Inflammation, overhydration and cardiac biomarkers in haemodialysis patients: a longitudinal study

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Abstract

Background. Inflammation, overhydration and elevated cardiac biomarkers are related to outcome in haemodialysis (HD) patients. Here, we explored the relationship between the body composition (BC), inflammation and cardiac biomarker concentrations in HD patients longitudinally.

Methods. A total of 44 HD patients were followed for 6 months. BC was assessed by multifrequency bioimpedance (BIA). Serum concentrations of cardiac troponin T (cTnT), high-sensitive C-reactive protein (hsCRP), brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) were assessed at 2 monthly intervals. The longitudinal data analysis was conducted with a marginal model.

Results. During the follow-up, the parameters describing the BC were highly predictive of both BNP and NT-proBNP and independent of gender, time, hsCRP and cTnT concentrations. The intracellular water (ICW)/body weight (BW) ratio (reflecting malnutrition) exerted a negative effect, whereas the extracellular water (ECW)/BW ratio (reflecting overhydration) had a positive effect on BNP and NT-proBNP concentrations. HsCRP and cTnT concentrations were significantly associated with each other. Furthermore, NT-proBNP concentrations were predictive of cTnT and hsCRP concentrations.

Conclusions. In the present study, we find a significant relation between BIA-derived BC parameters and natriuretic peptide concentrations. This relationship was independent of the cardiac history of the patient and suggests that the natriuretic peptide levels are to some degree modifiable by changing a patient's fluid distribution. Moreover, cTnT, BNP, NT-proBNP and hsCRP were significantly related, showing a complex relation between overhydration, malnutrition, inflammation and cardiac biomarkers in dialysis patients.

Keywords: bioimpedance; haemodialysis; natriuretic peptides; overhydration; troponin

Introduction

In dialysis patients, various risk markers for the vastly increased mortality risk have emerged. Among them, both parameters related to abnormalities in body composition (BC) (fluid distribution) [1–3] and various biochemical markers, such as such C-reactive protein (CRP), cardiac troponin T (cTnT), brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) were shown to be powerful predictors of mortality [4–6].

While the predictive power of these parameters has clearly been shown, they represent different biological processes. High-sensitive C-reactive protein (hsCRP) is thought to reflect inflammation, NT-proBNP concentrations are generally considered to reflect the cardiac wall stress and cTnT concentrations are thought to describe ischaemic cardiac damage. While NT-proBNP and cTnT have proven to be accurate and sensitive markers for assessing heart failure and ischaemic cardiac damage, respectively, their levels might additionally be influenced by a decreased renal clearance in patients with severe renal disease [7–9]. Moreover, cTnT may be elevated due to increased cardiac wall stress leading to micro-ischaemia, as reflected by the relation between left ventricle hypertrophy (LVH) and cTnT concentrations in haemodialysis (HD) patients [10,11]. Finally, elevated cTnT concentrations in dialysis patients have also been associated with inflammation and are possibly related to endothelial damage and an increase in the oxidative stress [12,13]. Hence, for subjects with renal failure, a clear-cut delimitation of the biomarker's individual functions and their apparent interdependence are seemingly more intricate. Empirical evidence so far suggests that their already
known and established biological roles may also be affected by a dysfunctional state of the dialysis patient’s BC status. The parameters describing the BC, such as the amount of intracellular (ICW) or extracellular water (ECW) have been linked to varying underlying causes. ICW is often used to estimate the body cell mass (BCM) and as such, reflects nutritional status, and ECW has been shown to reflect overhydration [14]. Over the years, several approaches using bioelectrical impedance (BIA) measurements in HD patients have been described to accurately assess volume distributions [15,16].

To date, few studies have assessed the relationship between the BC and cardiac and inflammatory biomarkers. In two recent studies, overhydration was found to be related to inflammation, as reflected by a relation between extracellular volume (or inferior caval vein diameter) and CRP concentrations [17,18]. Although the mechanism behind the relation between overhydration and inflammation has not yet been elucidated, an increased passage of endotoxins from the intestines into the blood due to gut oedema might be involved [19]. Another explanation might be a reduction in ‘dry weight’ because of loss of BCM in the inflammatory state, which leads to progressive overhydration if changes in dry weight are not appropriately detected and treated [20,21]. More recently, a relation between overhydration, NT-proBNP and cTnT has been suggested [22]. Mechanistically, an increase in ECW might induce left ventricular dilatation and subsequent increases in NT-proBNP (and cTnT) concentrations.

Focus of the present study is on the relationship between cardiac and inflammation biomarkers and abnormalities in body fluid distribution, as characteristically found in dialysis patients. While a few studies have assessed these interrelations, to the best of our knowledge, no study as yet assessed these relations in a longitudinal way.

Patients and methods

Study design

The study consisted of both a cross-sectional and a longitudinal part. Patients were studied for 6 consecutive months. Blood samples were collected pre-dialysis at the start of the study and subsequently every 2 months for a period of 6 months. Bioimpedance measurements were performed on the same day as blood sampling.

Patients

A cohort of 44 chronic HD patients participated in this 6-month longitudinal study. The study protocol was approved by the medical ethical review committee of the University Hospital Maastricht. All patients provided written informed consent. Exclusion criteria were the presence of a history of cardiovascular disease (CVD), coronary artery bypass grafting (CABG), or suffered from congestive cardiac failure.

Laboratory analysis

CRP concentrations were measured using the CardioPhase hsCRP assay (Dade Behring Inc., Newark, USA). According to the manufacturer’s protocol, the limit of detection (LOD) of the assay was 0.175 mg/L and the coefficient of variation (CV) was <10% for concentrations between 0.5 mg/L and 62 mg/L. HsCRP concentrations >3 mg/L were considered elevated. cTnT was measured on the Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) using a pre-commercial high-sensitive cTnT (hs-cTnT) assay. Precision profiles for this assay were established in our laboratory, and the 99th percentile reference cut-off value was determined in a population of 501 healthy subjects [23]. The LOD for the hs-cTnT assay was established at <0.001 µg/L, and the 10% CV cut-off concentration was set at 0.09 µg/L. The 99th percentile measured at 0.016 µg/L. CRP concentrations above the 99th percentile measured in the healthy reference population were considered elevated (as recommended in recent NACB guidelines [24]). NT-proBNP was measured using the proBNP assay for the Elecsys 2010. According to the package insert, the LOD is 0.6 pmol/L and the CV at 20.7 pmol/L is 3.2% and is 2.3% at 586 pmol/L. Elevated NT-proBNP concentrations were defined as values above the 99th percentile measured in a reference population of 501 healthy subjects (unpublished results). The 99th percentile value was found to be 31.28 pmol/L for the male- and 35.55 pmol/L for the female participants. BNP was measured in plasma with the SHIONORIA BNP immunoradiometric assay (Cis-Bio International, Gif-sur-Yvette, France). This kit, according to its package insert, has a LOD of 2.0 pg/mL and a within- and between-run CV of 2.7% and 4.3% at 22.1 pg/mL and 21.1 pg/mL, respectively (1 pg/mL = 0.289 pmol/L). BNP concentrations above the 95% confidence interval are considered elevated. According to the package insert this value is 18.4 pg/mL (18.6 pg/mL for women and 18.2 pg/mL for men). Blood samples were taken before dialysis, in order to prevent possible influences of the dialysis process itself on the concentrations of the different biomarkers [13,25].

Bioimpedance analysis

BC, described as ECW, and ICW were assessed by multifrequency bioimpedance (BIA) measurements (Xitron 4002 Hydra), as described previously [15,16, 26]. For each BIA measurement pre- and post-dialytic values of ECW and ICW were measured and reported. Additional parameters used to assess the volume and nutritional status were also recorded, among them the ICW:body weight ratio (ICW:BW) and the ECW:body weight ratio (ECW:BW). Furthermore, the phase angle (PA), which is related to the ratio between ICW and ECW, and is thought to represent cellular membrane integrity [27], is recorded. Briefly, the BIA device measures the overall opposition, the impedance, to an alternating and/or direct electrical current. The impedance is the resultant of two components, the resistance and the capacitive reactance. So, the impedance is the angle that can be drawn from the resistance and the reactance. PA is the angle between the impedance and resistance vectors. The resistance represents the restriction to the flow of an electrical current through the body and it is associated with the amount of water present in the tissues. The capacitive reactance represents the opposition encountered by the current as a result of the capacitance function of tissue interfaces and cell membranes [27,28]. Note that the PA thus depends on both the amount of tissue hydration and the amount of intact cell membranes.

In subsequent calculations, we used the BIA-derived parameters that were gathered post-dialysis. This was done because volume state after dialysis is less variable than pre-dialytic values. In addition to bioimpedance analysis, both pre- and post-dialytic blood pressures (taken 5 min after the end of dialysis) were recorded.

Statistics

Data analyses were performed using Statistical Package for Social Sciences (SPSS), Version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).
Inflammation, overhydration and cardiac biomarkers in HD patients

Table 1. Patient characteristics at the start of the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient characteristics at baseline</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 (total range 35–91)</td>
<td>30 (total range 1–102)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>37 (84%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (34%)</td>
<td></td>
</tr>
<tr>
<td>History of ischaemic heart disease</td>
<td>13 (30%)</td>
<td></td>
</tr>
<tr>
<td>History of cardiac failure</td>
<td>13 (30%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (22.2–26.0)</td>
<td>7.0 (1.7–15.9)</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L) (mean ± SD)</td>
<td>7.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>cTnT (µg/L)</td>
<td>0.056 (0.036–0.09)</td>
<td></td>
</tr>
<tr>
<td>BNP (pmol/L)</td>
<td>82.6 (30.1–252.0)</td>
<td>92.2 (55.0–244.8)</td>
</tr>
<tr>
<td>NT-proBNP (pmol/L)</td>
<td>843.8 (362.7–5544.3)</td>
<td>1259.5 (218.5–3808.0)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>63 ± 16</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135 ± 35</td>
<td>133 ± 32</td>
</tr>
<tr>
<td>ICW/BW (L/kg)</td>
<td>0.25 ± 0.05</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>ECW/BW (L/kg)</td>
<td>0.25 ± 0.04</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Phase angle (degrees)</td>
<td>4.97 ± 1.50</td>
<td>4.44 ± 1.24</td>
</tr>
</tbody>
</table>

The biomarker concentrations are displayed as median (IQR) concentrations. Body composition parameters (ICW/BW, ECW/BW and PA) are displayed as mean ± SD.

To accommodate the longitudinal nature of the data and investigate whether the biomarker concentrations changed over time, a marginal model was fit. All outcome parameters (either cardiac or inflammation biomarkers) showed a skewed distribution, the reason for which they were log transformed (ln). Main determinants were the BC parameters and the biomarkers themselves. Multivariable adjustment took place for a number of additional covariates, namely gender, occurrence of an event (hospitalization), diabetes, time (duration of study), cardiac history, time on dialysis, systolic blood pressure and vascular access (native fistula or PTFE graft). Non-significant predictors were eliminated via the backward procedure, except for gender and event, which were always retained in the model, irrespective of their significance. This was done to warrant adjusted effect of the main determinants for possible gender and/or event confounding. Parameters were estimated via the restricted maximum likelihood (REML) and the significance level was set at 5%. For those parameters whose P-values were <0.1, additional likelihood ratio (LR) tests were carried out, equally at the 5%, to determine whether to retain them in the final model.

Results

Patient characteristics at baseline

The patient characteristics at the start of our study are given in Table 1. Our population consisted of 30 male and 14 female patients with an average (±SD) age of 66 (±10.50) years. The 44 patients showed a large amount of cardiovascular complications, 55% of the patients had a history of ischaemic cardiac disease and many of our patients showed elevated biomarker concentrations. At baseline, 95% of the patients showed cTnT concentrations above the AMI cut-off concentration of 0.016 µg/L (23% had cTnT concentrations above 0.1 µg/L). Natriuretic peptide concentrations were also frequently increased and 91% and 98% of our patients had elevated BNP and NT-proBNP concentrations, respectively. In addition, hsCRP concentrations were elevated in 64% of the patients.

Marginal models

Cardiac and inflammatory biomarkers were taken as outcome variables individually and their associations with BC parameters and other biomarkers were tested. For all models, likelihood ratio tests confirmed that the unstructured covariance matrix of observations was the most appropriate. As BNP and NT-proBNP are strongly correlated (and originate from the same precursor), multicollinearity problems would arise, if both were to be fitted into the model together. Therefore, the models were fitted with either BNP or NT-proBNP, but never both of them simultaneously. Results are henceforth mainly shown for NT-proBNP. Similarly, the PA was found to be strongly associated with the ECW/BW and ICW/BW in a linear fashion (linear regression, averaged over the time points, PA = 3.44 + 23.12 ICW/BW – 17 ECW/BW with R² = 0.93). Accordingly, the models were fitted either with the PA only or ECW/BW and ICW/BW, not with the three of them concurrently. Hereafter, the models fitted with the phase angle will be referred to as PA-approach, whereas the models fitted with ECW/BW and ICW/BW are referred to as ICW–ECW approach.

Tables 2–5 present the marginal models’ regression coefficients for each biomarker as outcome variable separately. Table 2, with NT-proBNP as outcome, illustrates the results for both approaches (either PA or ICW–ECW), which yielded similar log likelihoods, had the same variables selected via the backward procedure and comparable estimates for the significant main effect. For parsimonious reasons, only the regression coefficients for the PA approach are presented in the other tables.

Time effect

For virtually all outcome variables, no significant time effect was detected. Thus, no significant time-related trends
Fig. 1. (ln)NT-proBNP concentrations as a function of the phase angle during the 6-month follow-up. Regression lines and correlation coefficients are shown for each of the four visits separately.

Table 3. Regression parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.16</td>
<td>2.84, 1.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.58</td>
<td>0.25, 0.90</td>
<td>0.001</td>
</tr>
<tr>
<td>Event (none)</td>
<td>−0.22</td>
<td>−0.55, 0.12</td>
<td>0.194</td>
</tr>
<tr>
<td>Diabetes</td>
<td>−0.39</td>
<td>−0.70, −0.07</td>
<td>0.017</td>
</tr>
<tr>
<td>(ln) NT-proBNP</td>
<td>0.07</td>
<td>0.014, 0.131</td>
<td>0.016</td>
</tr>
<tr>
<td>(ln) hsCRP</td>
<td>0.06</td>
<td>0.013, 0.113</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Outcome variable ln(cTnT).

were observed in the parameters under investigation. The exception was BNP that seemed to be slightly higher at visit two and four compared to visit one.

BC effect

In general, the BC parameters were highly predictive of both natriuretic peptide levels. As can be seen in Tables 2 and 5, the PA and ICW/BW were shown to exert a negative effect, whereas the ECW had a positive effect on the NT-proBNP and BNP concentrations. The effects of the BC on the BNPs were independent of gender, time, and other biomarker levels. Figure 1 illustrates the relationship between the PA and the NT-proBNP concentrations (regression lines are given for each visit separately). It can be seen that as the PA values increase, ln(NT-proBNP) levels will linearly decrease. The same rate of decrease for all time points, as given by similar regression slopes, indicates that the effect remains constant over all visits.

As can be interpreted from Tables 3 and 4, the parameters describing the BC were not significantly associated with cTnT and hsCRP concentrations, after adjustment for NT-proBNP. However, though omitted, it is noteworthy that, if NT-proBNP was taken out of the multivariable model, strong negative associations between PA and cTnT and hsCRP concentrations were detected (parameter estimates −0.104 and −0.306 with adjusted P-values 0.014 and 0.007, for cTnT and hsCRP, respectively). Similarly, ICW/BW showed a significant negative effect. In contrast, ECW/BW values were neither significantly predictive of cTnT nor hsCRP, even after deletion of NT-proBNP.

Table 4. Regression parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−2.45</td>
<td>−4.29, −0.60</td>
<td>0.010</td>
</tr>
<tr>
<td>Gender</td>
<td>−0.53</td>
<td>−1.25, 0.19</td>
<td>0.142</td>
</tr>
<tr>
<td>Event (none)</td>
<td>0.19</td>
<td>−0.51, 0.89</td>
<td>0.582</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.60</td>
<td>−0.06, 1.27</td>
<td>0.074</td>
</tr>
<tr>
<td>Vascular access</td>
<td>−0.90</td>
<td>−1.60, −0.19</td>
<td>0.014</td>
</tr>
<tr>
<td>(ln) NT-proBNP</td>
<td>0.29</td>
<td>0.12, 0.46</td>
<td>0.001</td>
</tr>
<tr>
<td>(ln) cTnT</td>
<td>0.53</td>
<td>0.18, 0.88</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Outcome variable ln(BNP).

Relations between biomarker concentrations

NT-proBNP levels were significantly predictive of all biomarkers. The opposite did not always apply. For instance, while elevated NT-proBNP concentrations were predictive of hsCRP, elevations in hsCRP were not predictive for NT-proBNP levels. cTnT and hsCRP concentrations were significantly associated with each other, irrespective of the modelling direction (i.e. which one is dependent or independent variable). In addition, cTnT concentrations were predictive of NT-proBNP concentrations, but not for BNP concentrations.

Other covariates

No gender-related differences were observed, except for cTnT, which was significantly lower in the female subjects. NT-proBNP and BNP concentrations were significantly higher in patients who had a history of cardiovascular disease, whereas for cTnT and hsCRP no cardiac history effect was observed (Tables 2–5). The patients who had suffered a clinical event during our study had similar cTnT and hsCRP concentrations as those who did not suffer from an event. We did find that non-event patients had, on average, lower natriuretic peptide levels. Vascular access was only significantly predictive for hsCRP concentrations, which were lowest when vascular access was achieved by PTFE graft. Finally, both higher systolic blood pressure and the
longer time on dialysis were predictive of increased NT-proBNP concentrations.

**Discussion**

The main findings of the present study were the significant relation between the BIA-derived parameters describing BC and natriuretic peptides, and the relation between inflammation and cardiac biomarkers. Earlier studies showed equivocal results with regard to the relation between volume status and (NT-pro)BNP concentrations [22,29]. This is likely due to the fact that elevated concentrations of BNP indirectly reflect overhydration due to left ventricular dilatation, whereas intrinsic cardiac disease may have the same effect. In the present study, higher natriuretic peptide concentrations (on the log scale) were related to malnutrition (negative association with ICW/BW) and/or overhydration (positive association with ECW/BW), additively and independently from each other. Importantly, this relation was independent of the cardiac history of the patient. Nonetheless, patients with a history of cardiac disease, patients with higher systolic blood pressure, as well as those who have been on dialysis for a longer period of time, had on average higher NT-proBNP concentrations. Taken together, our data suggest that elevations in NT-proBNP can be explained by abnormalities in a patient’s BC and/or by the presence of cardiac disease. This is in agreement with earlier data regarding ANP [30] in dialysis patients, in whom, after correction for overhydration, ANP concentrations only decreased in patients without cardiac disease but remained largely elevated in patients with intrinsic cardiac disease.

The PA is a complex variable due to its composite nature. On the one hand, it is highly correlated with the ratio between ICW and ECW. On the other, it can also be expressed as a linear function of both parameters, instead of only their ratio \( R^2 \sim 0.9 \). In this sense, lower PA levels may be the result of malnutrition and overhydration, or a combination of both simultaneously. The present findings suggest that the predictive power of PA alone on cardiac and inflammation biomarkers was comparable to that of ICW and ECW, once taken together. As such, they buttress the value of the PA as a general marker for illness in dialysis patients. Previous studies showed a relation between the PA and mortality in various patient groups, including dialysis patients [3]. Herein, the PA was independently related to the BNP, NT-proBNP, as well as to cTnT and hsCRP concentrations (under the premise that it is not corrected for NT-proBNP).

cTnT and hsCRP were found to be related to the PA and the ICW/BW ratio, but not to the ECW/BW ratio suggesting that malnutrition, rather than overhydration is the most likely reason underlying their increased concentrations. The fact that the statistical significance of these associations disappears after adjustment for NT-proBNP might be the result of a statistical artefact, resulting from the strong linear association between the BC and the natriuretic peptides, making the former redundant. We do, however, not rule out a potential role for NT-proBNP in mediating a BC effect of cTnT and hsCRP. At any rate, cTnT and NT-proBNP were significantly related, suggesting a relation between increased cardiac wall stress and micro-ischaemia [31]. Of note, increased cTnT concentrations, may occur even in the absence of coronary artery disease in dialysis patients [6,32–34], but were found to be related to left ventricular hypertrophy in dialysis patients [35]. Left ventricular hypertrophy may increase the cardiac wall stress and thus the oxygen demand of the ventricular wall [36].

Throughout the present study, the biomarker concentrations, as well as the BC parameters, remained relatively stable over time, nevertheless the natriuretic peptide levels were highly correlated with the BC parameters. This suggests that the natriuretic peptide concentrations are, to some degree, modifiable by changing the BC status. Based on current knowledge, it appears prudent to perform detailed assessment of the BC, as well as cardiac evaluation, in patients with clearly elevated NT-proBNP concentrations. Additionally, due to the effects of a reduced renal clearance per se on NT-proBNP concentrations [37], it is of great importance that appropriate cut-off concentrations for NT-proBNP concentrations in dialysis patients are developed, as was recently attempted by David et al. [38]. At any rate, it would appear sensible to assess cardiac biomarker concentrations at regular points in time, which would serve both as a baseline level, and as a possible tool for intervention and increased clinical vigilance in patients with largely increased values or variations. Finally, this study also confirms the usefulness of the PA as a general indicator for illness in dialysis patients.

A drawback of the study is the relatively small number of patients, and the absence of echocardiographic data. Strong points of the study are the longitudinal design, and the presence of detailed measurements of volume status.

In conclusion, in this longitudinal study, we found a significant relation between natriuretic peptides and the BC assessed by BIA. Also, cTnT, natriuretic peptides and hsCRP were significantly related, showing a complex relation between overhydration, malnutrition, inflammation and cardiac biomarkers in HD patients.

Acknowledgements. We would like to thank Dr Etienne Michielsen for his help during the initial conception of this study and for providing us with a convenient database, in which patient data could easily be entered and retrieved. We would also like to thank Roche Diagnostics for providing the hs-cTnT reagents used in this study.

Conflict of interest statement. None declared.

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16. Lowbeer C, Otto-Berger A, Gustafsson SA et al. Increased cardiac troponin T and endothelin-1 concentrations in dialysis patients may indicate heart disease [In Process Citation]. *Nephrol Dial Transplant* 1999; 14: 1948–1955