Elevated concentrations of cardiac troponins are associated with severe coronary artery calcification in asymptomatic haemodialysis patients

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Abstract

Background. Elevated concentrations of cardiac biomarkers, such as troponins and natriuretic peptides, have been shown to be predictive of poorer long-term cardiovascular outcomes in stable patients with end-stage renal disease (ESRD). However, little is known about the relationship between elevated concentrations of these cardiac markers and underlying coronary artery pathology in these patients. The aim of the present study was to investigate associations between coronary artery calcification (CAC) and the concentrations of cardiac biomarkers in ESRD patients.

Methods. We conducted a cross-sectional study of 38 asymptomatic patients (median age, 54 years; 26 males, 12 females; diabetic, 39%) who were undergoing chronic haemodialysis. In these patients, pre-dialysis circulating concentrations of cardiac troponin T (cTnT), cardiac troponin I (cTnI), creatine kinase-MB (CK-MB) and B-type natriuretic peptide (BNP) were measured. We quantified the level of CAC by multirow spiral computed tomography to obtain a CAC score. CAC scores ≥ 400 were defined as being indicative of severe CAC.

Results. Severe CAC was detected in 17 patients (45%). The degree of CAC severity was positively associated (P < 0.05) with cTnT concentrations. Thus, 15% of patients had severe CAC in the lowest tertile of cTnT, 50% had severe CAC in the middle third, and 69% in the highest third. Similarly, the degree of severity of CAC was positively associated (P < 0.01) with cTnI concentrations across concentration categories. In contrast, there was no association between the degree of CAC severity and the concentrations of either BNP or CK-MB. A logistic regression analysis revealed that elevated concentrations of cTnT (≥ median vs <median, P < 0.05) and cTnI (≥ 0.1 ng/ml vs < detection limit, P < 0.05) were independently associated with severe CAC after adjusting for age, duration of dialysis, diabetes and previous cardiovascular events.

Conclusions. Elevated concentrations of cTnT and cTnI, but not BNP or CK-MB, were independently associated with the degree of severity of CAC in asymptomatic haemodialysis patients.

Keywords: brain natriuretic peptides; coronary artery calcification; haemodialysis; troponin

Introduction

End-stage renal disease (ESRD) patients who are undergoing dialysis have an extraordinarily high risk of death. In such patients, fatalities are largely due to cardiac disease, which accounts for ~45% of all-cause mortality. Moreover, the incidence and prevalence of coronary artery disease in dialysis patients is 10–20 times that in the general population [1]. The high mortality rate and high burden of cardiac disease make identification of patients who are at risk for cardiac disease essential for the management of ESRD.

Recently, non-invasive imaging techniques have been used to diagnose coronary artery disease. These techniques include the use of electron-beam computed tomography (EBCT) and multirow spiral computed tomography (MSCT) to quantify coronary artery calcification (CAC). The degree of arterial calcification is measured to obtain a CAC score, which can have diagnostic value. CAC in ESRD patients has attracted increasing attention owing to its greatly increased prevalence in these patients [2,3]. In addition, CAC is associated with abnormal mineral metabolism [3], which contributes to increased cardiac and all-cause mortality [4]. Furthermore, the calcium-free, phosphate-binding compound sevelamer hydrochloride attenuates the progression of CAC [5].
Cardiac troponins are cardiac muscle proteins that serve as serum markers of myocardial necrosis. Cardiac troponin levels are commonly elevated in patients with ESRD even in the absence of clinically suspected acute myocardial ischaemia [6]. Despite persistent uncertainty about the aetiology of elevated serum troponins in these patients, several studies have suggested that elevated concentrations of these proteins in asymptomatic ESRD patients are associated with increased risk, including an increase in mortality [6,7]. Furthermore, a recent study reported that concentrations of natriuretic peptides, which are useful diagnostic and prognostic cardiac markers in the general population, are associated with cardiovascular and all-cause mortality in the ESRD population [8]. The reason why elevated concentrations of cardiac markers are correlated with a poor prognosis among asymptomatic ESRD patients is unclear, and little is known about the relationship between the concentrations of cardiac biomarkers and the underlying cardiac pathology in these patients.

Here, we conducted a cross-sectional study of patients who were undergoing long-term haemodialysis and who had no symptoms of myocardial ischaemia. The concentrations of cardiac biomarkers were determined and MSCT was used to derive CAC scores for the enrolled patients. Our goal was to investigate the possible association between the concentrations of cardiac biomarkers and coronary artery pathology.

**Subjects and methods**

**Subjects**

This study was carried out in the haemodialysis unit of Kangwon National University Hospital in South Korea. Thirty-eight of 42 screened patients were enrolled. To be enrolled, the patients were required to be (i) older than 18 years; (ii) lacking any metallic objects (e.g. stents, clips) in their chest; and (iii) clinically stable (i.e. no symptoms of ischaemic heart disease or infectious disease) for >2 months. The four excluded patients had, respectively, cancer with pneumonia, angina, coronary angioplasty with the presence of stents, and no blood drawn secondary to transfer. The study protocol was approved by the institutional review board of Kangwon National University Hospital, and written informed consent was obtained from each enrolled patient.

The initial clinical evaluation included a medical history questionnaire, a physical examination and a review of medical records. The causes of ESRD in the analysed group were as follows: chronic glomerulopathy, 12 patients; diabetic nephropathy, 15 patients; hypertensive nephropathy, three patients; and polycystic kidney disease, one patient. The cause of renal failure was unknown for seven patients. All patients were treated with conventional bicarbonate haemodialysis using Polyflux 6L (Gambro Dialysatoren GmbH & Co. KG, Hechingen, Germany) or F6 HPS (Fresenius Medical Care AG, Bad Homburg, Germany) synthetic membranes. The duration of each dialysis session was 4 h.

**Blood sampling and analysis**

Blood samples were obtained prior to the first dialysis session of each week. The concentration of B-type natriuretic peptide (BNP) was determined in EDTA-anticoagulated whole blood within 2 h of sample collection. BNP concentrations were measured using a fluorescence immunoassay (Triage BNP test; Biosite, San Diego, CA) with a detection range of 5–5000 pg/ml. BNP concentrations ≤100 pg/ml are representative of normal values in patients without congestive heart failure (CHF), whereas concentrations >100 pg/ml are considered abnormal and suggestive of CHF.

Blood samples were collected without an anticoagulant for the measurement of cardiac enzyme levels. The samples were allowed to coagulate for at least 30 min and then centrifuged at 520 g for 10 min. The resulting serum was divided into aliquots, frozen, and stored at −70°C until completion of cardiac marker concentration measurements.

Cardiac troponin T (cTnT) concentrations were measured using an electrochemiluminescence immunoassay (Elecsys Troponin T STAT Immunoassay; Roche Diagnostics, Indianapolis, IN) with a detection limit of 0.01 ng/ml. The 99th percentile concentration of a reference population was <0.01 ng/ml. The cTnT concentration at the receiver operator characteristic (ROC) curve cut-off for acute myocardial infarction (AMI) is 0.1 ng/ml. Cardiac troponin I (cTnI) concentrations were measured with the AxSYM cTnl microparticle enzyme immunoassay (Abbott Laboratories, Abbot Park, IL). According to the manufacturer, cTnI concentrations ≥2.0 ng/ml are indicative of AMI. The 95th percentile concentration of healthy controls was ≤0.5 ng/ml. Creatine kinase-BM (CK-MB) concentrations were measured using an electrochemiluminescence immunoassay (Elecsys CK-MB STAT Immunoassay; Roche Diagnostics) with a detection limit of 0.10 ng/ml. Routine clinical blood chemistry variables, including albumin and creatinine, were analysed using standard methods.

**Spiral computed tomography scan**

All patients underwent MSCT (LightSpeed Plus, GE Medical Systems, Milwaukee, USA) with retrospective gating to calculate a CAC score. Data were acquired with a rotation time of 500 ms and a table feed of 4 × 2.5 mm/rotation. The tube current was 370 mA at 120 kVp. During the scan, a digitized electrocardiogram was recorded continuously, and image acquisition was performed with prospective electrocardiogram gating. Data obtained during the diastolic phase of the heart cycle were used for image reconstruction.

The software for this system was able to detect calcified lesions at a density of at least 130 Hounsfield units with a minimal area of 0.5 mm². Calcification scores were calculated using measurement formulae for the total volume and area of calcified lesions, as well as the mean and maximum density of the lesions. Individual calcification scores were calculated for the left main coronary artery, descending branch of the left coronary artery, circumflex branch of the left coronary artery, and right coronary artery. The individual calcification scores were summed to calculate the total coronary calcification score. The final CAC score was expressed in modified Agatston units [9].

CAC scores ≥400 were defined as severe and were indicative of an extensive atherosclerotic plaque burden. Patients with calcification scores in this range are very likely...
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(> 90%) to have obstructive coronary artery disease and are at high risk of developing symptomatic myocardial ischaemia [9].

Statistical analysis

Analysis of the data was performed with the SPSS software package (version 10.0; SPSS, Chicago, IL). Continuous clinical variables are presented as median and interquartile range (IQRs). Comparisons between patients with or without severe CAC (groups I and II, respectively) were made using the Mann–Whitney U-test. The Spearman’s rank test was used to assess correlations among the cardiac biomarker levels, and the data were categorized into tertiles of biomarker levels. Because cTnI concentrations in 24 patients were below the lower detection limit of the assay, the values for these patients were categorized as the lowest third; the data for the remaining patients were split evenly between the middle and highest third categories. The distribution of categorical variables among the tertiles was assessed using a χ² test. Comparisons of CAC scores among these three categories were made using a Jonckheere–Terpstra test. Logistic regression was used to test whether cTnT or cTnI concentrations were an independent predictor of severe CAC. Two-sided P-values < 0.05 were considered statistically significant.

Results

Patient characteristics

The baseline clinical characteristics of the study population are shown in Table 1. Thirty-eight patients (26 men, 12 women) with a median (IQR) age of 54 (47–64) years underwent haemodialysis for a median (IQR) of 34 (17–58) months. Fifteen (39%) patients had a previous history of cardiovascular disease, including AMI, CHF and stroke. Aspirin had been administered to 14 (37%) patients, and anti-hypertensive drugs had been administered to 34 (89%) patients. Almost all (except one) patients had received calcium carbonate or calcium acetate as phosphate binders.

Cardiac biomarkers

The median (IQR) concentration of cTnT was 0.040 (0.017–0.131) ng/ml. A cTnT concentration > 0.1 ng/ml (the diagnostic cut-off for AMI) was found in 11 (29%) of the 38 patients. The cTnI concentration was below the assay detection limit in 24 (74%) patients, but the remaining 14 patients had cTnI concentrations of 0.1–0.3 ng/ml or ≥ 0.4 ng/ml (seven patients each, 18%). One patient had a cTnI concentration > 2.0 ng/ml (the diagnostic cut-off for AMI). A greater percentage of patients had elevated cTnT concentrations (> 0.01 ng/ml, 34 patients, 89%) than patients with elevated cTnI concentrations (> 0.5 ng/ml, three patients, 8%) at values that were greater than the cut-off for the reference range. The median (IQR) concentration of BNP was 591 (180–1080) pg/ml. Thirty-six (95%) patients had elevated concentrations of BNP (> 100 pg/ml, the diagnostic cut-off for CHF). The median (IQR) concentration of CK-MB was 2.55 (2.08–3.68) ng/ml.

Positive correlations were evident between the serum concentrations of each of the three cardiac biomarkers. The Spearman correlation coefficients for cTnI concentrations with cTnI and CK-MB concentrations were 0.58 (P < 0.001) and 0.78 (P < 0.001), respectively. The correlation coefficient for cTnI concentrations with CK-MB concentrations was 0.32 (P < 0.05). There was no significant correlation between BNP concentrations and the serum concentrations of each of the other three cardiac proteins.

Coronary artery calcification scores

On MSCT examination, eight patients (21%) had no evidence of CAC, whereas 30 patients (79%) had CAC of differing severities. Seventeen patients (45%) had severe CAC (CAC score ≥ 400, group I), while 21 (55%) patients did not have severe CAC (group II). The characteristics of these patients are presented in Table 2. Patients in group I were significantly older than those in group II, and more patients in group I had a previous history of cardiovascular disease. The prevalence of diabetes, the sex ratio, smoking habits, systolic and diastolic blood pressures, the length of time undergoing haemodialysis and the dialysis dose were not significantly different between the two groups. The patients in group I had significantly higher serum concentrations of cTnT and cTnI than did patients in group II. There were no differences in blood concentrations of CK-MB, BNP, albumin or total cholesterol, and haematocrit was the same in both groups.
The relationships between the concentrations of the cardiac biomarkers and the degree of severity of CAC and the presence of LVH in the study population are shown in Table 3. An increased degree of severity of CAC was associated ($P<0.05$) with increased concentrations of cTnT (from the lowest through the highest of the three categories). A similar association was noted between the degree of severity of CAC and the concentration of cTnI ($P<0.01$). Furthermore, the mean CAC score was significantly greater when categorized according to the concentrations of cTnT and cTnI (Figure 1). There was no significant association between the degree of severity of CAC and the concentration of either BNP or CK-MB, nor was there an association between the concentrations of the cardiac biomarkers and the presence of LVH.

Multiple logistic regression analysis revealed that elevated concentrations of cTnT ($>/=0.03$ vs $<0.03$, $P<0.05$) and cTnI ($>/=0.1$ ng/ml vs $<0.1$ ng/ml, $P<0.05$) were independently associated with severe CAC, after adjusting for age, duration of dialysis, diabetes and previous cardiovascular events.

**Discussion**

In the present study, we examined associations between serum concentrations of biomarkers of myocardial damage and the severity of CAC in asymptomatic patients who were undergoing long-term haemodialysis. All patients underwent MSCT in order to quantify CAC. This method is more readily available than EBCT and is considered comparable with or even...
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Fig. 1. Coronary artery calcification scores according to cardiac troponin (cTnT) concentrations. Data are presented as means±SEM. The study population was divided into lowest, middle and highest thirds of cTnT concentrations.

superior to EBCT for assessing CAC [10]. We found that even small elevations in cTnT and cTnI concentrations, to levels that were lower than those traditionally used to diagnose acute myocardial infarction, were associated with an increased severity of CAC in stable patients with ESRD, even after adjustment for confounders of CAC, including age, duration of dialysis, diabetes and previous cardiovascular events [3]. In contrast, there was no association between BNP concentrations and the severity of CAC. To our knowledge, this is the first study to report an association between elevated concentrations of troponins and the severity of CAC in patients with ESRD.

In the current study, a high percentage of patients exhibited concentrations of cardiac troponins (particularly cTnT) that were greater than the normal reference range. Elevated concentrations of serum troponins are often observed in ESRD patients who do not exhibit clinical signs of myocardial ischaemia. In a large study that included 733 asymptomatic patients with ESRD, an extremely high percentage of the patients had elevated concentrations of troponins despite the use of the latest assay technology [6]. The causes of these elevated concentrations are unclear, but may include injury to skeletal muscle, heart failure, LVH, apoptosis or impaired renal clearance.

It is also possible that elevated concentrations of serum troponins resulted from clinically silent myocardial necrosis. Indeed, there is documented pathological evidence of micro-infarctions in patients with increased serum concentrations of troponins. In one study of 78 autopsied patients without clinical myocardial infarction, all patients that had elevated antemortem concentrations of cardiac troponins also had myocardial pathology that included an old myocardial infarct or patchy fibrosis, a recent myocardial infarct, a healing myocardial infarct, degenerative changes consistent with heart failure and other cardiac pathology [11]. These infarctions may not be clinically recognized and may occur in the absence of elevated concentrations of CK-MB. It is possible that patients with ESRD are more likely to experience repeated episodes of clinically silent micro-infarctions secondary to the high incidence of coronary artery disease that characterizes these patients.

A key element in the present study was our analysis of associations between CAC severity and the concentrations of each of the biomarkers of myocardial necrosis. Severe CAC was associated with high concentrations of cTnT and cTnI. This finding is of potential significance because it links this preventable cardiovascular parameter (i.e. CAC) with validated and prognostically important cardiac biomarkers (i.e. troponins).

Arterial calcification occurs in both the intima and media of an artery, and these two sites of calcification represent distinct entities. Intimal calcification occurs only within atherosclerotic plaques, whereas medial calcification occurs independently of both intimal calcification and atherosclerosis. Medial calcification commonly occurs in otherwise healthy elderly patients and in younger patients with diabetes and chronic renal failure. Intimal and medial calcifications are frequently found together in patients with ESRD.

Although CAC scores may provide an indication of atherosclerotic plaque burden (area) in coronary arteries [12], there are complex relationships between CAC and the presentation of acute coronary events. In several studies that included intravascular ultrasoundography, findings relating to CAC have been conflicting. As compared with patients with stable angina, patients with unstable angina had more echolucent lesions but fewer calcified plaques [13]. A more recent study demonstrated that a larger plaque area and larger outer boundary area at the target lesion, rather than a smaller lumen area, were associated with unstable coronary syndromes [14]. Together, these findings indicate that echolucent and larger plaques, rather than calcified plaques with severe luminal stenosis, are more vulnerable and are associated with an unstable clinical presentation, whereas an elevated CAC score may indicate the presence of larger but calcified plaques. Nevertheless, there is evidence that CAC scores predict subsequent coronary events in patients with known coronary artery disease [15].

Because CT scanning detects calcium independent of its location, CAC provides an assessment of risk that is not related to the degree of luminal stenosis. Therefore, the most pertinent application of CAC is the identification of arterial wall calcification instead of luminal stenosis. Arterial calcification may be equivalent to arterial stiffness and may be related to vascular non-compliance, but is mechanistically different from obstructive coronary artery disease. It has been shown that arterial wall stiffness, as assessed by aortic pulse wave velocity, is correlated independently with vascular calcification [16]. Conceptually, increased arterial stiffness as a result of calcification can increase the cardiac load. In addition, a reduction in aortic compliance may decrease diastolic coronary
perfusion, because coronary perfusion depends on the recoil of the aorta after it has been stretched during systole.

In the general population, CAC correlates strongly with future cardiac events, even in asymptomatic patients [17]. Arterial stiffness and the extent of common carotid calcification assessed by ultrasonography are strong predictors of all-cause and cardiovascular mortality in ESRD patients [16,18]. It has therefore been suggested that the high cardiovascular mortality rates in ESRD patients may be partly attributable to increased arterial calcification. However, there have been no reports that compared CAC with future cardiovascular events in ESRD patients. Our findings support the possibility that severe CAC may represent an additional cardiovascular risk factor in the ESRD population.

Although the mechanism of arterial calcification development in ESRD patients is unknown, several studies have identified elevations in serum phosphate and in the serum calcium x phosphate product, as well as increased calcium load as risk factors for vascular calcification [2,3]. Arterial calcification is progressive, especially when mineral metabolism is not well controlled [19], and sevelamer, a non-calcium-containing polymer, attenuates its progression more than calcium-based phosphate binders [5]. This protective effect of sevelamer may be related to its action on calcium load or to favourable effects on lipid profiles.

Patients with elevated serum troponin concentrations, even those that are asymptomatic, should be treated by aggressive risk factor modifications. Overall, the results of the present study suggest that it may be useful to incorporate CAC scores into diagnostic and therapeutic strategies that are aimed at the earlier detection and management of clinically silent myocardial damage in patients with ESRD. Patients with CAC scores that are >400 should be considered as being at high risk for cardiac damage. Sevelamer administration causes attenuation of CAC progression as well as improvement of altered mineral metabolism, and this would be helpful in preventing further myocardial damage in patients with elevated concentrations of troponins. Clearly, future interventional and prospective studies will be required to confirm these potential benefits of sevelamer treatment.

We did not find an association between elevated BNP concentrations and CAC severity. Several studies have revealed a correlation between elevated natriuretic peptide concentrations and increased rates of multivessel coronary artery disease in patients with acute coronary syndromes [20]. At present, there have been no studies comparing natriuretic peptide concentrations and severity of coronary artery disease in ESRD patients, and data from non-ESRD patients with acute coronary syndromes may not apply to asymptomatic patients with ESRD.

There were several limitations in the present study. First, the sample size was relatively small and the power to detect associations between clinical parameters (such as dialysis duration, diabetes and LVH) and the severity of CAC was limited. Secondly, this cross-sectional study was not able to establish a causal relationship between severity of CAC and elevated concentrations of cardiac troponins. Finally, we used electrocardiograms, which are insensitive for the detection of LVH, and did not assess arterial stiffness by pulse wave velocity, which is prognostically and haemodynamically important in arterial calcification.

In conclusion, we found that severe CAC was significantly associated with increased serum concentrations of cTnT and cTnI in asymptomatic ESRD patients who were undergoing long-term haemodialysis. The relatively small number of patients who participated in the present study necessitates the confirmation of these findings in additional and larger scale studies.

Conflict of interest statement. None declared.

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