Cardiac troponin T in patients with end-stage renal disease: absence of expression in truncal skeletal muscle

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In patients with end-stage renal disease (ESRD), the serum concentration of cardiac troponin T (cTnT) may be increased without cardiac ischemia. One reason for this unexplained increase could be the extracardiac expression of cTnT. However, truncal skeletal muscle biopsies of five patients with ESRD showed no evidence of the expression of either cTnT mRNA (reverse transcription-PCR) or protein (immunoblot, immunofluorescence). We also measured the serum concentration of cTnT in 97 patients with ESRD. The serum cTnT concentration determined in both first and second generation cTnT assays was significantly lower $P < 0.01$ in patients with a low cardiac risk than in patients with positive indicators of coronary artery disease. The correlation between cTnT and indicators of coronary artery disease is consistent with the hypothesis that cTnT in the serum of patients with ESRD originates from the heart.

Cardiovascular complications are the most important cause of morbidity and mortality in patients with end-stage renal disease (ESRD)3 (1, 2). The diagnosis and risk stratification of coronary artery disease (CAD) are key issues in the clinical management of these patients (3).

Recently the myofibrillar proteins of the troponin complex (4) have been introduced as novel serodiagnostic markers of myocardial cell injury (5, 6). The high concentration in the myocardium and the tissue-specific expression of the cardiac troponins (7–11) are the basis for the excellent sensitivity and specificity of the respective immunoassays in a variety of clinical settings, including minor cardiac muscle damage (12, 13). In patients with renal failure, the diagnostic utility of the cardiac troponins has been challenged by the observation that both troponin T (14–20) and troponin I (17–20) may be increased in the serum of uremic patients without clinical evidence of acute cardiac ischemia. The cause of the increased serum concentration of cardiac troponins in patients with ESRD is not clear. Because this increased serum troponin concentration is particularly prominent for cardiac troponin T (cTnT), it has been suggested that this may reflect an altered regulation of tissue-specific cardiac troponin expression that leads to the expression of cTnT in skeletal muscle. Indeed, such an extracardiac expression of cTnT has been reported in diseased/regenerating skeletal muscle (21–23). Therefore, it has been speculated that the increased concentration of cTnT in the serum of some dialysis patients without evidence of cardiac ischemia results from the extracardiac expression of cTnT in uremic skeletal muscle (24, 25). Recently McLaurin et al. (26) reported cTnT expression in the skeletal muscle of dialysis patients, but the evidence is not conclusive (27). To investigate the potential expression of cTnT in skeletal muscle, we analyzed the expression of cTnT in skeletal muscle specimens of five hemodialysis patients at the RNA or protein level. We also investigated the incidence of an increased cTnT concentration in the blood of 97 patients on chronic maintenance hemodialysis, using both a first (28) and a second generation cTnT assay (29). The main difference between these two assays is that the first generation test used a combination of a cardiac-specific and a nonspecific antibody, whereas the second assay

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3 Nonstandard abbreviations: ESRD, end-stage renal disease; CAD, coronary artery disease; cTnT, cardiac troponin T; RT-PCR, reverse transcription-PCR; SDS, sodium dodecyl sulfate; and CK, creatine kinase.

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cTnT, 0.07

(normal related donor for kidney transplantation; plasma assay) and one control patient with healthy renal function patients

biopsy specimens according to the method of Chirgwin et al.

Total RNA was isolated from the snap-frozen muscle in skeletal muscle reverse transcription–PCR (RT-PCR) of troponin T in skeletal muscle.

**Expression of cTnT in Skeletal Muscle**

Five patients with ESRD (all with an increased plasma concentration of cTnT; 0.25–5.32 μg/L, first generation assay) and one control patient with healthy renal function (living related donor for kidney transplantation; plasma cTnT, 0.07 μg/L, first generation assay) underwent elective surgery. Skeletal muscle specimens were obtained from the operation site (abdominal wall or back muscles) under direct vision while the patients were under general anesthesia. The tissue samples were divided in the operating room into two aliquots: One aliquot was snap-frozen in liquid nitrogen; the other aliquot was immediately immersion-fixed in 30 g/L paraformaldehyde and 10 g/L glutaraldehyde in phosphate-buffered saline (140 mmol/L sodium chloride, 4 mmol/L sodium phosphate, 1.5 mmol/L potassium phosphate, and 2.7 mmol/L potassium chloride), pH 7.4. All chemicals were purchased from Sigma Chemical Co. unless indicated otherwise.

**Reverse Transcription–PCR (RT-PCR) of Troponin T in Skeletal Muscle**

Total RNA was isolated from the snap-frozen muscle biopsy specimens according to the method of Chirgwin et al. (30). One hundred milligrams of the muscle tissue yielded ~80 μg of RNA. The RNA was reverse transcribed using M-MLV reverse transcriptase (Life Technologies) according to the supplier’s protocol. The reverse-transcribed cDNAs were amplified by PCR using the following oligonucleotide primers:

(a) human slow skeletal muscle troponin T amplification (31),

forward: CCTCGAGATTCCAGCATCTC

reverse: AGCTGCAGGTTCTTTTCTC; or

(b) human cTnT amplification (32),

forward: GGAGACAGAGACTATG

reverse: ACTCTCTCTCAGCGGGATC.

The amplified DNA fragments were visualized by agarose gel electrophoresis combined with ethidium bromide staining.

**Immunoblot**

Frozen muscle specimens were ultrasonically lysed in 50 mmol/L KCl, 2 mol/L urea, 50 mmol/L Tris-HCl, pH 7.5, and immediately transferred into sodium dodecyl sulfate (SDS) sample buffer (33), boiled for 15 seconds, and subjected to SDS-polyacrylamide gel (15%) electrophoresis. The separated proteins were electrophoretically blotted onto a polyvinylidene difluoride membrane (Millipore), using a semidyly transfer apparatus.

The membranes were blocked with 30 g/L casein in incubation buffer (150 mmol/L NaCl, 10 mmol/L Tris-HCl, 10 g/L Tween 20, pH 8) for 1 h at room temperature. The membranes were then incubated for 1 h at room temperature with a mouse monoclonal antibody (1B10) that recognizes both the skeletal and cardiac muscle isoforms of human troponin T (34) at a dilution of 1:10 000. Alkaline phosphatase-conjugated goat antimouse IgG (Dianova) was used at a dilution of 1:20 000 for the detection of specific antibody binding with a chemoluminescence kit (Tropix) and an exposure time of 10 min. The cardiac and skeletal muscle isoforms could be discerned by their different molecular masses by means of their distinctive migration pattern on SDS-polyacrylamide gel electrophoresis. For both the RT-PCR and immunoblot experiments, postmortem human myocardium was used as a positive control. The cardiac specimen of a previously healthy, violent crime victim was obtained 6 h postmortem from the Department of Forensic Pathology at the University of Heidelberg.

**Indirect Immunofluorescence**

Small pieces of immersion-fixed skeletal muscle were epon-embedded and sectioned with an ultramicrotome. Epon sections (0.5 μm) on microscopy slides were blocked at room temperature with 10 g/L bovine serum albumin and 0.5 g/L saponin in phosphate-buffered saline, pH 7.4, for 20 min. The sections were incubated with cTnT-specific mouse monoclonal antibody 1H10 overnight at 4 °C at a dilution of 1:5000 in blocking buffer. After the sections were washed with phosphate-buffered saline, they were incubated for 1 h at room temperature in Cy3-conjugated anti-mouse IgG (Dianova). Immersion-fixed right ventricular biopsies from cardiac transplant...
recipients were used as positive controls. The muscle sections were examined with a Zeiss photomicroscope.

**cTnT measurement in blood**

Plasma samples derived from EDTA-treated blood obtained during routine monthly predialysis blood draws were stored at −20 °C for later batch analysis of the cTnT concentration. cTnT was measured in plasma using a first (35) and a second generation (29) cTnT sandwich ELISA and an ES 300 (Boehringer Mannheim). A discriminator value of 0.2 μg/L was chosen in the first generation assay (35); 0.15 μg/L was the cutoff value in the second generation assay. Total creatine kinase (CK) activity was measured in the clinical chemistry laboratory of the University of Heidelberg Medical School, using a standard colorimetric assay (Beckmann).

**Statistical analysis**

All measurements are presented as median and quartiles denoting the 25th and the 75th percentile of the distribution. The overall differences between the cTnT concentrations in the three patient groups (global hypothesis) were tested with the nonparametric Kruskal–Wallis test. Because there was a significant difference ($P < 0.01$) between the groups, pairwise comparisons were carried out with the nonparametric Wilcoxon signed rank test.

The prevalence of increased cTnT values ($\geq 0.2 \mu g/L$, first generation assay; $\geq 0.15 \mu g/L$, second generation assay) in the different patient groups was compared using contingency table analysis. Contingency table analysis of the three groups rejected the global hypothesis that there was no difference between the groups. Therefore, pairwise comparisons of the groups were carried out using contingency table analysis with continuity correction.

**Results**

RT-PCR analysis using oligonucleotide primers specific for the cardiac and skeletal muscle isoform of troponin T showed the respective isoform-specific amplification products of reverse-transcribed and PCR-amplified RNA in both cardiac and skeletal muscle tissue. However, the cardiac isoform was expressed only in the (positive) control myocardial tissue, not in skeletal muscle whether it was uremic or control skeletal muscle (Fig. 1). The detection of cTnT in the cardiac tissue and the skeletal muscle of hemodialysis patients by immunoblot is shown in Fig. 2. The anti-troponin T antibody 1B10 recognizes both cardiac and skeletal muscle isoforms of troponin T (34), which can be distinguished clearly by their different migration patterns on SDS-polyacrylamide gel electrophoresis. cTnT was absent in the truncal skeletal muscle of five dialysis patients with increased plasma cTnT concentrations, as well as in a patient with healthy renal function.

The results of a representative immunofluorescence experiment on a truncal skeletal muscle specimen from a hemodialysis patient with a serum troponin T concentration of 0.41 μg/L (first generation assay) is shown in Fig. 3. The mouse monoclonal antibody specific for the cardiac isoform of human troponin T (1H10) failed to stain the skeletal muscle sections of the uremic patient (data not shown), whereas there was specific sarcomeric staining for cTnT in human cardiac control tissue. The cardiac-specific antibody also failed to bind to the truncal skeletal muscle of four other patients with ESRD who had serum cTnT concentrations of 0.37, 5.32, 0.25, and 0.5 μg/L (first generation assay).

The measurements of cTnT in the blood of 97 patients on chronic maintenance hemodialysis showed that cTnT is detectable in a substantial subgroup of these patients. Table 1 shows the demographic characteristics of the hemodialysis patients from the two dialysis centers. Patient age and comorbidity were similar in the two centers. The patients were stratified into three groups according to cardiac risk (Table 2). There were no major differences between the three patient groups with regards to renal diagnosis, duration of hemodialysis, or dialysis protocols/efficiency. However, because of the stratification criteria, the prevalence of cardiac risk factors was lower in group C. Because the patients in group C were younger and generally less sick, it can be inferred that the medications were different between the groups. No history of uremic myopathy was available, but there was no difference in the serum parathyroid hormone concentrations that are generally regarded as a marker for uremic musculoskeletal disease. The prevalence of cardiac risk factors was similar in groups A and B, and most
patients in these two groups had echocardiographic evidence of concentric left ventricular hypertrophy.

When the serum samples were analyzed with the first generation cTnT ELISA, 39% of the patients had a serum concentration of cTnT \( \geq 0.2 \, \mu g/L \), which is the upper limit of the reference interval determined in patients with intact renal function. At the chosen cutoff value of the second generation cTnT ELISA (cTnT \( \geq 0.15 \, \mu g/L \)), 29% of the dialysis patients had an increased serum concentration, but only 24% exceeded 0.2 \( \mu g/L \). The increase in serum concentration of cTnT did not correlate with the patients’ age or sex, the duration of hemodialysis, the cause of renal failure, residual urine production, or indicators of dialysis efficiency, such as pre- and postdialysis blood urea nitrogen concentrations, \( \beta_2 \)-microglobulin concentrations, parathyroid hormone concentrations, or nutritional status (data not shown).

As can be seen in Table 3), in both the first and the second generation cTnT assays, the prevalence of increased cTnT concentrations correlated with cardiac risk, as indicated by the significant difference between groups A and C \(( P = 0.0196 \) in the first generation assay and \( P = 0.0074 \) in the second generation assay). There was a trend towards a difference between groups B and C, which reached borderline significance \(( P = 0.0510 \) in the first generation cTnT assay.

Table 4 shows the median cTnT concentration in the three groups. In both assays, the patients in group A with established CAD had a significantly higher median plasma concentration of cTnT compared with patients in the low risk group C. The median cTnT concentration in group B was similar to that of group A, but significantly different from group C.

**Discussion**

The results of the present study show an increased steady-state plasma cTnT concentration \(( \geq 0.2 \, \mu g/L \) in 39% (first generation cTnT ELISA) of a large and representative population of hemodialysis patients. When the second generation cTnT ELISA was used, 29% of the hemodialysis patients had increased cTnT concentrations, even when a cutoff value \(( \geq 0.15 \, \mu g/L \) higher than the discriminator value recommended for patients without renal failure \(( \geq 0.1 \, \mu g/L \) \(29) \) was used. The higher discriminator value was chosen to increase the specificity of the measurement with the second generation cTnT ELISA.

Although the reasons for the increased cTnT concentrations in the blood of dialysis patients without clinical evidence of myocardial cell injury are not clear, the present results are consistent with the hypothesis that the cTnT measured in the blood of dialysis patients may be of cardiac origin. The two main findings of the present study are the absence of cTnT expression in the truncal skeletal muscle of five hemodialysis patients with an increased
serum cTnT concentration and the correlation between the plasma cTnT concentration and indicators of CAD.

**cTnT assays**

cTnT has been systematically evaluated as a serodiagnostic tool for myocardial cell injury in patients without renal disease (12). Specific monoclonal antibodies that do not cross-react with extracardiac TnT isoforms are available and have been extensively characterized (28). The sensitivity and specificity of assays using cTnT as a diagnostic marker has been demonstrated in a variety of clinical settings, such as acute myocardial infarction (35), unstable angina (36), and perioperative myocardial infarction (37), which poses a particular challenge for a test to differentiate between skeletal and cardiac muscle cell injury.

In patients with end-stage renal disease—a population with a high prevalence of ischemic heart disease—the diagnostic performance of cTnT as a serodiagnostic marker for myocardial ischemia has not been systematically evaluated. Several case reports and reports from small patient series have suggested that cTnT concentrations may be increased in the serum of hemodialysis patients without myocardial ischemia (14–17). In theory, this could be a laboratory artifact of the cTnT assay in the presence of uremic serum. Interferences between uremic serum and the cTnT assay have not been evaluated. However, the theoretical possibility of artifactual interference between blood factors present only in dialysis patients and cTnT measurements is not likely because there was no apparent correlation between dialysis duration, residual urine production, and other standard indicators of dialysis efficiency. Only a subgroup of patients had increased cTnT concentrations, whereas other patients exhibited no increase in serum cTnT concentrations despite a similar uremic/dialytic state. Thus, neither the uremic state itself nor the hemodialysis procedure accounts for the increased cTnT values; rather, some patient-related factor appears to be involved. Both the first and second generation cTnT assays yielded a high prev-

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**Table 1. Demographic characteristics of patients from the two dialysis centers.**

<table>
<thead>
<tr>
<th></th>
<th>Center A</th>
<th>Center B</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>n</td>
<td>62 (64%)</td>
<td>35 (36%)</td>
<td>34 (68%)</td>
</tr>
<tr>
<td>Median (Q1; Q3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>63 (55; 68)</td>
<td>68 (62; 73)</td>
<td>66 (53; 70)</td>
</tr>
<tr>
<td>Duration of ESRD, months</td>
<td>92 (90; 93)</td>
<td>60 (24; 96)</td>
<td>62 (53; 70)</td>
</tr>
<tr>
<td>Causes of ESRD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>14 of 50 (28%)</td>
<td>16 of 47 (34%)</td>
<td>16 of 47 (34%)</td>
</tr>
<tr>
<td>Chronic GN</td>
<td>12 of 50 (24%)</td>
<td>9 of 47 (18%)</td>
<td>12 of 50 (24%)</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>4 of 50 (8%)</td>
<td>6 of 47 (13%)</td>
<td>4 of 50 (8%)</td>
</tr>
<tr>
<td>APCKD</td>
<td>6 of 50 (12%)</td>
<td>4 of 47 (9%)</td>
<td>6 of 50 (12%)</td>
</tr>
<tr>
<td>Others</td>
<td>14 of 50 (28%)</td>
<td>12 of 47 (26%)</td>
<td>14 of 50 (28%)</td>
</tr>
</tbody>
</table>

*Q1, 25th percentile; Q3, 75th percentile.

The most common cause for ESRD in both centers was diabetes mellitus (DM), followed by chronic glomerulonephritis (GN). Because there were no substantial differences between the two patient groups, both populations were combined for additional evaluation.

APCKD, adult polycystic kidney disease.

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**Table 2. Stratification of patients from both dialysis centers according to risk of cardiac ischemia.**

<table>
<thead>
<tr>
<th></th>
<th>Center A</th>
<th>Center B</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Group A</td>
<td>8</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Group B</td>
<td>17</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Group C</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

*Group A, documented CAD (previous myocardial infarction and/or at least one vessel stenosis >50% on coronary angiopathy); group B, two or more cardiac risk factors in addition to ESRD; group C, one or no cardiac risk factors in addition to ESRD.
alence of increased serum cTnT concentrations, even when the discriminator value of the second generation assay was adjusted from the recommended value of 0.1 \( \mu g/L \) in patients without renal disease to 0.15 \( \mu g/L \) in patients with ESRD. This argues against the interpretation that an increased cTnT concentration in dialysis patients merely represents an assay artifact, because the newer assay with a different antibody combination and an

<table>
<thead>
<tr>
<th>Table 3. Prevalence of pathological cTnT plasma concentrations(^a) in ESRD patients on chronic maintenance hemodialysis.</th>
<th>Group A(^b)</th>
<th>Group B</th>
<th>Group C</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT &lt;0.2 ( \mu g/L )</td>
<td>10 (45%)</td>
<td>27 (56%)</td>
<td>22 (81%)</td>
<td>59 (61%)</td>
</tr>
<tr>
<td>cTnT ( \geq )0.2 ( \mu g/L )</td>
<td>12 (55%)</td>
<td>21 (44%)</td>
<td>5 (19%)</td>
<td>38 (39%)</td>
</tr>
<tr>
<td>Totals</td>
<td>22 (100%)</td>
<td>48 (100%)</td>
<td>27 (100%)</td>
<td>97 (100%)</td>
</tr>
<tr>
<td>Contingency table analysis</td>
<td>( \chi^2 ) test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global hypothesis (groups A–C)</td>
<td>( P = 0.0243 )</td>
<td></td>
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<tr>
<td>Group A vs group B</td>
<td>( P = 0.5605 ) (with continuity correction)</td>
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<tr>
<td>Group A vs group C(^c)</td>
<td>( P = 0.0196 ) (with continuity correction)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B vs group C</td>
<td>( P = 0.0510 ) (with continuity correction)</td>
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</table>

<table>
<thead>
<tr>
<th>Second generation cTnT assay</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT &lt;0.15 ( \mu g/L )</td>
<td>11 (50%)</td>
<td>33 (69%)</td>
<td>24 (89%)</td>
<td>68 (70%)</td>
</tr>
<tr>
<td>cTnT ( \geq )0.15 ( \mu g/L )</td>
<td>11 (50%)</td>
<td>15 (31%)</td>
<td>3 (11%)</td>
<td>29 (30%)</td>
</tr>
<tr>
<td>Totals</td>
<td>22 (100%)</td>
<td>48 (100%)</td>
<td>27 (100%)</td>
<td>97 (100%)</td>
</tr>
<tr>
<td>Contingency table analysis</td>
<td>( \chi^2 ) test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global hypothesis (groups A–C)</td>
<td>( P = 0.0121 )</td>
<td></td>
<td></td>
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<tr>
<td>Group A vs group B</td>
<td>( P = 0.2147 ) (with continuity correction)</td>
<td></td>
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<tr>
<td>Group A vs group C(^c)</td>
<td>( P = 0.0074 ) (with continuity correction)</td>
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<tr>
<td>Group B vs group C</td>
<td>( P = 0.0932 ) (with continuity correction)</td>
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</table>

\( a \) \( \geq \)0.2 \( \mu g/L \), first generation assay; \( \geq \)0.15 \( \mu g/L \), second generation assay.

\( b \) Group A, documented CAD; group B, high risk of CAD; group C, low risk of CAD (see text).

\( c \) In both cTnT assays, the incidence of increased cTnT was significantly higher in group A than in group C.

<table>
<thead>
<tr>
<th>Table 4. Median plasma concentrations of cTnT in patients on chronic maintenance hemodialysis.</th>
<th>cTnT, ( \mu g/L ) (First generation assay)</th>
<th>cTnT, ( \mu g/L ) (Second generation assay)</th>
<th>CK, U/L(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 22)</td>
<td>0.215 (0.08; 0.46)</td>
<td>0.155 (0.06; 0.27)</td>
<td>19 (13; 27)</td>
</tr>
<tr>
<td>Group B (n = 48)</td>
<td>0.135 (0.06; 0.43)</td>
<td>0.10 (0.05; 0.21)</td>
<td>19 (12; 27)</td>
</tr>
<tr>
<td>Group C (n = 27)</td>
<td>0.050 (0.02; 0.2)</td>
<td>0.04 (0.002; 0.127)</td>
<td>14 (12; 22)</td>
</tr>
<tr>
<td>First generation assay</td>
<td>Overall comparison of groups A–C (Kruskal–Wallis test)</td>
<td>( P &lt;0.01 )</td>
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<tr>
<td>Pairwise comparisons (Wilcoxon signed rank test):</td>
<td></td>
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<tr>
<td>Group A vs B(^b)</td>
<td>( P = 0.78 )</td>
<td></td>
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<tr>
<td>Group A vs C(^c)</td>
<td>( P = 0.03 )</td>
<td></td>
<td></td>
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<tr>
<td>Group B vs C</td>
<td>( P = 0.014 )</td>
<td></td>
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<tr>
<td>Second generation assay</td>
<td>Overall comparison of groups A–C (Kruskal–Wallis test)</td>
<td>( P &lt;0.01 )</td>
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<tr>
<td>Pairwise comparisons (Wilcoxon signed rank test):</td>
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<tr>
<td>Group A vs B(^b)</td>
<td>( P = 0.90 )</td>
<td></td>
<td></td>
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<tr>
<td>Group A vs C(^c)</td>
<td>( P = 0.006 )</td>
<td></td>
<td></td>
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<tr>
<td>Group B vs C</td>
<td>( P = 0.01 )</td>
<td></td>
<td></td>
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</tbody>
</table>

\( a \) The median plasma activity of CK was within reference intervals without differences between groups.

\( b \) Q1, 25th percentile; Q3, 75th percentile.

\( c \) The cTnT concentrations in groups A and B were not significantly different.

\( d \) The cTnT concentration in group C was significantly lower than in groups A and B in both cTnT assays.
increased specificity (29) produced a prevalence of increased cTnT concentrations similar to that of the first generation assay. Thus, it is not unreasonable to speculate that both assays measure cTnT circulating in the blood of a substantial subgroup of hemodialysis patients.

CORRELATION WITH CARDIAC RISK FACTORS
We hypothesized that the increased cTnT concentration in the serum of some dialysis patients might be related to subclinical myocardial cell injury, which could occur during the hemodynamic “stress” of hemodialysis. cTnT has a serum half-life of 1.5 h in patients without renal failure. Hence, the increased steady-state concentration of cTnT in the serum of some patients with ESRD could be a cumulative result of (repetitive) subclinical myocardial cell injury during hemodialysis.

Our results show a correlation between the cTnT concentration measured with the first generation cTnT assay and indicators of CAD. This is consistent with the hypothesis that the increased cTnT concentration may indeed be of cardiac origin. Hence, the high sensitivity of the cTnT assay may allow the detection of subclinical myocardial cell injury in the setting of hemodialysis.

In addition to CAD, the uremic state itself could predispose the heart to myocardial cell injury. Experimental studies in uremic animals have shown a pronounced, blood pressure-independent microvascular rarefaction and fibrosis in cardiac muscle, which was presumed to be produced by chronic ischemia in the absence of epicardial stenotic lesions (38–40). It could be speculated that this structural alteration of cardiac muscle during uremia, especially when combined with hypertensive left-ventricular hypertrophy and/or CAD, could be the cause of subclinical, slowly ongoing cardiac cell damage that leads to the release of small amounts of cTnT into the circulation, which may be detected with the sensitive cTnT assays. In this context, it may be relevant that most of the patients in this study had echocardiographic evidence of left-ventricular hypertrophy, because echocardiographic abnormalities were associated with increased cTnT concentrations in a pediatric hemodialysis population (41).

Taken together, the available data are consistent with the hypothesis that cTnT in the serum of dialysis patients is of cardiac origin rather than an assay artifact or a result of extracardiac expression. Nevertheless, to exclude the latter possibility, we investigated the expression of cTnT in skeletal muscle biopsies of hemodialysis patients.

ABSENCE OF EXTRACARDIAC EXPRESSION OF cTnT
The tightly regulated tissue-specific expression of cTnT (42, 43) is the basis for its diagnostic specificity as a marker for myocardial cell injury. Unlike the conventional cardiac enzymes, cTnT is undetectable with the available assays in the serum under nonischemic conditions in patients without renal failure. Thus, no ambiguity exists in the interpretation of cTnT serum concentrations, because circulating cTnT necessarily derives from injured cardiomycocytes unless there is extracardiac expression of cTnT in skeletal muscle. Because of the large mass of skeletal muscle and the propensity for minor trauma during daily activities, the expression of cTnT in uremic skeletal muscle could evidently explain an increased steady-state serum concentration of cTnT. It has been reported that injured or regenerating skeletal muscle can express different protein isoforms compared with quiescent/fully differentiated muscle. Recent work has demonstrated decreased enzymatic activities in energy-providing metabolic pathways in uremic patients (44). The extracardiac expression of cTnT in injured or diseased skeletal muscle has been reported in animals (23) and in humans with polymyositis/dermatomyositis (22).

Recently McLaurin et al. (26) reported evidence of cTnT expression in skeletal muscle of dialysis patients. However, this evidence is not conclusive for a variety of reasons (27). These authors used an anti-cTnT antibody that reportedly cross-reacted with some human skeletal muscle troponin T isoforms. The reduced specificity of the antibody and the low resolution of the immunoblot shown in their article limit the interpretation that cTnT may be expressed in the skeletal muscle of dialysis patients.

In our study, we used a specific antibody against the cardiac isoform of troponin T, which did not react with skeletal muscle tissue but showed a specific band in cardiac control tissue. One limitation of both the present study and the study performed by McLaurin et al. is that proximal extremity muscles, which are the most common target of uremic myopathy, were not examined. Because the muscle biopsies in the present study were obtained from the surgical site during elective surgery, it was not possible, for obvious reasons, to study limb girdle muscles or other muscles from the same patient, although the expression of troponins may vary between different muscle groups (21). Despite the increased serum cTnT concentration, none of the specimens showed any evidence of cTnT expression, neither at the RNA nor at the protein level.

No history of myopathic symptoms was available. Because uremic myopathy mostly affects proximal limb girdle muscles, which were not sampled in the present study, we theoretically could have missed the expression of cTnT in muscle groups that may be more likely to reveal extracardiac cTnT expression. Because the surgical muscle biopsies were not subjected to routine light-microscopy examination, we cannot comment on histopathological evidence of myopathy. It should be noted, however, that the prevalence of an increased cTnT concentration in the blood of dialysis patients is higher than the incidence of clinical uremic myopathy. Therefore, clinical myopathy alone may not explain the increased cTnT concentrations in the blood of dialysis patients. Moreover, parathyroid hormone as a marker for musculoskeletal uremic disease was not different between the patient groups in the present study. Therefore, it appears
that blood concentrations of cTnT may be increased in
dialysis patients in the absence of myopathy.

The absence of extracardiac cTnT expression in
truncal skeletal muscle lends additional support to the hypothesis
that the cTnT found in the serum of a substantial sub-
group of hemodialysis patients originates from the heart.
The clinical importance of this finding is not clear, but it
could be speculated that the low-level release of cTnT
from cardiac myocytes signifies cardiac cell injury in the
setting of the poorly defined “uremic cardiomyopathy”.
Further studies are warranted to test the hypothesis that
the serum concentration of cTnT could be clinically useful
to identify a subpopulation of dialysis patients with high
cardiac risk.

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