Cardiac troponin T and malondialdehyde modified plasma lipids in haemodialysis patients

Benjamin Scott¹, An Deman¹, Patrick Peeters¹, Christiane Van den Branden³, Jean-Claude Stolear⁴, Guy Van Camp² and Dierik Verbeelen¹

¹Department of Nephrology, ²Department of Cardiology, Academisch Ziekenhuis Vrije Universiteit Brussel, ³Laboratory for Anatomy, Vrije Universiteit Brussel and ⁴Department of Nephrology, Réseau hospitalier de médecine sociale (RHMS), Tournai, Belgium

Abstract

Background. In patients with end-stage renal disease (ESRD), treated with haemodialysis, a high overall mortality is observed. A previous study showed that cardiac troponin T (cTnT) is a strong independent predictor of outcome in this population. In this study we investigated possible causes of cTnT increase and its relationship with a marker of oxidative stress.

Methods. In a group of 71 haemodialysis patients (36 male, 35 female, mean age 68.7±1.5 years) we determined cTnT and compared its presence with several biochemical parameters and with malondialdehyde (MDA), which is an indicator of oxidative stress. None of the patients suffered an acute coronary event during the observation period. Three measurements of cTnT and MDA were performed with a 2-week interval. Forty-nine patients underwent a transthoracic echocardiography.

Results. Twenty-nine patients (or 40.8%) had a positive cTnT determination (defined as cTnT >0.10 ng/ml). cTnT positive patients had significantly higher levels of MDA (P=0.0125), C-reactive protein (CRP) (P=0.04) and pre-dialysis urea (P=0.04). Regression analysis showed that both pre-dialysis urea and MDA independently influenced cTnT. No correlation was found with age, dialysis adequacy, post-dialysis urea, total cholesterol, white blood cell count, fibrinogen or any of the echocardiographical parameters. Presence of heart failure, diabetes or use of medication could not discriminate between cTnT positive and cTnT negative patients. MDA levels correlated positively with time on haemodialysis (P=0.0021). Echocardiography showed left ventricular hypertrophy in 88% of the examined patients and impaired wall motion in 35%. Patients with clinical signs of heart failure had a lower ejection fraction and worse wall motion score index. No correlation existed between echocardiographic findings and cTnT or MDA. Survival was independently predicted by cTnT (P=0.0025), MDA (P=0.0007), CRP (P=0.006) and age (P=0.0143). Patients with both cTnT and CRP increase had a survival of <50% at 1 year, compared with 90% in patients with both cTnT and CRP within the normal range and 80% when either CRP or cTnT was increased (χ²=12.127; P=0.0023).

Conclusions. This study confirms that the presence of cTnT predicts prognosis in ESRD. The presence of cTnT is linked to oxidative stress, inflammation and uraemia. The absence of specific findings on EKG and echocardiography points towards subclinical myocardial damage caused by endothelial disturbances.

Keywords: cardiac troponin T; haemodialysis; malondialdehyde; oxidative stress

Introduction

Cardiovascular disease is a major cause of mortality and morbidity in patients with end-stage renal disease (ESRD). Mortality from cardiovascular disease is ~9% per year, which is about 32 times the risk in the general population. This higher risk is explained partly by a higher prevalence of risk factors for cardiovascular disease in the ESRD population, such as diabetes mellitus, hypertension, hyperlipidaemia and physical inactivity. On the other hand haemodynamic and metabolic factors, characteristic for the ESRD population, are also held partly responsible for the increased prevalence of cardiovascular disease: for example, proteinuria, fluid overload, electrolyte imbalance, anaemia and higher levels of thrombogenic factors and homocysteine [1]. Macrovascular disease develops faster in ESRD patients than in an age- and blood pressure-matched non-uraemic population. As a consequence there are increased systolic and pulse
pressures, left ventricular hypertrophy and altered coronary circulation [2]. These factors could also obviously play a role in the higher prevalence of cardiovascular disease in this population.

Cardiac troponin T (cTnT) is the troponym-binding subunit of the cardiac actin–myosin complex. A small pool also exists in the cytosol of myocardial striated muscle cells. A transient leakage of cTnT of the cytosolic pool might occur as a consequence of the loss of membrane integrity during severe reversible ischaemia (as in unstable angina pectoris), but a prolonged leakage is due to degradation of myofilaments in irreversibly damaged cells. At present cTnT is broadly used for risk stratification in the event of acute coronary syndromes [3–5]. Furthermore, low cTnT values in the absence of cardiac troponin I (cTnI) are highly suggestive of minor myocardial damage [3]. Rises in serum cTnT without apparent concurrent myocardial damage have been reported in renal failure [6–8]. cTnT has also been identified as a strong independent predictor of outcome in patients on haemodialysis [9]. The origin of this rise in troponin T in these patients is still unknown. We reported previously that cTnT is increased in a large number of haemodialysis-treated patients with ESRD. We also found that patients with an increased cTnT had a poor prognosis [9]. As the presence of cTnT points towards cardiovascular disease, and as others have also shown a relationship between cTnT and cardiac and vascular problems, we investigated patients on regular haemodialysis with echocardiography and also measured TBARS (thiobarbituric acid reactive substances) modified lipids.

Serum malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to definite oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid (n6 series) and thus serves as a reliable marker of lipid peroxidation [10,11].

Serum MDA is a predictor of cardiovascular disease in patients on haemodialysis, which may underscore the role of oxidative stress as a cardiac risk factor in these patients [12].

The goal of this study was to try to identify parameters that help to understand this increased cardiovascular morbidity and mortality in haemodialysis patients. Identifying these parameters may contribute to risk assessment and therapeutic possibilities for this population.

Subjects and methods

Subjects

All patients with ESRD on intermittent haemodialysis treatment at the dialysis centres of the AZ-VUB, who agreed to enter the study, were included. No specific selection criteria were used, in order to obtain a representative population of haemodialysis patients.

The following characteristics were observed or calculated: age, weight, gender, height, body-mass index, body surface area, medication taken by the patient such as angiotensin converting enzyme-inhibitors, β-lytics, calcium channel blockers, α-lytics, anticoagulants, aspirin, digoxin, nitrates, lipid-lowering drugs, corticosteroids, i.v. iron, erythropoetin, presence of hypertension, heart failure, angina pectoris, history of myocardial infarction and renal disease.

The following biochemical parameters were included: urea before and after dialysis, albumin, ionized calcium measured with an ion-specific electrode, total cholesterol, triglycerides, C-reactive protein (CRP) (normal value <10 mg/l), serum iron, transferrin, ferritin, intact PTH and complete blood count. Adequacy of dialysis was determined by the Kt/V formula using a formal single compartment model.

Three pre-dialysis blood samples were obtained with a 2-week interval for cTnT and MDA determination.

Measuring cTnT

Troponin T was measured using the Cardiac reader from Boehringer Mannheim. This quantitative immunological test for specific detection of cTnT was carried out by adding 150 μl of anticoagulated venous whole blood via a packaged syringe directly to the application well of the test device. The test contains two antibodies, one labelled with gold and one with biotin. These antibodies form a sandwich with any cTnT that is present in the sampled blood. The cTnT–antibody complexes migrate through the detection zone and accumulate along a line of streptavidin, appearing as a red (signal) line. Excess gold-labelled antibodies gather to form a second (control) line. The signal line increases proportionately in intensity as the concentration of cTnT rises. The optical system of the device recognizes the two lines and measures the intensity of the signal line, which is converted to a quantitative result. The sensitivity was 0.01 μg/l, the detection range was 0.10–3.00 μg/l. cTnT value ≥0.10 μg/l was interpreted as a positive cTnT test.

Evaluation of lipid peroxidation products (MDA)

The method for determination of MDA was based upon its reaction with thiobarbituric acid (TBA) [13]. Blood was collected by venipuncture with heparin as anticoagulant and centrifuged at 4°C for 10 min at 1500 g. EDTA (final concentration 1.34 mmol/l) and GSH (final concentration 0.65 mmol/l) were added to the plasma. Samples were processed for TBARS immediately. The standard assay was performed according to Yagi. The concentration of the TBA reaction products was determined fluorometrically using excitation at 525 nm and emission at 547 nm. The calibration curve was prepared with MDA standards of 0–0.5 mmol/tube. Plasma lipoperoxide concentrations were calculated as micromole MDA per litre plasma.

Echocardiography

Forty-nine haemodialysis patients were willing to undergo transthoracic echocardiography. The echocardiographic examination was performed immediately after haemodialysis treatment with a SSH 380 (Powervision 7000) from Toshiba. To record M mode and pulsed Doppler echocardiograms, a 3.7 MHz probe was used. We measured posterior wall thickness during diastole and systole, left ventricular diameter during diastole and systole and fractional shortening of the septum. Wall motion was scored and wall motion score index (WMSI) was calculated (WMS/# segments scored). Left ventricular mass (LVM) and left ventricular
mass index (LVMi) were calculated. Left ventricular hypertrophy was defined as LVMi > 125 g/m².

**Statistical analysis**

Parametric or non-parametric tests were used for comparison of data according to the distribution of the data. Comparison of survival in cTnT positive and negative subjects was calculated with a log rank test. Effect of different parameters on survival was tested with a proportional hazards regression model. Statistical significance was defined as \( P < 0.05 \). The statistical package of Statview version 4.5 (Abacus, Berkeley, CA, USA) was used for all calculations. Results are expressed as mean±SD or median and range, according to their distribution.

**Results**

Thirty-five of the patients were female, 36 were male. The underlying causes of ESRD were categorized as primary renal (33.3%), diabetes mellitus (22.5%), hypertension and renal vascular (19.7%), systemic disease (14.1%) and unknown (11.3%). Basic data and time on dialysis are shown in Table 1.

Twenty-nine patients or 40.8% had a positive cTnT determination. The baseline biochemical and haematological characteristics of the study population are shown in Table 2.

Using the Mann–Whitney \( U \)-test, we found that MDA levels \( P = 0.125 \), CRP \( P = 0.04 \) and pre-dialysis urea levels \( P = 0.04 \) were significantly higher in those patients with a positive cTnT test. A Spearman Rank test showed a correlation between cTnT and pre-dialysis urea (rho 0.359, \( P = 0.0029 \)) and MDA rho 0.369, \( P = 0.0020 \). No correlation was found between cTnT and post-dialysis urea, Kt/V, cholesterol, triglycerides, creatinin, fibrinogen, haematocrit or any of the electro- or echocardiographical parameters. Stepwise regression analysis showed that two factors independently influenced cTnT: MDA and pre-dialysis urea. The results of this regression are shown in Table 3.

**Determinants of MDA**

Multiple regression with MDA as dependent variable showed that only the time on dialysis correlated

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**Table 1. Basic characteristics of the study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean/median</th>
<th>Standard deviation/ range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>68.70</td>
<td>12.55</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.84</td>
<td>13.55</td>
</tr>
<tr>
<td>Time on HD (month)</td>
<td>18.3</td>
<td>2.4-257.1</td>
</tr>
</tbody>
</table>

*Indicates median, range values.

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**Table 2. Biochemical and haematological characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean/median</th>
<th>Standard deviation/ range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>126.63</td>
<td>57.81</td>
</tr>
<tr>
<td>Urea pre HD (mg/dl)</td>
<td>133.07</td>
<td>36.80</td>
</tr>
<tr>
<td>Urea post HD (mg/dl)</td>
<td>42.23</td>
<td>15.19</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>8.29</td>
<td>2.72</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.31</td>
<td>0.17</td>
</tr>
<tr>
<td>K⁺ (mEq/l)</td>
<td>4.94</td>
<td>0.44</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.48</td>
<td>0.36</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>190</td>
<td>111-361</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>136</td>
<td>60-849</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>407.61</td>
<td>87.41</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>9.0</td>
<td>6.8-88.0</td>
</tr>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>3.721</td>
<td>0.392</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.55</td>
<td>1.04</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>35.38</td>
<td>3.29</td>
</tr>
<tr>
<td>WBC (mm³)</td>
<td>7300</td>
<td>4110</td>
</tr>
<tr>
<td>Platelets (mm³)</td>
<td>225 714</td>
<td>61 305</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>59.63</td>
<td>25.03</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>369.09</td>
<td>338.90</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>174.50</td>
<td>39.73</td>
</tr>
<tr>
<td>Epo dose (IU/kg/week)</td>
<td>171.27</td>
<td>106.23</td>
</tr>
</tbody>
</table>

*Indicates median, range values.
Table 3. Stepwise regression: cTnT vs two independent parameters (MDA and pre-dialysis urea)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Standard coefficient</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/l)</td>
<td>0.036</td>
<td>0.016</td>
<td>0.259</td>
<td>2.313</td>
<td>0.0238</td>
</tr>
<tr>
<td>Pre-dialysis urea (mg/dl)</td>
<td>0.001</td>
<td>3.175E-4</td>
<td>0.296</td>
<td>2.648</td>
<td>0.0101</td>
</tr>
</tbody>
</table>

P-value of the regression = 0.0021.

Significantly with MDA levels (P = 0.0021). Using a stepwise regression, no other biochemical, echocardiographic or anamnestic parameter remained in the regression model.

Survival analysis

During the 1-year follow-up period 17 patients died, 11 of whom where cTnT positive. This means that 41% of cTnT positive patients died, vs 17% of cTnT negative patients. The Kaplan–Meier survival analysis of the 71 patients divided into cTnT positive and negative shows a 1-year survival of 86% for the cTnT negative group vs 62% for the cTnT positive group (P = 0.0273 with a Mantel–Cox Logrank test, \( \chi^2 = 5.273 \)). We also divided patients in to three groups: cTnT and CRP negative (defined as CRP < 10 mg/l), cTnT or CRP positive and cTnT and CRP positive. Then we performed a Kaplan–Meier survival analysis with these groups. The survival curve is shown in Figure 1. Survival was 90% (2/23) in patients with both CRP and cTnT within the normal range, 80% (6/30) when either CRP or cTnT was abnormal and < 50% (9/16) when both tests were elevated.

Survival was independently predicted by cTnT (P = 0.0025), MDA (P = 0.0007), CRP (P = 0.006) and age (P = 0.0143) in a global null hypothesis test. The results of this test are shown in Table 4.

Discussion

The study population consists of patients with ESRD treated with haemodialysis. This is a population with a high cardiovascular mortality and morbidity. Our study population has a high prevalence of cardiovascular risk factors: hypertension (56%), sequellae of infarction on ECG (31%), left ventricular hypertrophy on echocardiogram (88.4%), left ventricular contractility disorders (34.7%).

Other co-morbid conditions are frequent: diabetes mellitus (22.5%), hypercholesterolaemia (45.7%) and hypertriglyceridaemia (27.5%).

The high prevalence of left ventricular hypertrophy is in accordance with findings of other authors. Left ventricular hypertrophy is in part due to the high prevalence of hypertension. It is also known that the arterial wall in patients with ESRD undergoes structural remodelling, characterized by dilation and hypertrophy of conduit arteries, resulting in stiffening of the aorta and major arteries. Although the arterial alterations can largely be explained by haemodynamic factors, non-haemodynamic factors more or less specific for renal insufficiency could also play an important role. Arterial stiffness causes diminished arterial impedance and increased pulse wave velocity with early wave reflections, which leads to increased pulse and systolic pressures, left ventricular hypertrophy and altered coronary circulation [2].

In our study population, > 41% of patients have a cTnT level \( > 0.10 \mu g/ml \). cTnT positive haemodialysis patients have been shown to have a significantly poorer 1-year survival rate than those without cTnT \( > 0.10 \mu g/ml \) [9]. The troponins have undergone rigorous testing in the area of cardiac risk stratification. The prognostic significance of a positive troponin level in patients with electrocardiographic ST-segment elevation and myocardial infarction is well established. From the Global Use of Strategies to Open Coronary Arteries (GUSTO) it appears that patients with these electrocardiographical findings and a positive cTnT

Table 4. Survival analysis: global null hypothesis test for 1-year survival

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>( \chi^2 )</th>
<th>Exp (coef)</th>
<th>P-value</th>
<th>95% lower</th>
<th>95% upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/l)</td>
<td>0.813</td>
<td>0.240</td>
<td>11.516</td>
<td>2.254</td>
<td>0.0007</td>
<td>1.410</td>
<td>3.605</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.036</td>
<td>0.013</td>
<td>7.585</td>
<td>1.037</td>
<td>0.0059</td>
<td>1.011</td>
<td>1.064</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.088</td>
<td>0.036</td>
<td>6.001</td>
<td>1.092</td>
<td>0.0143</td>
<td>1.018</td>
<td>1.172</td>
</tr>
<tr>
<td>cTnT (µg/l)</td>
<td>4.988</td>
<td>2.185</td>
<td>5.210</td>
<td>146.668</td>
<td>0.0225</td>
<td>2.023</td>
<td>10 635.934</td>
</tr>
</tbody>
</table>
level experienced a 300% increase in 30-day mortality. Moreover, troponin T positive patients lacking electrocardiographical ST changes consistently experienced a worse short-term outcome than did those with ST-segment elevation and negative cTnT levels at presentation [4]. In several studies, elevated cTnT levels in the absence of clinically detectable acute myocardial damage have been reported in patients with ESRD [3–5]. It is now well established that the troponins are reliable biological markers for myocardial damage, even in patients with renal failure [14].

We found that patients with a positive cTnT determination had significantly higher MDA, CRP and pre-dialysis urea levels. Regression analysis showed that both urea and MDA independently and significantly predicted cTnT. The relationship between cTnT and MDA is not surprising. Boaz et al. [12] conducted a study with 76 haemodialysis patients. They found that MDA levels were significantly higher in patients with prevalent cardiovascular disease and that MDA was a strong independent predictor of cardiovascular disease in patients on haemodialysis. Holvoet et al. [15] showed in a population of 168 patients with coronary artery disease that MDA-modified low-density lipoprotein (LDL) is released into the blood stream from disrupted or unstable atherosclerotic plaques, which may cause unstable angina or myocardial infarction. They also showed an association between myocardial infarction and unstable angina pectoris on the one hand and MDA-modified LDL on the other. The MDA-modified LDL is probably not released constantly, but intermittently from unstable atherosclerotic plaques, which may produce large amounts of aldehydes during the process of platelet activation. The correlation between pre-dialysis urea levels and cTnT is less well documented. No correlation was found with post-dialysis urea levels or with adequacy of dialysis (expressed by Kt/V). Patients with chronic renal failure have been shown to have significantly impaired endothelium dependent dilatation, even in mildly decreased renal function and independently of the presence of atherosclerosis [16]. Uraemia could alter nitric oxide (NO) activity via endothelial dysfunction, decreased arginine synthesis by the kidney and arginine analogues that act as NO-synthase inhibitors [17]. Endothelial injury in some patients on haemodialysis is supported by the work of L"owbeer et al. [18]. They showed a correlation between cTnT and circulating endothelin-1 concentrations in dialysis patients and concluded that it may indicate heart disease.

The increase of oxidative with stress with time on dialysis has recently been confirmed and is probably primarily due to inflammatory conditions [19].

In this study, outcome is predicted by four parameters. Age is the most logical factor influencing outcome. Several studies have shown a relationship between outcome and CRP in dialysis patients [9]. We observed previously the different outcome of cTnT positive patients [9]. This finding has been extensively confirmed by others [8]. In this study we also show that MDA predicts mortality in a dialysis population. MDA is a marker of lipid peroxidation. It has been shown that there is a narrow link between inflammation, oxidative stress and endothelial injury [20]. In the present study those three parameters can be related to outcome. It seems therefore that future research should aimed at unravelling this intriguing relationship in order to determine whether inflammation induces vascular instability and increased susceptibility to oxidative damage or whether oxidative damage causes endothelial dysfunction and induces inflammation. Few studies are available in patients with ESRD. The finding that oxidative stress is closely related to inflammation and endothelial dysfunction and the possibility to reduce cardio-vascular morbidity with vitamin E point towards a critical role of oxidation in these patients [20,21].

These results show a narrow relationship between cardiovascular disease, inflammation and oxidative stress in patients on haemodialysis. These three factors strongly influence survival in this population.

Further larger studies are necessary to determine the temporal relationship of these factors as well as the influence of possible therapeutic strategies (e.g. antioxidant or anti-inflammatory strategies) on these markers.

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