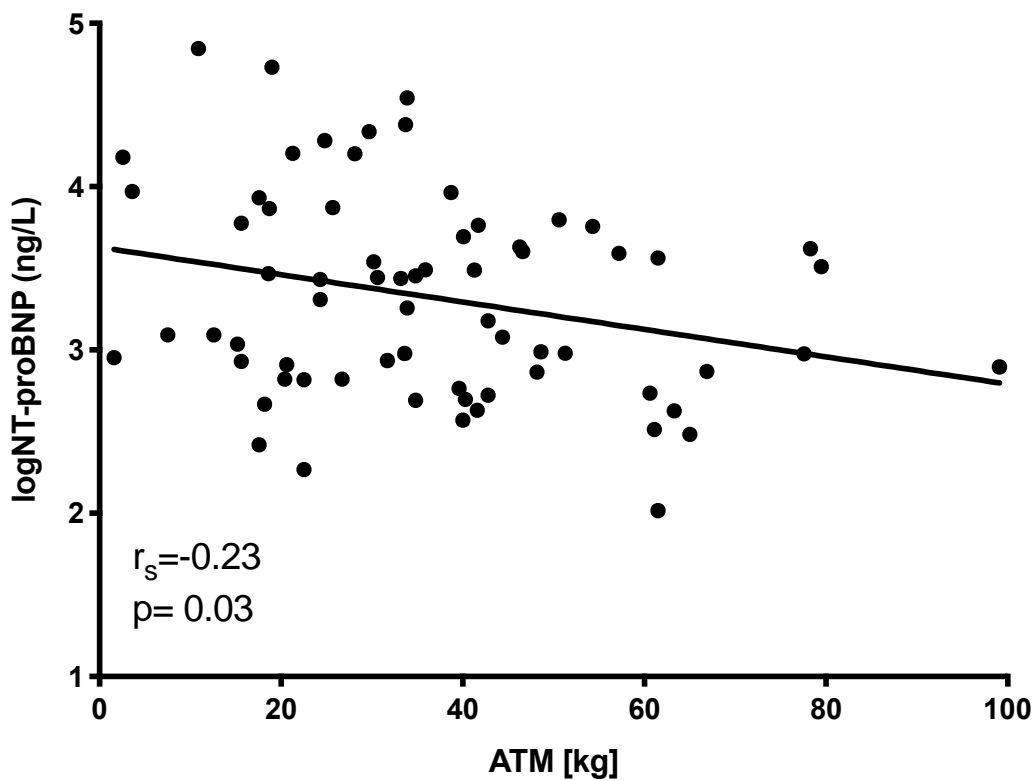
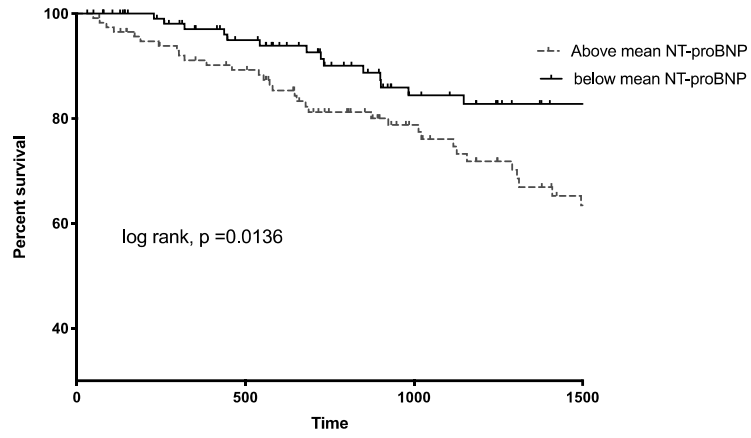


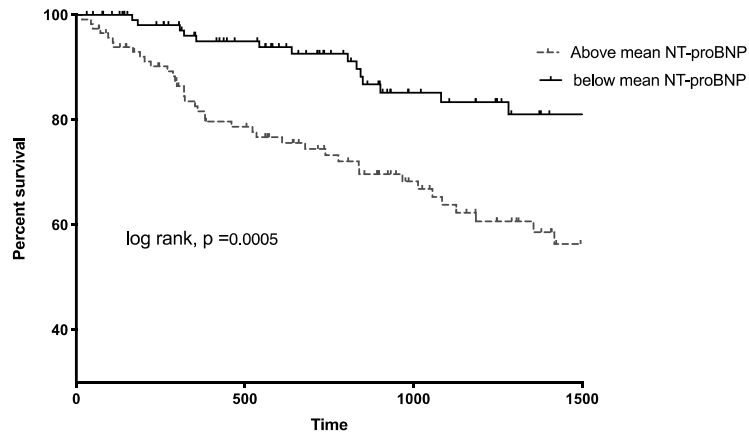
Figure 3

A



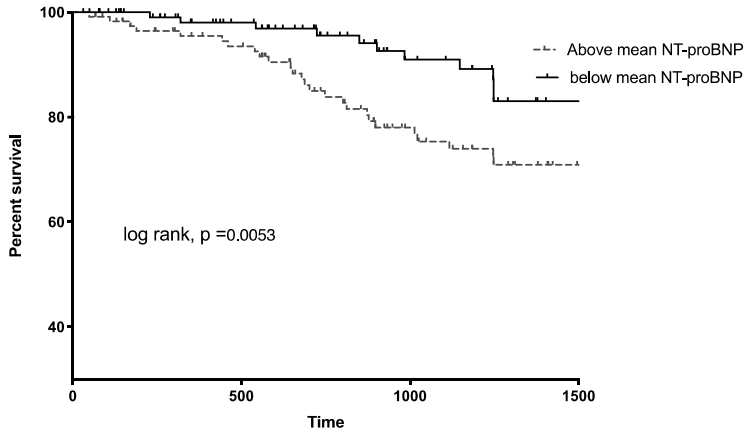
	Number of patients at risk			
	0 day	500 days	1000 days	1500 days
Above	116	97	60	37
Below	115	89	54	34

B



	Number of patients at risk			
	0 day	500 days	1000 days	1500 days
Above	116	86	49	31
Below	115	81	48	24

C



	Number of patients at risk			
	0 day	500 days	1000 days	1500 days
Above	116	97	60	37
Below	115	89	54	34

Figure 4

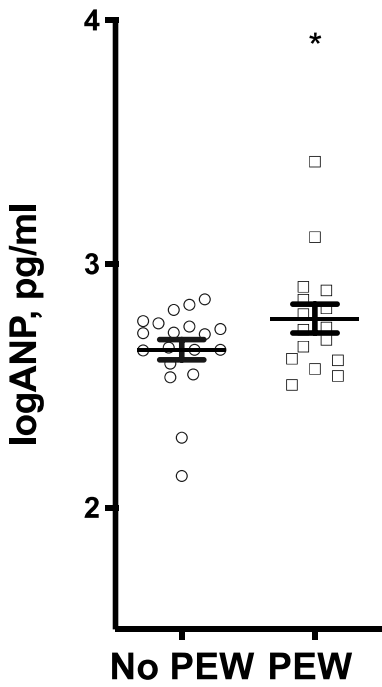


Table 1. Characteristics according to PEW levels in maintenance hemodialysis patients selected for NT-proBNP measurement (n= 231)

	Overall population, n=231	No PEW, n=161	PEW, n=70	P-value
Clinical characteristics				
Sex, male/female	142/89	99/62	43/27	< 0.01
Age, yrs	64.1[49.3-75.5]	61.6[48.6-71.8]	71.3[51.8-78.2]	< 0.01
BMI, kg/m ²	25.4[22.5-29.2]	25.7[23.4-29.8]	24.0[20.6-27.5]	< 0.01
Dialysis vintage, yrs	2.21[1.07-4.73]	2.39[1.07-4.96]	1.99[1.03-4.22]	0.25
nPCR, g/kg/day	1.09[0.90-1.29]	1.10[0.96-1.30]	0.98[0.76-1.24]	< 0.01
Dialysis dose, Kt/V	1.69[1.44-1.90]	1.63[1.44-1.80]	1.76[1.45-2.00]	0.04
Predialysis SBP, mmHg	130[117-140]	130[119-140]	127[112-149]	0.48
CV history, %	35	32	42	0.12
Diabetes, %	32	30	34	0.56
Smoking, %	37	36	39	0.43
Hypertension, %	90	88	93	0.12
Dyslipidemia, %	57	57	58	0.77
Causes of nephropathy				
Diabetes, %	26	25	28	0.71
Vascular, %	19	16	24	0.19
Glomerulopathy, %	23	21	26	0.44
ADPKD (n)	9	12	3	0.01
Interstitial nephritis, %	6	7	3	0.23
Urology, %	9	8	11	0.42
Unknown, %	10	11	6	0.19
Laboratory Tests				
NT-ProBNP, ng/L	1883[830-4940]	1602[735-3999]	2801[1179-6839]	< 0.01
Log NT-proBNP	3.27[2.92-3.69]	3.20[2.87-3.60]	3.45[3.07-3.7]	< 0.01
Hemoglobin, g/dL	11.2[10.5-12.1]	11.3[10.5-12.2]	11.1[10.3-12.1]	0.17
Albumin, g/L	39.0[36.0-41.3]	40.4[38.5-41.9]	35.3[33.0-36.9]	< 0.01
Prealbumin, mg/L	330[280-390]	360[310-410]	270[230-290]	< 0.01
Creatinine, µmol/L	737[579-898]	787[607-941]	636[528-804]	< 0.01
Urea, mmol/L	20.2[16.1-23]	20.8[17.9-23.7]	18.0[13.3-21.7]	< 0.01
Bicarbonate, mmol/L	21.0[19.0-23.0]	21.0[19.0-23.0]	22.0[20.0-24.0]	0.01
Calcium, mmol/L	2.25[2.12-2.35]	2.27[2.14-2.35]	2.22[2.08-2.31]	0.01
Phosphorus, mmol/L	1.44[1.15-1.75]	1.50[1.22-1.78]	1.22[1.02-1.72]	< 0.01
PTH, pmol/L	234[106-512]	260[121-522]	184[67.3-495]	0.04
25OH Vitamin D, nmol/L	32.4[24.8-40.4]	33.6[26.0-40.9]	30.8[20.3-36.0]	0.02
Hemoglobin A1c, %	5.60[5.10-6.50]	5.60[5.10-6.30]	5.70[5.13-7.38]	0.12
Total cholesterol, mmol/L	1.63[1.40-1.94]	1.60[1.40-1.90]	1.65[1.31-2.01]	0.32
Triglycerides, mmol/L	1.51[1.00-2.20]	1.57[1.00-2.30]	1.50[1.00-2.10]	0.23
CRP, mg/L	5.00[1.80-12.2]	4.10[1.50-9.30]	9.80[3.35-19.5]	< 0.01
Treatment				
Diuretics, %	46	54	45	0.67
Anti-diabetic therapy, %	26	25	27	0.71
Lipid-lowering therapy, %	52	50	54	0.13
RAA, %	42	42	42	0.93
Beta blocking agents, %	46	45	48	0.59

Data are expressed as medians [IQR]. Differences between groups were tested using Mann & Whitney U tests or Fisher exact tests as appropriate. eGFR was estimated according to the CKD EPI formula. Abbreviations: AAP, Antiplatelet agents, ADPKD, Autosomal dominant polycystic kidney disease, BMI, body mass index, CRP, C-reactive protein, CV, cardiovascular, eGFR, estimated glomerular filtration rate, nPCR, normalized protein catabolic rate, NT-proBNP, N-terminal pro-brain natriuretic peptide, PTH, parathormone, SBP, systolic blood pressure, RAA, renin angiotensin aldosterone, yrs, years,.

Table 2. Unadjusted Spearman correlation coefficients (r_s) between NT-pro-BNP and relevant variables in hemodialysis patients

Variable	r_s	95% CI	p
Dialysis vintage, yrs	0.280	0.15 to 0.40	< 0.01
Age, yrs	0.260	0.13 to 0.38	< 0.01
Predialysis systolic BP, mmHg	0.034	-0.11 to 0.18	0.32
Hemoglobin, g/dL	-0.140	-0.27 to -0.0068	0.02
Urea, mmol/L	-0.069	-0.20 to 0.064	0.15
Creatinine, mmol/L	0.011	-0.12 to 0.14	0.43
Bicarbonate, mmol/L	0.047	-0.088 to 0.18	0.24
Dialysis dose, Kt/V	0.210	0.079 to 0.34	< 0.01
Calcium, mmol/L	-0.043	-0.17 to 0.091	0.26
Phosphorus, mmol/L	-0.041	-0.17 to 0.093	0.27
PTH, pmol/L	-0.061	-0.20 to 0.075	0.18
25-OH Vitamin D3, nmol/L	0.059	-0.078 to 0.19	0.19
CRP, mg/L	0.230	0.096 to 0.35	< 0.01
Hemoglobin A1c, %	-0.180	-0.33 to -0.018	0.01
Total cholesterol, mmol/L	0.090	-0.065 to 0.24	0.12
Triglycerides, mmol/L	-0.170	-0.31 to -0.011	0.02
Serum albumin, g/dL	-0.130	-0.26 to 0.0024	0.02
Serum prealbumin, mg/L	-0.290	-0.41 to -0.16	< 0.01
nPCR, g/kg/day	0.056	-0.081 to 0.19	0.20
BMI, kg/m²	-0.200	-0.33 to -0.069	< 0.01

Spearman rank correlation test was performed in the population of 231 hemodialysis patients. Abbreviations: BMI, body mass index, BP, blood pressure, CRP, C-reactive protein, eGFR, estimated glomerular filtration rate, nPCR, normalized protein catabolic rate, NT-proBNP, N-terminal pro-brain natriuretic peptide, PTH, parathormon, SBP, systolic blood pressure, yrs, years.

Supplementary Table 1. Multiple linear regression of association between NT-pro-BNP concentration and BMI.

Outcome variable: BMI (kg/m²)			
Predictor Variable	β Coefficient	Standard Error	<i>p</i>-Value
Intercept	25.27	4.918	6.69e-07***
NT-proBNP, ng/L	-9.731e-05	4.522e-05	0.0326 *
Age, yrs	7.958e-02	2.342e-02	0.0008 ***
Albumin (g/L)	-0.1728	0.1191	0.1482
Prealbumin (g/L)	1.481e-02	5.081e-03	0.0040***
nPCR, g/kg/day	-1.692	1.293	0.1921

Adjusted R-squared: 0.09042, p-value= 0.00027

Abbreviations: nPCR, normalized protein catabolic rate, NT-proBNP, N-terminal pro-brain natriuretic peptide, yrs, years.

Supplementary Table 2. Unadjusted Spearman Correlation Coefficients (r) of NT-proBNP and bioimpedance parameters.

Variable	r_s	95% CI	<i>p</i> -Value
BMI (kg/m²)	-0.290	-0.50 to -0.05	0.01
Lean tissue mass, kg (LTM)	-0.088	-0.33 to 0.16	0.24
Lean tissue index, kg/m ² (LTI)	-0.068	-0.31 to 0.18	0.29
Adipose tissue mass, kg (ATM)	-0.230	-0.45 to 0.02	0.03
Fat tissue index, kg/m² (FTI)	-0.220	-0.44 to 0.03	0.04
Body cell mass, kg (BCM)	-0.085	-0.33 to 0.17	0.25
Extracellular water, L (ECW)	-0.098	-0.34 to 0.15	0.21

Spearman rank correlation test was performed in a subgroup of 68 hemodialysis patients.

Abbreviations: ATM, Adipose tissue mass, BCM, Body cell mass, BMI, body mass index, ECW, Extracellular water, FTI, Fat tissue index, LTI, Lean tissue index, LTM, Lean tissue mass.

Supplementary Table 3. Characteristics according to NT-proBNP quartile in maintenance hemodialysis patients selected for NT-proBNP measurement (n=231)

	Below mean NT-proBNP, n=115	Above mean NT-proBNP, n=116	P-value
Clinical characteristics			
Sex, male/female	71/44	71/45	0.93
Age, yrs	60.4[44.9-70.6]	69.3[54.5-78.4]	< 0.01
BMI, kg/m²	27.1[23.3-31.1]	25.0[22.0-27.0]	< 0.01
Dialysis vintage, yrs	1.71[0.98-3.55]	3.10[1.24-6.31]	< 0.01
nPCR, g/kg/day	1.10[0.90-1.26]	1.08[0.91-1.30]	0.46
Dialysis dose, Kt/V	1.60[1.37-1.79]	1.74[1.50-1.95]	0.00
Predialysis SBP, mmHg	130[118-140]	130[114-145]	0.33
CV history, %	26	45	< 0.01
Diabetes, %	35	28	0.30
Smoking, %	30	44	0.02
Hypertension, %	89	91	0.65
Dyslipidemia, %	51	63	0.07
Causes of nephropathy			
Diabetes, %	33	20	0.05
Vascular, %	12	25	0.01
Glomerulopathy, %	18	27	0.23
ADPKD (n)	10	8	0.48
Interstitial nephritis, %	5	6	0.79
Urology, %	10	8	0.48
Unknown, %	12	7	0.17
Laboratory Tests			
NT-ProBNP, ng/L	830[504-1219]	4904[2882-9158]	< 0.01
Log NT-ProBNP, ng/L	2.92[2.70-3.09]	3.69[3.46-3.96]	< 0.01
Hemoglobin, g/dL	11.4[10.8-12.2]	11.1[10.2-12.0]	0.04
Albumin, g/L	39.6[36.6-41.6]	38.5[36.0-38.5]	0.04
Prealbumin, mg/L	360[300-403]	310[270-370]	0.00
Creatinine, μmol/L	760[570-901]	704[591-883]	0.27
Urea, mmol/L	20.9[17.7-23.5]	19.4[15.4-22.2]	0.04
Bicarbonate, mmol/L	21.0[19.0-23.0]	21.0[19.0-23.0]	0.27
Calcium, mmol/L	2.25[2.13-2.35]	2.25[2.10-2.34]	0.31
Phosphorus, mmol/L	1.48[1.16-1.77]	1.42[1.08-1.74]	0.24
PTH, pmol/L	238[123-515]	231[845-493]	0.31
25OH Vitamin, nmol/L	31.0[23.8-40.4]	32.6[25.7-39.4]	0.26
Hemoglobin A1c, %	5.70[5.23-7.15]	5.45[5.00-6.00]	0.00
Total cholesterol, mmol/L	1.60[1.30-1.89]	1.70[1.40-2.00]	0.13
Triglycerides, mmol/L	1.80[1.06-2.40]	1.44[1.00-2.10]	0.06
CRP, mg/L	4.20[1.70-10.3]	6.30[2.10-17.7]	0.02
Treatment			
Anti-diabetic therapy, %	30	22	0.10
Lipid-lowering therapy, %	49	54	0.39
RAA, %	40	45	0.46
Beta blocking agents, %	34	58	0.00

Data are expressed as medians [IQR]. Differences between groups were tested using Mann & Whitney U tests or Chi-squared tests as appropriate. eGFR was estimated according to the CKD EPI formula. Abbreviations: AAP, Antiplatelet agents, ADPKD, Autosomal dominant polycystic kidney disease, BMI, body mass index, CRP, C-reactive protein, CV, cardiovascular, eGFR, estimated glomerular filtration rate, nPCR, normalized protein catabolic rate, NT-proBNP, N-terminal pro-brain natriuretic peptide, PTH, parathormone, SBP, systolic blood pressure, RAA, renin angiotensin aldosterone, yrs, years.

Supplementary Table 4A. Multivariable Cox regression analysis of NT-pro-BNP as a predictor all-cause mortality, cardiovascular mortality and cardiovascular events

Events	HR (95% CI)	p-value
Overall mortality		
Unadjusted	1.000031 (1.000014 - 1.000048)	<0.001***
Model 1 ^a	1.000027 (1.000009 - 1.000045)	0.003***
Model 2^b	1.000032 (1.000009- 1.000055)	0.006**
Cardiovascular mortality		
Unadjusted	1.000044 (1.000021 - 1.000067)	<0.001***
Model 1 ^a	1.000032 (1.000005 - 1.000060)	0.019*
Model 2^b	1.000051 (1.000018 - 1.000083)	0.002**
Cardiovascular events		
Unadjusted	1.000030 (1.000014- 1.000046)	<0.001***
Model 1 ^a	1.000024 (1.000007 - 1.000041)	0.006**
Model 2^b	1.000028 (1.000008 - 1.000048)	0.006**

CI, confidence interval; HR, hazard ratio.

^aModel 1 was adjusted for demographic characteristics (age, sex, and dialysis vintage).

^bModel 2 was adjusted for cardiovascular risk factors (age, smoking, presence of diabetes, dyslipidemia and history of cardiovascular disease, *residual renal function*).

Supplementary Table 4B. Multivariable Cox regression analysis of log (NT-pro-BNP) as a predictor all-cause mortality, cardiovascular mortality and cardiovascular events

Events	HR (95% CI)	p-value
Overall mortality		
Unadjusted	1.572 (1.263- 1.957)	<0.001***
Model 1 ^a	1.506 (1.212- 1.872)	<0.001***
Model 2^b	1.405 (1.107- 1.783)	0.006**
Cardiovascular mortality		
Unadjusted	1.753 (1.204 – 2.552)	0.003**
Model 1 ^a	1.607 (1.122 – 2.301)	<0.010**
Model 2^b	1.521 (1.023 – 2.260)	0.038*
Cardiovascular events		
Unadjusted	1.572 (1.290 - 1.916)	<0.001***
Model 1 ^a	1.529 (1.261 - 1.854)	<0.001***
Model 2^b	1.412 (1.147 - 1.738)	0.002***

CI, confidence interval; HR, hazard ratio.

^aModel 1 was adjusted for demographic characteristics (age, sex, and dialysis vintage).

^bModel 2 was adjusted for cardiovascular risk factors (age, smoking, presence of diabetes, dyslipidemia and history of cardiovascular disease, *residual renal function*).

Supplementary Table 5. Characteristics according to PEW levels in a subgroup of maintenance hemodialysis patients selected for ANP measurement (n= 35)

	Overall population, n=35	No PEW n=19	PEW n=16	P-value
Clinical characteristics				
Sex, male/female	15/20	8/11	7/9	0.92
Age, yrs	55.7[37.7-69.5]	55.8[37.7-67.2]	54.3 [35.8-77.5]	0.49
BMI, kg/m ²	25.4[23.6-29.4]	25.2[24.2-29.6]	25.6[23.1-28.2]	0.19
Dialysis vintage, yrs	1.83[1.08-3.40]	2.14[1.51-4.14]	1.67[0.99-1.99]	0.06
nPCR, g/kg/day	1.03[0.90-1.22]	1.06[0.94-1.24]	0.91[0.72-1.21]	0.08
Dialysis dose, Kt/V	1.70[1.47-2.01]	1.68[1.47-1.75]	1.88[1.46-2.17]	0.08
Predialysis SBP, mmHg	131[117-144]	131[112-159]	131[118-150]	0.23
CV history, %	0	0	0	1.00
Diabetes, %	25	21	31.25	0.49
Smoking, %	29	36.8	18.75	0.24
Hypertension, %	89	84.2	93.75	0.38
Dyslipidemia, %	51	47.3	50	0.88
Causes of nephropathy				
Diabetes, %	20	21	18.75	0.87
Vascular, %	17	21	12.5	0.50
Glomerulopathy, %	23	15.7	31.25	0.28
ADPKD (n)	9	10.5	6.25	0.65
Interstitial nephritis, %	6	5.2	6.25	0.90
Urology, %	11	10.5	12.5	0.85
Unknown, %	14	15.7	12.5	0.83
Laboratory Tests				
ANP, pg/mL	582[401-647]	475[389-570]	708[402-761]	0.04
Log ANP , pg/ml	2.76[2.60-2.81]	2.68[2.59-2.76]	2.85[2.60-2.88]	0.04
NT-ProBNP, ng/L	1341[705-3079]	942[483-1613]	2521[1003-6937]	<0.01
Hemoglobin, g/dL	11.0[10.1-12.1]	11.0[10.1-12.1]	11.1[10-12.2]	0.44
Albumin, g/L	38.4[34.4-41.0]	41[38.8-41.4]	34.25[30.8-36.2]	< 0.01
Prealbumin, mg/L	310[269-390]	380[327-412]	268[225-280]	< 0.01
Creatinine, μmol/L	778[556-955]	881[570-1031]	605[524-802]	0.02
Urea, mmol/L	19.9[15.3-23.5]	22[18.3-23.7]	17.6[12.9-21.0]	0.01
Bicarbonate, mmol/L	21.0[20.0-23.0]	21.0[20.0-23.0]	21[20.0-25.8]	0.29
Calcium, mmol/L	2.12(2.07-2.27)	2.2[2.1-2.4]	2.1[2.0-2.3]	0.13
Phosphorus, mmol/L	1.44[1.08-1.88]	1.5[1.1-1.7]	1.3[1.0-1.9]	0.24
PTH, pmol/L	301[83-367]	306[133-428]	296[42-341]	0.08
25OH Vitamin, nmol/L	32.4[24.4-36.9]	36.4[26.0-40.0]	32.0[20.4-32.8]	0.05
Hemoglobin A1c, %	5.45[5.00-6.25]	5.0[5.0-6.0]	5.7[5.0-7.9]	0.09
Total cholesterol, mmol/L	1.88[1.50-2.18]	1.79[1.52-2.18]	2.10[1.48-2.18]	0.40
Triglycerides, mmol/L	1.37[1.00-2.12]	1.49[1.06-2.65]	1.34[0.99-1.74]	0.16
CRP, mg/L	5.30[2.33-15.5]	5.4[1.7-7.6]	5.2[3.6-20]	0.07
Treatment				
Anti-diabetic therapy, %	20	15.7	25	0.50
Lipid-lowering therapy, %	25	26.3	25	0.93
RAA, %	35	36.8	31.25	0.73
Beta blocking agents, %	25	26.3	31.25	0.75
AAP, %	35	36.8	25	0.45

Data are expressed as medians [IQR]. Differences between groups were tested using Mann & Whitney U tests or Fisher exact tests as appropriate. Abbreviations: AAP, Antiplatelet agents, ADPKD, Autosomal dominant polycystic kidney disease, ANP: atrial natriuretic peptide, BMI, body mass index, CRP, C-reactive protein, CV, cardiovascular, nPCR, normalized protein catabolic rate, NT-proBNP, N-terminal pro-brain natriuretic peptide, PEW, protein-energy wasting, PTH, parathyroid hormone, RAA, Renin-angiotensin-aldosterone system antagonists, SBP, systolic blood pressure, yrs, years.