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 of these 63 hemodialysis patients. Although creatine kinase isoenzyme MB values excluded recent ischemic myocardial events in 55 of the 63 cases, cTnT fragments ranging in size from 8 to 25 kDa were present in the serum samples of all dialysis patients.

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 of skeletal troponin T in the first-generation cTnT assay. 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.

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
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 (Analyse-it software, Ltd).

Results
From 63 dialysis patients, 55 (87%) had measurable serum cTnT concentrations. Only 7 patients had slightly elevated CK-MB\textsubscript{mass} values, whereas all patients had increased myoglobin concentrations (Table 2). All upper reference limits are based on the 97.5th percentiles (cTnT, \(<0.01\ \mu\text{g/L};\) CK-MB\textsubscript{mass}, men \(<4.94\ \mu\text{g/L};\) women \(<2.88\ \mu\text{g/L};\) myoglobin, men \(<72.0\ \mu\text{g/L};\) women \(<58.0\ \mu\text{g/L};\) Roche Diagnostics GmbH).

Visualization of serum cTnT showed that various fragments are present in the circulation, ranging in size from 8 to 25 kDa (Figure, lanes 5 through 7). Despite variations between the relative amounts of different fragments, all 63 patient sera displayed bands with comparable molecular weights, even the samples of dialysis patients with a measured cTnT concentration \(<0.01\ \mu\text{g/L} (Figure, lane 3).

Intact cTnT (39 kDa) was found only in the cTnT-spiked, pooled serum from healthy subjects (Figure, lane 1). A sample of the same pooled serum, to which no cTnT was added, served as a negative control and showed no bands at all (Figure, lane 4).

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-MB\textsubscript{mass}</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 \(<0.01\ \mu\text{g/L} are arbitrarily replaced by 0.005 \mu\text{g/L}.

The duration of dialysis varied between 1 month and 9 years and was divided into quartiles for additional evaluation. A statistically significant trend was only present in the time course of cTnT, whereas no trend at all was present for CK-MB\textsubscript{mass} and myoglobin (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\textsuperscript{a} 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.\textsuperscript{b}

Löwbe et al.\textsuperscript{c} 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-MB\textsubscript{mass} values were normal in 56 of 63 patients, and the 7 remaining patients had only slightly increased concentrations. With these normal concentrations...
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-MBmass 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 reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity. Differences between the quartile reports have indicated that this is caused by the decrease in renal clearance capacity.

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 cardiomycocytes 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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