Impaired Renal Clearance Explains Elevated Troponin T Fragments in Hemodialysis Patients

Jart H.C. Diris, PhD; Christian M. Hackeng, PhD; Jeroen P. Kooman, MD, PhD; Yigal M. Pinto, MD, PhD; Wim T. Hermens, PhD; Marja P. van Dieijen-Visser, MD, PhD

Background—Patients with severe renal dysfunction often have unexplained elevated serum concentrations of cardiac troponin T (cTnT). We investigated whether in vivo fragmentation of cTnT could explain these increases.

Methods and Results—cTnT, creatine kinase isoenzyme MB, and myoglobin serum concentrations were measured in all 63 dialysis patients of our in-hospital dialysis department. A highly sensitive immunoprecipitation assay, followed by electrophoresis and Western blotting, was used to extract and concentrate cTnT and its possible fragments from serum samples sent in for routine analysis. All 63 samples were also subjected to cTnT fragmentation analysis. Patients’ characteristics are listed in Tables 1 through 3.

Conclusions—cTnT is fragmented into molecules small enough to be cleared by the kidneys of healthy subjects. Impaired renal function causes accumulation of these cTnT fragments and is very likely the cause of the unexplained elevations of serum cTnT found in patients with severe renal failure. (Circulation. 2004;109:23–25.)

Key Words: kidney ■ troponin T ■ plasma

The origin and clinical significance of elevated concentrations of cardiac troponin T (cTnT) in patients with renal failure and no signs of recent myocardial damage have been a point of confusion and discussion in recent years. Various groups have reported these findings, but no satisfying explanation has been given yet. In the mid 1990s, some hypothesized that fetal expression of cTnT in skeletal muscle was the cause of the increase, but others have proven this hypothesis to be wrong and suggested it to be caused by cross-reactivity.

However, the second- and third-generation cTnT assays still demonstrate the unexplained elevated cTnT concentrations in patients with renal failure. A third option for the unexplained elevations of cTnT concentration has also been mentioned: fragmentation of cTnT and subsequent (impaired) renal clearance. Van Eyk and colleagues have published several reports about the fragmentation of troponin I, but some figures also show a fragment of cTnT of 25 kDa. However, these findings have not been discussed in the light of renal function impairment. Recently, Ziebig et al reported findings that strongly suggest significant renal contribution to the elimination of plasma troponin T, but no definite answer has been given.

We used a highly sensitive immunoprecipitation assay to isolate and concentrate cTnT fragments subsequently separated by gel-electrophoresis and visualized by Western blotting. With this method, we were able to visualize cTnT fragments (8 to 25 kDa) even in serum with cTnT concentrations below 0.01 µg/L according to the third-generation assay.

Methods

Patients

After obtaining informed consent, serum samples of all 63 patients from the in-hospital dialysis department of the University Hospital Maastricht were collected before dialysis and stored at −20°C. cTnT, creatine kinase isoenzyme MB (CK-MBmass), and myoglobin (Roche Diagnostics GmbH) were measured in separate serum samples sent in for routine analysis. All 63 samples were also subjected to cTnT fragmentation analysis. Patients’ characteristics are listed in Tables 1 through 3.

Pooled serum samples from healthy subjects, checked for normal creatine values (men <110, women <97 µmol/L) and no history of cardiac disease, were used as a negative control. Spiked with purified human cTnT (Advanced Immunochemical), they were used as positive control.

Immunoprecipitation of Serum Samples

A mix of 5 anti-cTnT antibodies (clones 9G6, 7F4, 1C11, 1F11, and 7A9, Research Diagnostics Inc) directed against specified epitopes throughout the cTnT molecule were used to catch as many cTnT fragments as possible. These antibodies were covalently immobilized on protein A–Sepharose (Pharmacia) using dimethyl pimelimidate (Sigma) as a cross-linking agent. Human serum was incubated with the Sepharose beads to extract cTnT and its fragments. After

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From the Departments of Clinical Chemistry (J.H.C.D., C.M.H., M.P.v.D.-V.), Nephrology (J.P.K.), and Cardiology (Y.M.P.), University Hospital Maastricht, and the Cardiovascular Research Institute Maastricht (W.T.H), The Netherlands.

Correspondence to J.H.C. Diris, Department of Clinical Chemistry, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail Diris@klinchem.azm.nl

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incubation, the beads were washed and incubated in sample buffer containing 0.6% SDS to elute the cTnT for application onto an SDS-polyacrylamide gel.

**Separation and Detection**

Prepared cTnT precipitates were separated by gel-electrophoresis (BioRad) and transferred to nitrocellulose (BioRad) before incubation with a monoclonal mouse antibody against cTnT (clone 4C5, FORTRON). A peroxidase-conjugated polyclonal goat anti-mouse antibody (DAKO) was used for visualization by chemoluminescence (Perkin-Elmer LifeScience).

**Statistics**

Relations between history of dialysis therapy and the serum concentrations of cardiac markers were investigated using the nonparametric Kruskal-Wallis rank-sum test, incorporated in the Analyse-it software package (Analyze-it software, Ltd).

**Results**

From 63 dialysis patients, 55 (87%) had measurable serum cTnT concentrations. Only 7 patients had slightly elevated CK-MBmass values, whereas all patients had increased myoglobin concentrations (Table 3). The same was found after adjustment for age, gender, smoking status, diabetes, hypertension, and history of cardiac disease.

**Discussion**

In 1999, Missov and De Marco determined the plasma value for cTnT in healthy subjects with an ELISA based on the antibodies used in the second and third generation of the cTnT assay. The origin of low cTnT concentrations in healthy subjects has not been discussed, but recent results from a large population (n = 1951) suggest that even a slight increase in cTnT, with measurable plasma values between 0.01 and 0.10 μg/L, might indicate the presence of minor, subclinical myocardial events.

Löwbeer et al. reported a high association between cTnT serum concentrations (>0.1 μg/L) and poor outcome in patients starting dialysis treatment. However, the prognostic value of serum cTnT concentrations between 0.01 and 0.10 μg/L, often encountered in dialysis patients without signs of myocardial events is less clear.

We found that CK-MBmass values were normal in 56 of 63 patients, and the 7 remaining patients had only slightly increased concentrations. With these normal concentrations in dialysis patients, the prognostic value of low cTnT concentrations is less clear.

**Table 1. Patient Data, Hemodialysis Population (n=63)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>70 (58–76)*</td>
</tr>
<tr>
<td>Men, %</td>
<td>54</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>35</td>
</tr>
<tr>
<td>Diabetic, %</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>71</td>
</tr>
<tr>
<td>History of cardiac disease, %</td>
<td>51</td>
</tr>
<tr>
<td>Duration of dialysis, mo</td>
<td>26 (15–51)*</td>
</tr>
</tbody>
</table>

*Median value (interquartile range).

**Table 2. Patient Data, Overall Values of Cardiac Markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Values, μg/L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT†</td>
<td>0.051 (0.027–0.097)</td>
</tr>
<tr>
<td>CK-MBmass</td>
<td>1.85 (1.22–2.51)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>157.0 (117.8–204.1)</td>
</tr>
</tbody>
</table>

*Median value (interquartile range).

†Eight values of <0.010 μg/L are arbitrarily replaced by 0.005 μg/L.

**Table 3. Patient Data, Median Values of Cardiac Markers in Relation to Quartiles of Dialysis Duration**

<table>
<thead>
<tr>
<th>Median Values, μg/L</th>
<th>Quartile 1 (n=16)</th>
<th>Quartile 2 (n=14)</th>
<th>Quartile 3 (n=17)</th>
<th>Quartile 4 (n=16)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT</td>
<td>0.0285</td>
<td>0.0305</td>
<td>0.0710</td>
<td>0.0915</td>
<td>0.005</td>
</tr>
<tr>
<td>CK-MBmass</td>
<td>1.86</td>
<td>1.51</td>
<td>1.91</td>
<td>1.83</td>
<td>0.77</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>131</td>
<td>188</td>
<td>143</td>
<td>199</td>
<td>0.041</td>
</tr>
</tbody>
</table>

*P value obtained from Kruskal-Wallis test for a difference between the medians.
of a relatively specific cardiac marker, recent ischemic myocardial events are unlikely. This was confirmed by the results listed in Table 3, which strongly indicate that slowly increasing cTnT levels (medians of quartiles still below 0.10 μg/L) are not caused by individually increased release from the myocardium because of a deteriorating cardiovascular status (indicated by constant CK-MB mass medians) but are solely the result of accumulating cTnT fragments. Although myoglobin concentrations are elevated in all patients, earlier reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile median myoglobin concentrations are statistically significant (Table 3), but because of the absence of a trend, it is evident that these differences merely reflect a large variance of the measured concentrations.

We believe that the plasma value for cTnT (0.0002±0.0001 μg/L) in healthy subjects is the result of a continuous microloss of cardiomycytes during normal life. This results in an estimated negligible loss of 27 mg cardiac tissue per year, based on a renal clearance rate of 1.2 hour⁻¹, a plasma volume of 3 L, and a cardiac tissue content of 234 μg/g. Clearance of cTnT normally happens with such a speed that serum concentrations are well below the current detection limit of 0.01 μg/L. However, impaired renal function leads to a diminished clearance rate that can cause the measurable increase in basal cTnT concentrations. So although intact cTnT is too large to be effectively cleared, because of fragmentation, actual clearance rates are normally much higher than previously estimated. These findings are supported by the reports of a rapid decrease of cTnT concentrations in renal transplantation patients. The prognostic factor of increased cTnT concentrations in patients with renal failure has been discussed in several articles. A recent article showed that the short-term predictive value of cTnT concentrations remains useful despite decreased renal function. A large study, recently conducted among 224 dialysis patients, showed that higher levels of cTnT are associated with long-term increased risk of death. The major difference between earlier studies and our results is based on the fact that in the present study the duration of dialysis therapy, instead of cTnT concentration, was used for group classification. This way, individually occurring ischemic myocardial events are evenly distributed among the quartiles of therapy duration.

With our findings regarding the relation between duration of dialysis therapy and increasing cTnT concentrations, we do not question the predictive value of cTnT, but we show the presence of an additional underlying phenomenon contributing to increased cTnT concentrations in patients with renal failure.

The present study proposes an explanation for the unexplained cTnT elevations in dialysis patients by showing that accumulated cTnT fragments are present in their serum, even in samples with measured cTnT concentrations below 0.010 μg/L. These findings clarify the cause of the unexplained elevations of serum cTnT found in patients with severe renal failure.

Limitations of the Study
We now have demonstrated that cTnT is split into fragments ranging in size from 8 to 25 kDa, small enough to be cleared by the kidneys of healthy persons. Unfortunately, because of the unavailability of the unlabeled antibodies from the third-generation Roche cTnT assay, no comparison could be made between the intensity of the detected fragments and the measured cTnT concentrations. Only when fragmentation analysis can be performed using the same antibodies as used in the Roche assay can the single fragments contributing to the measured cTnT concentrations be identified, quantified by band intensity, and compared.

References
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