Increased cardiac troponin T and endothelin-1 concentrations in dialysis patients may indicate heart disease

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Abstract
Background. Cardiac troponin T (cTnT) is a highly sensitive marker for the detection of myocardial damage. However, patients maintained on chronic dialysis often have increased serum cTnT concentrations without evidence of acute myocardial injury. The reason for this is unclear. In chronic haemodialysis patients, elevated plasma concentrations of big endothelin-1 (big ET-1) and endothelin-1 (ET-1) have been reported which may be associated with ischaemic heart disease. The aim of the present study was to investigate possible associations between cTnT, big ET-1, ET-1, other cardiac markers and cardiac disease in dialysis patients.

Methods. Thirty-six haemodialysis (HD) patients and 26 peritoneal dialysis (PD) patients without symptoms of acute myocardial ischaemia were investigated. In all patients, serum concentrations of cTnT (2nd generation ELISA), cardiac troponin I (TnI) (Opus, Behring), creatine kinase MB (CKMB) mass and creatine kinase (CK) were determined, in HD patients before and after dialysis. Additionally, in HD patients, plasma ET-1 and big ET-1 were measured. In 27 HD patients, left ventricular mass index (LVMI) was determined. Patients with ischaemic heart disease (IHD) were compared with non-IHD patients.

Results. Serum cTnT was elevated (≥0.10 µg/l) in 20 of 36 HD patients and in eight of 26 PD patients. cTnI was elevated (≥0.5 µg/l) in four of 62 dialysis patients. HD + PD patients with IHD showed higher cTnT than HD + PD patients without IHD, and ET-1 concentrations were higher in HD patients with than without IHD. In HD patients, there was a positive correlation between cTnT and big ET-1. In HD patients with left ventricular hypertrophy (LVH), serum cTnT, CKMB mass and post-dialysis plasma big ET-1 were higher than in patients with normal LVMI. Furthermore there was a positive correlation between cTnT levels and LVMI.

Conclusion. These findings suggest that circulating cTnT may reflect left ventricular hypertrophy and/or myocardial ischaemia in dialysis patients, and indicate that ET-1 and big ET-1 might be associated with these conditions.

Key words: renal failure; troponin; endothelin; cardiac markers; creatine kinase isoenzymes; left ventricular hypertrophy

Introduction
Cardiac disease is the leading cause of morbidity and mortality in patients with end-stage renal disease (ESRD). Myocardial infarction accounts for 30–50% of cardiac deaths [1,2]. Ischaemic heart disease appears to be only partially due to coronary atherosclerosis since in a group of dialysis patients with symptomatic ischaemic heart disease more than 40% were shown to have patent coronary arteries [3]. In these patients, ventricular hypertrophy, interstitial myocardial fibrosis and endothelial dysfunction, which all limit the coronary flow reserve available to the myocardium during stress and which are frequent findings in ESRD, may play a pathophysiological role.

The diagnosis of ischaemic myocardial injury is difficult in the ESRD population. Several studies have demonstrated that the older cardiac marker CK and the more cardiac specific isoform CKMB are frequently elevated or indeterminate in ESRD patients without other signs of acute ischaemic heart disease [4,5].

The cardiac muscle isoforms of troponin T (cTnT) and troponin I (cTnI) belonging to the myofibrillar troponin complex have been claimed to be superior markers of myocardial injury. Several studies have shown that the cardiac troponin T ELISA is a very sensitive method for the detection of myocardial damage and cTnT is a powerful, independent risk
marker in patients with acute myocardial ischaemia [6]. In the first generation assay there was a slight cross-reactivity with skeletal muscle troponin T but the second generation cardiac troponin T ELISA shows no such cross-reactivity [7].

However, increased serum concentrations of cTnT and, to a much lesser extent, of cTnI have been reported in ESRD patients without any signs of acute myocardial ischaemia [8]. This problem has previously been discussed by Haller et al. [9]. The mechanism of this troponin elevation is unclear. It is possible that cTnT is so sensitive that it detects subclinical myocardial cell injury or myocardial remodelling in the development of left ventricular hypertrophy whereas the cTnI assays are less sensitive. On the other hand, the elevated serum concentrations of cTnI could also originate from diseased skeletal muscle.

Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor peptide known to date which is produced by conversion of the propeptide big ET-1. Increased plasma levels of ET-1 and big ET-1 have been demonstrated in states of myocardial ischaemia and heart failure in humans [10,11]. ET-1 and big ET-1 have also been shown to induce a marked reduction of coronary blood flow in healthy humans [12]. Further, ET-1 is assumed to play a role in the development of coronary atherosclerosis and left ventricular hypertrophy [13,14]. Increased plasma levels of ET-1 and big ET-1 have been found in ESRD patients [15] and may play a role in the development of cardiac disease in this patient group.

In order to investigate the relationship between serum concentrations of cTnT, other cardiac markers and left ventricular hypertrophy/ischaemic heart disease in ESRD, we measured serum concentrations of cTnT, cTnI, CK and CKMB mass in chronic haemodialysis and peritoneal dialysis patients without acute cardiac symptoms. Additionally, in haemodialysis patients, plasma levels of ET-1 and big ET-1 were determined and electrocardiography (ECG) and echocardiography was performed.

The aim of the study was to investigate possible associations between concentrations of cTnT, other cardiac markers, big ET-1, ET-1, and cardiac disease in dialysis patients.

Subjects and methods

Patients and protocol

Sixty-two chronic dialysis patients (28 men, 34 women) without symptoms of acute myocardial ischaemia were studied. Thirty-six patients (age: median 74, range 29–88 years) were treated with haemodialysis (HD) and 26 patients (age: median 58, range 32–80 years) with peritoneal dialysis (PD). Eighteen patients (13 HD, 5 PD) had chronic ischaemic heart disease (IHD), which was defined as a history of angina pectoris and/or previous myocardial infarction. Fourteen patients (9 HD, 5 PD) had diabetes mellitus (DM). Six patients (4 HD, 2 PD) had IHD and DM. In all patients, serum concentrations of cTnT, cTnI, CKMB mass, CK and creatinine were determined, in HD patients before the start and at the end of a routine haemodialysis treatment. Additionally, in the HD patients, plasma levels of ET-1 and big ET-1 were measured before and after dialysis. In HD patients 12-lead ECG and two-dimensional echocardiography were performed within 2 h after the end of the haemodialysis. In 27 randomly selected HD patients left ventricular mass index (LVMI) was determined using the Penn-cube formula (16). Left ventricular hypertrophy (LVH) was defined as LVMI > 125 g/m² [17]. The study protocol was approved by the local ethics committee and the subjects were informed of the purpose of the study before giving their voluntary consent.

Biochemical assays

For analysis of serum from PD patients, peripheral venous blood was collected in tubes without additives. In HD patients, blood samples were taken from the venous side of the arteriovenous fistula. Tubes with EDTA were used for the preparation of plasma and tubes without additives for the preparation of serum. Specimens were centrifuged and serum or plasma was aliquoted for subsequent analysis. Serum CKMB mass and CK were determined immediately. Serum for cTnT was stored at −20 °C and analysed within 24 h. Serum specimens for cTnI and creatinine were stored at −70 °C and analysed in a batch manner.

Samples for ET-1 and big ET-1 were immediately placed on ice until centrifuged at 4 °C. Plasma was stored at −70 °C until analysed by radioimmunoassay (RIA) technique. Plasma aliquots (1 ml) were extracted with acid ethanol and dried under a nitrogen stream. For determination of big ET-1, big ET-1 antiserum (B6) and 125I-labelled big ET-1 (Amersham, UK) were used. The detection limit was 0.78 fmol per tube. Expressing the big ET-1 value as 100%, the cross-reactivity of the used antiserum (B6) was < 0.007% for ET-1 (1–21), < 0.03% for ET-2, < 0.03% for ET-3 and 35% for big ET-1 fragment (22–38). The intra-assay variation was 5.6% at a plasma concentration of 50 pmol/l [18]. For determination of ET-1, rabbit ET-1 antiserum (E1), which was diluted to give a specific binding of 35–40%, and 125I-labelled ET-1 (Amersham, UK) were used. The detection limit for the assay was 0.39 fmol per tube. The cross-reactivity for the E1 antiserum when the ET-1 (1–21) value is expressed as 100% was: ET-1 (16–21), 16%; ET-2, 27%; ET-3, 8%; and big ET-1, 0.45%. No cross-reactivity with big ET-1 (22–38) was observed at concentrations up to 1 pmol/l. The intra- and inter-assay variations were 6 and 14%, respectively [18].

Cardiac TnT was determined with the second generation troponin T ELISA (Enzymun-Test Troponin-T) on ES 300 system (Boehringer Mannheim GmbH, Germany). This assay uses the two cardiac-specific monoclonal antibodies M11.7 and M7 [7]. cTnT was measured with the Opus Troponin I assay (Behring Diagnostics, Westwood, MA, USA) performed on the Opus analyser. CKMB mass was measured by Microparticle Enzyme Immunoassay (MEIA) technology with AxSYM system (Abbott Diagnostics, Abbott Park, IL, USA). A relative index (% CKMB) for CKMB mass defined as [CKMB mass (µg/l)]/CK (U/l) × 100 was calculated. Serum CK and creatinine were measured with dry chemistry using Ektachem 950ICR System (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY, USA). The total imprecisions (CV%) of the assays for cardiac markers were: 8.0% at 0.16 µg/l for cTnT; 8.4% at 7.1 µg/l for cTnI; 8.3% at 3.8 µg/l for CKMB mass, and 8.6% at 192 U/l for the CK assay.
Values exceeding the upper reference limits were considered elevated: cTnT \( \geq 0.10 \mu g/l \), cTnI \( \geq 0.5 \mu g/l \), CKMB mass \( > 5 \mu g/l \), CK (males) \( > 14 \mu g/l \), and CK (females) \( > 14 \mu g/l \). The discrimination limits for acute myocardial infarction (AMI) were cTnT \( \geq 0.20 \mu g/l \), cTnI \( \geq 0.5 \mu g/l \), CKMB mass \( > 15 \mu g/l \) and %CKMB \( > 5\% \).

**Statistical analysis**

Data are given as median [interquartile range]. Non-parametric tests were used. Correlations between variables were tested by Spearman rank correlation test (\( \rho \) = correlation coefficient). The Mann-Whitney U-test was employed to compare unpaired data between groups, and the Wilcoxon rank sum test was used to compare paired data. The 97.5th percentiles for cTnI, cTnT, CKMB mass and %CKMB were determined. If not otherwise stated, calculations for haemodialysis patients were made by using data from pre-dialysis samples. Statistical significance was defined as \( P < 0.05 \).

**Results**

**HD + PD patients**

In HD + PD taken as one group (\( n = 62 \)), cTnT concentration was \( > 0.20 \) in 15 of 62 (24\%) and \( \geq 0.50 \mu g/l \) in 4 of 62 (6\%) patients. None of the dialysis patients had CKMB mass \( > 8 \mu g/l \). The 97.5th percentiles (range) were: cTnT 0.54 \( \mu g/l \) (\( < 0.02–1.6 \mu g/l \)), cTnI 1.3 \( \mu g/l \) (\( < 0.5–5.7 \mu g/l \)), CKMB mass 8 \( \mu g/l \) (\( < 1–8 \mu g/l \)) and %CKMB 28\% (\( 1–44\% \)).

Compared to dialysis patients without IHD (\( n = 44 \)), dialysis patients with IHD (\( n = 18 \)) had higher serum concentrations of cTnT (0.16 \[ 0.07–0.31 \] \( v s \) 0.05 \( [< 0.04–0.15] \); \( P = 0.004 \)) (Figure 1 A). %CKMB (11 \( [6–17] \) \( v s \) 5 \( [2–10] \); \( P = 0.02 \)) and lower CK (\( < 21 \) \( [21–66] \); \( P = 0.0003 \)). The 18 patients with IHD had a higher percentage of elevated cTnT concentrations (\( \geq 0.10 \mu g/l \)), than did the 44 patients without IHD, 67\% vs 36\%.

In patients with DM without IHD (\( n = 8 \)), cTnT and CKMB mass were significantly higher (\( P = 0.003 \) and \( P = 0.001 \), respectively) than in patients without DM and IHD (\( n = 36 \)). Serum concentrations of cTnT and CK did not differ in dialysis patients with and without DM.

Male dialysis patients demonstrated higher serum concentrations of cTnT and CKMB mass than female dialysis patients (\( P = 0.05 \) and \( P = 0.01 \), respectively). In dialysis patients with ischaemic heart disease, there was no difference in serum cTnT between males (\( n = 10 \)) and females (\( n = 8 \)). Age and serum creatinine showed no differences between gender.

Four patients (3 HD, 1 PD) had elevated cTnI concentrations of 0.9, 1.1, 1.3, and 5.7 \( \mu g/l \). The corresponding cTnT was 0.46, 0.12, 0.14 and 0.10 \( \mu g/l \), respectively. CKMB mass was \( < 5 \mu g/l \) in these patients. Two of them had IHD (cTnI 1.1 and 0.9 \( \mu g/l \)) and one had DM (cTnI 1.3 \( \mu g/l \)).

A positive correlation was found between serum concentrations of cTnT and the following variables: CKMB mass, %CKMB and age, whereas a negative correlation was found between cTnT and CK, and cTnT and creatinine (Table 1).

**HD patients**

Serum concentrations of the cardiac markers, together with LVMI and plasma concentrations of ET-1 and big ET-1 are presented in Table 2. None of the biochemical markers of myocardial damage showed any significant difference between pre- and post-dialysis samples. The number of patients with elevated cardiac markers and LVMI, are shown in Table 3.

None of the patients had electrocardiographic signs of acute myocardial infarction.

In 27 patients LVMI was determined. In 18 of these 27 (72\%) HD patients, left ventricular hypertrophy (LVMI \( > 125 \) g/m\(^2\)) was noted. In these patients serum concentrations of cTnT and CKMB mass were significantly higher than in HD patients with normal LVMI (Figure 2). Eleven of 13 patients with serum cTnT \( \geq 0.10 \mu g/l \) had left ventricular hypertrophy. All of five patients with heart valve disease had serum cTnT \( \geq 0.10 \mu g/l \). In the 18 patients with LVH, 61\% had elevated serum cTnT, whereas 22\% of the 9 patients without LVH had elevated cTnT concentrations. Plasma big ET-1 post-dialysis concentration was higher in patients with LVH than in patients without LVH (\( P = 0.01 \)).

Moreover there was a significant correlation between LVMI and cTnT (\( \rho = 0.40 \), \( P = 0.04 \)), LVMI and CKMB mass (\( \rho = 0.57 \), \( P = 0.004 \)), LVMI and %CKMB (\( \rho = 0.52 \), \( P = 0.008 \)) and between LVMI and big ET-1 post-dialysis (\( \rho = 0.45 \), \( P = 0.03 \)). LVMI did not correlate with CK, cTnI, creatinine or age.

In HD patients with IHD (\( n = 13 \)), significantly higher levels of ET-1 (Figure 1B) and lower levels of CK (\( < 21 \) \( [21–36] \); \( P = 0.002 \)) were found than in patients without IHD (\( n = 23 \)).

Significant correlations between cTnT, ET-1, big ET-1 and other data are summarized in Table 1. Serum cTnT did not correlate with cTnI or CK.

**PD patients**

The concentrations of the markers of myocardial damage are presented in Table 2. The number of patients with elevated cardiac markers are shown in Table 3. Compared to PD patients without IHD (\( n = 21 \)), PD patients with IHD (\( n = 5 \)) had higher serum concentrations of cTnT (0.15 \[ 0.08–0.46 \] \( v s \) \( < 0.04 \[ 0.04–0.08 \]; \( P = 0.01 \)) and higher %CKMB (11 \[ 6–11 \] vs 3 \[ 2–7 \]; \( P = 0.03 \)). The other markers of myocardial damage; cTnI, CKMB mass and CK, did not differ in PD patients with and without IHD.

Correlations between serum cTnT and other data are summarized in Table 1.

**Discussion**

The main findings in the present study were that, there was an association between serum cTnT and LVH and...
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**Fig. 1.** A. Serum concentrations of cTnT in haemodialysis + peritoneal dialysis (HD + PD) patients without ischaemic heart disease ($n=44$) and in HD + PD patients with ischaemic heart disease ($n=18$). B. Plasma ET-1 concentrations in haemodialysis patients without ischaemic heart disease ($n=23$) and in haemodialysis patients with ischaemic heart disease ($n=13$). No IHD: without ischaemic heart disease. IHD: with ischaemic heart disease. Column: median. Whisker: interquartile range.

**Table 1.** Correlations between serum cTnT, plasma ET-1, plasma big ET-1 and other data in dialysis patients

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Compared data</th>
<th>Correlation coefficient</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD patients ($n=36$)</td>
<td>cTnT and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.42</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>CKMB</td>
<td>0.53</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>big ET-1</td>
<td>0.45</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>%CKMB</td>
<td>0.48</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>LVMIC ($n=27$)</td>
<td>0.40</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>ET-1 and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK</td>
<td>−0.38</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>big ET-1 and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%CKMB</td>
<td>0.48</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>LVMIC ($n=27$)</td>
<td>0.45†</td>
<td>*</td>
</tr>
<tr>
<td>PD patients ($n=26$)</td>
<td>cTnT and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.43</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>CKMB</td>
<td>0.66</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>−0.40</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>%CKMB</td>
<td>0.67</td>
<td>**</td>
</tr>
<tr>
<td>HD + PD patients ($n=62$)</td>
<td>cTnT and:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.48</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>CKMB</td>
<td>0.57</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>CK</td>
<td>−0.33</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>−0.29</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>%CKMB</td>
<td>0.56</td>
<td>**</td>
</tr>
</tbody>
</table>

*HD: haemodialysis, PD: peritoneal dialysis, "LVMIC: left ventricular mass index. †Correlation between big ET-1 post-dialysis and LVMIC.

*P<0.05, **P<0.01, ***P<0.001. ET-1 and big ET-1 was only analysed in HD patients.

between serum cTnT and IHD in dialysis patients without acute myocardial infarction. Furthermore HD patients with IHD had higher plasma ET-1 than patients without IHD and there was a correlation between serum cTnT and plasma big ET-1 in HD patients.

The diagnosis of myocardial ischaemia is difficult in ESRD patients since they often demonstrate abnormal baseline ECGs and often are not able to perform adequate exercise tests due to limited exercise tolerance. Symptoms may be masked by underlying diseases, and peripheral neuropathies may alter the perception of chest pain. Therefore the use of reliable biochemical markers for the detection of myocardial damage is essential in these patients. Since serum baseline levels of CKMB can be elevated in ESRD without clinical signs of myocardial ischaemia [19], more specific markers are in great demand.
Table 2. Serum concentrations of markers of myocardial damage, plasma concentrations of ET-1 and big ET-1, and left ventricular mass index (LVMI) in haemodialysis (HD) and peritoneal dialysis (PD) patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HD patients (n = 36)</th>
<th>PD patients (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT (µg/l)</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>cTnI (µg/l)</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>CKMB (µg/l)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CK (U/l)</td>
<td>&lt;21</td>
<td>&lt;21–30</td>
</tr>
<tr>
<td>%CKMB (%)</td>
<td>10</td>
<td>4–17</td>
</tr>
<tr>
<td>ET-1 (pmol/l)</td>
<td>6.4</td>
<td>4.8–7.0</td>
</tr>
<tr>
<td>Big ET-1 (pmol/l)</td>
<td>14.2</td>
<td>11.4–16.2</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>n=27</td>
<td>142</td>
</tr>
</tbody>
</table>

%CKMB: [CKMB (µg/l)/CK (U/l)] × 100. *P < 0.05 and ***P < 0.001 for significant difference between HD- and PD patients. Data are given as median and interquartile range.

Table 3. Number of haemodialysis (HD) patients and peritoneal dialysis (PD) patients with elevated serum concentrations of markers of myocardial damage, and left ventricular mass index (LVMI)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value*</th>
<th>HD patients</th>
<th>PD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT</td>
<td>≥0.10</td>
<td>20/36 (56%)</td>
<td>8/26 (31%)</td>
</tr>
<tr>
<td>cTnI</td>
<td>≥0.5</td>
<td>3/36 (8%)</td>
<td>1/26 (4%)</td>
</tr>
<tr>
<td>CKMB</td>
<td>≥5 µg/l</td>
<td>3/36 (8%)</td>
<td>2/26 (8%)</td>
</tr>
<tr>
<td>CK</td>
<td>m≥174 U/l</td>
<td>0/36 (0%)</td>
<td>2/26 (8%)</td>
</tr>
<tr>
<td>%CKMB</td>
<td>&gt;5%</td>
<td>25/36 (70%)</td>
<td>11/26 (42%)</td>
</tr>
<tr>
<td>LVMI</td>
<td>&gt;125 g/m²</td>
<td>18/27 (72%)</td>
<td></td>
</tr>
</tbody>
</table>

*Elevated values were defined as values exceeding the upper reference limits.

Two proteins of the troponin complex, TnT and TnI, have been regarded as ideal serodiagnostic markers for myocardial damage since cardiac and extracardiac tissues have been assumed to express specific isoforms. The troponin T ELISA is more specific than the CKMB mass assays. Furthermore, cTnT is very sensitive for the detection of minor myocardial damage (MMD) and provides prognostic information in patients with unstable coronary artery disease [20]. However, in ESRD patients frequent and unexplained increases of serum cTnT [21] have raised questions about the cardiac specificity of this marker in patients with renal failure.

Our findings of increased serum cTnT with the second generation ELISA in 20 of 36 (56%) HD patients are consistent with other recent findings of a

Fig. 2. Serum concentrations of cTnT and CKMB mass in haemodialysis patients with normal left ventricular dimensions (n=9) and in haemodialysis patients with left ventricular hypertrophy (LVH) (n=18). Column: median. Whisker: interquartile range.
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High frequency of increased serum cTnT in ESRD patients without symptoms of acute myocardial ischaemia [22]. Furthermore, we showed that also PD patients have a high percentage (31%) of elevated serum cTnT.

The reasons for the cTnT elevations in renal disease are still unclear. Like Frankel et al. [23], we found no difference between cTnT before and after haemodialysis. This eliminates the possibility that a cross-reacting dialysable substance caused the cTnT elevation. In accordance with several previous reports, we did not find any positive correlation between serum cTnT and serum creatinine. On the contrary, we found a negative correlation between these two analytes. This could be explained by a lower muscle mass in the older and severely ill patients in our study population. This was indicated by a significant negative correlation between age and creatinine (data not shown).

Extracardiac expression of cTnT in ESRD patients is controversial. McLaurin et al. reported evidence of cTnT expression in skeletal muscle of dialysis patients [24]. However, they did not use the same antibodies (M11.7 and M7) that are used in the second generation cTnT ELISA. On the contrary, Haller et al., using reverse transcription-PCR and immunoblot, immunofluorescence methods, found no evidence of cTnT expression in skeletal muscle biopsies of ESRD patients [25]. Furthermore Ricciuti et al. showed that cardiac TnT isoforms expressed in renal diseased skeletal muscle will not cause false-positive results by the second generation cTnT assay [26]. Therefore it is most likely that serum cTnT in ESRD patients, as measured by the assay used in our study, originates from the heart.

In the present study we showed an association between serum cTnT and cardiac disease in dialysis patients without clinical or electrocardiographic signs of acute myocardial infarction. One explanation for this could be a high sensitivity of cTnT to detect subclinical myocardial injury and/or remodelling of a hypertrophic left ventricle in dialysis patients. In the present study 72% of the HD patients had left ventricular hypertrophy, which may cause an increased susceptibility of the myocardium to ischaemia. These patients had higher serum cTnT and CKMB mass than the patients with normal left ventricular dimensions. We also found a correlation between cTnT and LVMI in HD patients. To our knowledge this has not been shown before. Furthermore, in HD + PD patients without clinical evidence of acute myocardial ischaemia, those with a history of ischaemic heart disease showed higher serum cTnT than patients without IHD. The latter finding is in accordance with Haller et al. who reported a correlation between the plasma cTnT concentration and indicators of coronary artery disease in ESRD patients [25].

In hypertrophy more ultrastructural components are synthesized with increased synthesis of enzymes and increased number and size of the cardiac muscle fibres. This may lead to an increased leakage of cTnT and other structural proteins from the cells. Furthermore the membranes of injured myocytes may lose their integrity and allow the exposure of intracellular proteins to the extracellular environment. This has been discussed by Missov et al. who found increased serum troponin I in patients with severe congestive heart failure when using an assay with very high analytical sensitivity [27]. It has also been shown experimentally that cTnT concentration increases in rat left ventricular after trophic stimuli and pressure overload [28]. Furthermore Haller et al. reported a high prevalence of concentric left ventricular hypertrophy in a haemodialysis population with increased cTnT levels [9]. Thus cTnT may be an early marker of cardiac hypertrophy.

Another novel finding was the positive correlation between plasma big ET-1 and serum cTnT. Although it does not prove causal relationship, it might strengthen the hypothesis that endothelin is associated with cardiac disease in dialysis patients.

In the present study, there was a correlation between big ET-1 levels post-dialysis and LVMI in HD patients, and HD patients with a history of IHD showed higher ET-1 concentrations than HD patients without IHD. The latter finding is in accordance with a recent study investigating 53 HD patients which showed that haemodialysis patients with IHD had higher plasma concentrations of ET-1 and big ET-1 than haemodialysis patients without IHD [29]. The elevated plasma concentrations of ET-1, which were noted in ischaemic heart disease, may be due to episodes of ischaemia which is known to stimulate ET synthesis [30]. On the other hand, ET-1 may cause myocardial ischaemia since Pernow et al. demonstrated that exogenous ET-1 elicited coronary vasoconstriction in healthy humans [12]. In addition, ET-1 has been associated with the development of left ventricular hypertrophy [31] and Demuth et al. have demonstrated a significant positive correlation between elevated plasma ET concentration and an increased LV mass in ESRD patients [32].

Left ventricular hypertrophy may be a reflection of arterial hypertension and blood pressure per se might influence ET-1 and big ET-1. However, in the present study mean arterial blood pressure in HD patients post-dialysis did not correlate with plasma ET-1, big ET-1, serum cTnT or with CKMB mass. The correlation between blood pressure and LVMI showed a trend ($P = 0.07$) (data not shown).

Our findings of higher serum cTnT concentrations in diabetic dialysis patients without IHD than in patients without diabetes mellitus and IHD, are consistent with Ooi et al., who reported a high percentage (58%) of increased cTnT in haemodialysed patients with diabetes mellitus [33]. The authors speculate that advanced glycosylation end-products could induce cTnT expression in noncardiac cells and affect membrane integrity. However, diabetic patients are at high risk of developing ischaemic heart disease, which may be present but undiagnosed in these patients. We found that not only serum cTnT but also serum CKMB mass was higher in patients with diabetes.
Cardiac TnI has been claimed to be a more specific marker for myocardial injury than cTnT in serum from patients with chronic renal failure [8]. The explanation for this could be the use of different cut-off values for the markers, and the higher sensitivity of the cTnT assay. The analytical sensitivity for the second generation cTnT ELISA is 0.02 μg/l and for the Opus troponin I assay 0.5 μg/l. When we used the same discrimination limit (≥0.5 μg/l) for cTnT and cTnI, there were no differences in specificities for the diagnosis of AMI in our study (i.e. 4 of 62 false positive with both markers). Furthermore the 97.5th percentile was higher for cTnI than for cTnT. Therefore we argue that the specificity of cTnI has to be proven with more sensitive cTnI assays. It seems that slightly elevated serum levels of cTnT and to a much lesser extent cTnI and CKMB mass, can be expected in ESRD patients without evidence of acute myocardial infarction. The prognostic importance of these elevations should be further investigated. Although not specific for AMI, elevated serum cTnI may be valuable for identifying dialysis patients at high risk for future cardiac events.

All 62 patients had serum CKMB mass ≤8 μg/l. Thus none of the patients had CKMB mass >10 μg/l, a widely used cut-off for the diagnosis of AMI. %CKMB was elevated (>5%) in 70% of HD patients and 42% of PD patients which makes it inapplicable for the diagnosis of AMI in these patients. The high %CKMB could in some cases be explained by elevated serum CKMB mass, but the main reason was the markedly low serum CK activity in many dialysis patients. Low CK may reflect small muscle mass, sedentary lifestyle, or both. However, low CK could also be a marker of oxidative stress since in vivo loss of CK activity due to extracellular glutathione depletion has been reported [34]. Interestingly, an increased oxidative stress with decreased plasma glutathione peroxidase activity has been reported not only in haemodialysis patients, but also in peritoneal dialysis patients [35].

In summary, serum cTnT was higher in dialysis patients with IHD and in haemodialysis patients with LVAH than in patients without these diseases. There was a positive correlation between serum cTnT and LVMI in HD patients. Not only HD patients but also PD patients had a high percentage of elevated serum cTnT. Plasma ET-1 was higher in HD patients with than without IHD and there was a positive correlation between plasma big ET-1 and serum cTnT. In conclusion, these findings suggest that circulating cTnT may reflect left ventricular hypertrophy and/or subclinical myocardial cell damage in dialysis patients and support the hypothesis that cTnT in serum from dialysis patients originates from the heart.

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References
Increased cardiac troponin T and endothelin-1 in dialysis patients


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