The Extent of Coronary Atherosclerosis Is Associated With Increasing Circulating Levels of High Sensitive Cardiac Troponin T


Objective—This study explored the relationship between coronary atherosclerotic plaque burden and quantifiable circulating levels of troponin measured with a recently introduced high sensitive cardiac troponin T (hs-cTnT) assay.

Methods and Results—Cardiac patients suspected of having coronary artery disease (CAD) but without acute coronary syndrome were studied. Cardiac troponin T levels were assessed using the fifth-generation hs-cTnT assay. All patients (n=615) underwent cardiac computed tomographic angiography (CCTA). On the basis of CCTA, patients were classified as having no CAD or mild (<50% lesion), moderate (50% to 70% lesion), severe (>70% lesion), or multivessel CAD (multiple >70% lesions). As a comparison, high-sensitivity C-reactive protein levels were measured. Progressively increasing hs-cTnT levels were found in patients with mild (median, 4.5 ng/L), moderate (median, 5.5 ng/L), severe (median, 5.7 ng/L), and multivessel (median, 8.6 ng/L) CAD compared with patients without CAD (median, 3.7 ng/L) (all P<0.01). For high-sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide, no such relationship was observed. In patients without CAD, 11% showed hs-cTnT levels in the highest quartile, compared with 62% in the multivessel disease group (P<0.05). Multivariate analysis identified hs-cTnT as an independent risk factor for the presence of CAD.

Conclusion—In patients without acute coronary syndrome, even mild CAD is associated with quantifiable circulating levels of hs-cTnT. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: coronary artery disease ischemic heart disease computed tomography imaging troponin

The release of cardiac troponins (cTn) into the circulation occurs with cardiomyocyte injury. cTn is a preferred marker for diagnosing acute myocardial infarction (AMI) and is used as a key diagnostic tool for decision making in patients presenting with chest pain. However, the current commercially available assays do not have analytic precision for reliable detection of minor myocardial damage.1,2 The variation between measurements at the upper reference limit of a reference population, for instance, exceeds 10%.2 Furthermore, these immunoassays are unable to detect cTn in apparently healthy individuals harboring coronary artery disease (CAD). Recently, analytic improvements have been made in the lower part of the cTn measurement interval. Using the highly sensitive fifth-generation high sensitive cardiac troponin T (hs-cTnT) assay, we recently reported an almost gaussian distribution in a reference population (n=479).3 For cTnI, a similar distribution was seen using the adapted AccuTnI assay from Beckman Coulter.4 Recent studies in patients suspected of having an acute coronary syndrome (ACS) found that ultrasensitive cTn assays substantially improve the early diagnosis of AMI.5,6 Recently, Sabatine et al7 showed that transient stress test–induced ischemia is associated with increase of cTnI as detected with an ultrasensitive cTnI assay. The level of cTnI increase was proportionally related to the extent of ischemia as assessed by nuclear perfusion imaging.

We hypothesized that mismatch between supply and demand in the heart, and the resulting episodes of cardiac ischemia, may result in quantifiable circulating levels of cardiac troponin T (cTnT) in patients with CAD, especially in patients with significant lesions. To investigate this question, we assessed the extent of coronary plaque burden on cardiac computed tomographic angiography (CCTA) and associated this with hs-cTnT levels in a group of patients suspected for having CAD but without ACS. As a comparison, we used 2 other widely used cardiac serum biomarkers, high-sensitivity...
C-reactive protein (hsCRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP).

Methods

Study Population
A total of 646 patients were referred from the cardiology outpatient department for CCTA because of suspected CAD. Inclusion criteria were a recent history of cardiac typical or atypical chest pain, dyspnea, or collapse; at least 1 mL of serum for determination of biomarkers; and a diagnostic CCTA-scan, defined as 7 or more interpretable coronary segments. The exclusion criterion was an hsCRP concentration >10 mg/L, indicating underlying inflammatory disease. The institutional review board and ethics committee at the Maastricht University Medical Center approved the study, and all patients gave informed consent.

Risk Factor Assessment
Cardiac risk factors were assessed by the referring cardiologists. Risk factors were gathered just before CCTA. Patients were classified as having diabetes if they were treated with a hypoglycemic agent or if they had a fasting plasma glucose ≥6.7 mmol/L. Patients were classified as smokers if they had smoked in the 12 weeks before CCTA. A family history of CAD was defined as having a first-degree relative with a history of myocardial infarction or sudden cardiac death before the age of 60. We calculated the PROCAM risk score to estimate the 10-year risk of AMI and the Framingham risk score to estimate the 10-year risk of cardiovascular disease. The PROCAM risk score takes the following risk factors into account: age, gender, smoking status, diabetes mellitus, systolic blood pressure, myocardial infarction in first degree relatives before the age of 60, high-density lipoprotein (HDL), low-density lipoprotein, and triglycerides.

CCTA Acquisition
Patients received 5 to 20 mg of Metoprolol intravenously to lower the heart rate to <65 bpm, as well as sublingual nitroglycerin spray. Heart rate and ECG were monitored during CCTA.

A native scan was performed to determine the calcium score using the Agatston method. Subsequently, CCTA was performed using 85 to 110 mL of contrast agent (Kreonix 350; Guerbet), which was injected in the antecubital vein at a rate of 6.0 mL/s directly followed by 40 mL of intravenous saline (6.0 mL/s). In patients with a heart rate <65 bpm, a prospective-gated “step and shoot” protocol was used. In patients with a heart rate >65 bpm, a retrospective-gated “helical” protocol with dose modulation was used to obtain the best image quality at a minimal radiation dose.

CCTA was performed using a 64-slice MDCT scanner (Brilliance 64; Philips Healthcare) with a 64×0.625-mm slice collimation, a heart rate to 65 bpm, as well as sublingual nitroglycerin spray. The heart rate to 65 bpm, as well as sublingual nitroglycerin spray. The heart rate followed by 40 mL of intravenous saline (6.0 mL/s). In patients with a heart rate <65 bpm, a prospective-gated “step and shoot” protocol was used. In patients with a heart rate >65 bpm, a retrospective-gated “helical” protocol with dose modulation was used to obtain the best image quality at a minimal radiation dose.

CCTA was performed using a 64-slice MDCT scanner (Brilliance 64; Philips Healthcare) with a 64×0.625-mm slice collimation, a gantry rotation time of 420 ms, and a tube voltage of 80 to 120 kV depending on the patient’s height and weight.

CCTA Coronary Plaque Assessment
All CCTA-scans were independently analyzed by 2 cardiologists (M.H.M.W., E.M.L.), both blinded for patient details and both experienced in interpreting more than 1,000 CCTA scans. The interobserver agreement was excellent for the Agatston score (κ 0.96) and substantial for the CT plaque burden score (κ 0.79).

To indicate the location of coronary atherosclerosis, the 16-segment classification of the American Heart Association was used. The coronary artery tree was assessed using the source images on the Cardiac Comprehensive Analysis software (Philips Healthcare, Best, the Netherlands) and Terra Recon AQNet Client reconstruction software. Coronary plaques were defined as visible structures within or adjacent to the coronary artery lumen, which could be clearly distinguished from the vessel lumen and the surrounding pericardial tissue. The degree of stenosis of atherosclerotic lesions was classified as none (no luminal stenosis), mild (1 or more lesions with diameter stenosis of 20% to 50%), moderate (1 or more lesions with diameter stenosis of 50% to 70%), or severe (1 or more lesions with diameter stenosis of >70%).

CCTA Burden Scores
We used 3 scoring systems to assess coronary plaque burden.

CT plaque burden score
No CAD and mild, moderate, severe, and severe multivessel CAD were distinguished. The most diseased segment determined the final score.

Segment-Based Score
Segments with mild diameter stenosis (<50%) scored 1 point, segments with moderate diameter stenosis (50% to 70%) scored 2 points, and segments with severe diameter stenosis (>70%) scored 3 points. The total score was the sum of all points divided by the number of assessable segments.

Plaque Involvement Score
Segments with no CAD scored 0 points, and segments with CAD present scored 1 point. Total score ranged from 0 to 16.

Agatston Score
The Agatston score was calculated using the calcium scoring software of Philips Healthcare, with a threshold of 130 Hounsfield units.

Biomarker Measurement
Blood samples were obtained just before CCTA, after an overnight fast. Samples were processed within 2 hours and stored at −80°C until analysis. Total cholesterol, HDL, triglycerides, glucose, and creatinine concentrations were measured using the Synchron LX20 (Beckman Coulter). Low-density lipoprotein was calculated using the Friedewald equation, except for subjects with triglycerides >4.5 mmol/L and total cholesterol <1.3 mmol/L, in which case low-density lipoprotein was determined on the Cobas Mira Plus (Roche Diagnostics). hsCRP was measured on the BN ProSpec using the CardioPhase hsCRP assay (Siemens Diagnostics). As validated in our laboratory (NCCLS EP5-A guidelines), the between-runs coefficients of variation (CV) at 0.25 and 45 mg/L were 3.7% and 1.0%, respectively. cTnT was measured on the Elecsys 2010 using the precommercial highly sensitive fifth-generation cTnT assay (hs-cTnT) and the fourth-generation cTnT assay (Roche Diagnostics). The hs-cTnT assay was validated as reported previously with the upper reference limit (99th percentile) at 0.016 μg/L, limit of detection at 0.001 μg/L, and 10% CV cutoff at 0.009 μg/L. Interassay CVs were 3.0% and 1.4% at 0.021 and 3.03 μg/L, respectively. For the cTnT assay, the upper reference limit was <0.01 μg/L, limit of detection was <0.01 μg/L, and 10% CV cutoff was 0.03 μg/L (given by manufacturer). NT-proBNP was also measured on the Elecsys 2010, with the limit of detection at 0.6 pmol/L and the interassay CV 6.8% at 8.78 pmol/L (given by manufacturer), and in our reference population, we measured the upper reference limit (97.5th percentile) at 28 pmol/L.

Statistical Analysis
Data were analyzed using SPSS 15.0. To test for differences in patient characteristics, we used the Pearson χ² test for discrete variables and the 1-way analysis of variance test for continuous variables, including Bonferroni correction. The PROCAM, Framingham, cardiac marker concentration (hsCRP, hs-cTnT and NT-proBNP), and plaque burden (Agatston, CT plaque burden, plaque involvement, and segment-based score) scores were normalized by natural logarithm transformation. For natural log transformation of the plaque burden scores we used the score plus 1 to also include patients with a score of 0. Gender differences were tested using the Mann-Whitney U test. Correlations were calculated using Spearman’s correlation coefficient and tested with the 1-sample t test. Multinomial logistic regression analysis was performed using the stepwise method including variables with P<0.05. Receiver-
Results

Baseline characteristics of the study population are presented in Table 1. There was a positive correlation between the extent of CAD and the levels of hs-cTnT. Figure 1A shows a progressive increase \(P<0.01\) of hs-cTnT in patients with mild (median, 4.5 ng/L; interquartile range [IQR], 3.0 to 5.4 ng/L), moderate (median, 5.5 ng/L; IQR, 3.5 to 8.3 ng/L), severe (median, 5.7 ng/L; IQR, 3.7 to 8.4 ng/L), and multivessel (median, 8.6 ng/L; IQR, 5.3 to 14.3 ng/L) CAD compared with patients without CAD (median, 3.7 ng/L; IQR, 3.0 to 5.4 ng/L) as assessed with the CT plaque burden score. For the other plaque assessment scores, similar data were observed. hs-cTnT concentrations revealed a correlation with CT plaque burden score and Agatston score \(r=0.293\) and 0.353, respectively, both \(P<0.001\). Similar correlations were found using the involvement score and the segment-based score. Figure 1B shows gender differences in hs-cTnT concentrations, as described previously. Moreover, 41% of patients without CAD had hs-cTnT values in the lowest quartile, compared with 12% in the multivessel group (Figure 2). By contrast, 11% of patients without CAD showed hs-cTnT levels in the highest quartile, compared with 62% in the multivessel group \(P<0.05\).

hsCRP did not show a significant correlation with any of the plaque burden scores \(r=0.074\), \(P=0.06\) for CT plaque burden score; Figure 1C). NT-proBNP showed only modest correlation with plaque burden assessed by the different scores \(r=0.131, P<0.001\) for CT plaque burden score; Figure 1D).

Multivariance analysis (multinomial logistic regression, Table 2), using all significant risk factors from Table 1 \(P<0.05\), identified gender, smoking, age, body mass index, HDL, and hs-cTnT as independent predictors for the presence of CAD. In the second and third models, in which clinical risk profiling according to the PROCAM and Framingham risk scores was used, hs-cTnT was again identified as an independent risk factor. As shown in Table 2, for a 1-unit increase in natural log–transformed hs-cTnT, the odds ratio of having a severe lesion was 2.6, and the odds ratio of having multivessel disease was 7.8 \(P=0.01\) and \(P<0.001\), respectively.)
Comparable odds ratios were obtained in the second and third models (Table 2). Overall $\chi^2$ of the final regression model was 133.311 ($P<0.001$). The criteria for classification accuracy were satisfied, as the proportional by chance accuracy rate was below the classification accuracy rate (36.8% and 46.9%, respectively).

The AUCs for the presence of CAD for clinical risk profiling using the PROCAM and Framingham methods were 0.70 and 0.72, respectively, using receiver-operating-characteristic analysis (Figure 3). The AUC for hs-cTnT was 0.64. Addition of hs-cTnT to PROCAM risk profiling added significantly ($P=0.046$) to the AUC for the presence of CAD (AUC, 0.73), which was not the case for Framingham profiling (AUC, 0.72, $P=0.394$). For hsCRP and NT-proBNP, no significant addition to the AUC for the presence of CAD was observed for either the PROCAM method ($P=0.464$) or the Framingham method ($P=0.298$ and 0.192, respectively).

**Discussion**

In the present study, we assessed hs-cTnT levels and coronary plaque burden in 615 patients suspected of having CAD but without ACS. Our findings indicate that hs-cTnT correlates well with the CT plaque burden score and that even mild coronary atherosclerosis results in quantifiable circulating levels of hs-cTnT. In addition, hs-cTnT is an independent predictor for the presence of coronary atherosclerosis. For hsCRP, no such correlation was observed, which is in line with studies concluding that hsCRP may only have limited value in clinical risk profiling.18,19

The fourth-generation cTnT level has shown tremendous clinical value in identification patients with acute cardiac ischemia. The detection of myocardial injury is an important step in making the clinical decision to admit patients with chest pain to the coronary care unit. Studies evaluating the clinical value of cTnI were performed in patients with unstable angina pectoris20,21 and patients who underwent coronary artery bypass grafting22 or percutaneous coronary intervention.23 These studies unequivocally demonstrated that elevated levels of cTnI are associated with poorer prognosis and higher rates of major adverse cardiac events. The fourth-generation cTnT assay has a detection limit of 0.01 µg/L and is not sensitive enough to show variation in patients without cardiac ischemia. The novel, fifth-generation hs-cTnT assay
has shown a gaussian variation in healthy subjects, and it was able to measure an increase of cTnT levels after transient ischemia. The ability to measure hs-cTnT variation in normal subjects prompted us to investigate the relationship between the extent of coronary plaque burden and circulating hs-cTnT levels.

A question remains as to what the meaning of circulating cTnT levels is, in patients with variable degrees of CAD who do not have ACS. Traditionally, it was thought that release of cTn is equivalent to myocardial necrosis. However, some animal studies have suggested that short episodes of ischemia may result in the release of cTnT, without demonstration of cell death. Recently, Sabatine et al showed that a few minutes of exercise-induced ischemia in patients is sufficient to result in the release of quantifiable levels of cTnT. It is unlikely that only minutes of ischemia would have resulted in myocardial cell death in the patients exhibiting significant ischemia. Recent work of our group (unpublished data, 2009) demonstrated that 5-minute episodes of cardiac ischemia in a mouse model, followed by reperfusion, results in exposure of phosphatidylserine to the surface of cardiomyocytes and activation of caspase-3, both of which are indicative of apoptosis. However, no demonstration of cardiomyocyte cell death was made. In vitro studies have demonstrated that caspase-3 activation results in cleavage of cTnT and subsequent release. Therefore, elevated hs-cTnT levels observed in patients with CAD may be the result of activation of caspase-3 within cardiac myocytes and the resulting cleavage and release of cTnT, but not they do not necessarily implicate myocardial cell death. However, even if hs-cTnT does not reflect cell death, activation of the apoptotic program within cardiomyocytes would still be unwanted, and as such, increased release of hs-cTnT found in patients with variable degrees of CAD is undesirable.

The question is, what triggers sufficient ischemia to result in cell stress and caspase-3 activation or even cell death? In patients with severe lesions, transient cardiac ischemia could easily result from a mismatch in supply and demand during the day, triggered by physical exercise or emotional stress. However, it is puzzling how mild lesions would result in ischemia and cTnT release during
periods when the metabolic demand of the heart exceeds the supply. An alternative mechanism is suggested by the data of Rittersma et al,30 who demonstrated that in 50% of the cases, organized older thrombi were visible at the site of the culprit lesion in AMI patients admitted for thrombectomy. This suggests that thrombus formation at the site of atherosclerotic lesions is not a rare event and is not necessarily linked to clinically manifest plaque rupture and vessel occlusion. Dislodgement of these thrombi in small coronary vessels could be a potential cause for microinjury. Virmani et al30 reported that 25% to 40% of AMI cases were caused by plaque erosion, rather than rupture, and subsequent thrombus formation. Plaque erosion may therefore be an important cause of localized thrombus formation and subsequent dislodgement.

Whatever the mechanism of the cTnT release is, the fact remains that our data show that quantifiable circulating levels of hs-cTnT occur in patients with even mild CAD. Two important questions arise from this finding. The first question is whether measurement of this variation of hsTnT levels and its correlation with CAD may hold diagnostic importance or predictive value. Studies have shown that coronary atherosclerosis is responsible for at least two-thirds of ACS.30 Therefore, the outcomes of our data suggest that hs-cTnT has the potential to become a serum biomarker that will improve identification of patients at risk for developing cardiac events. The second question is whether we should put mild CAD in a different perspective and treat it more aggressively, as these conditions are associated with significantly increased circulating cTnT levels. Because percutaneous intervention for mild lesions would be out of the question, pharmacological intervention aiming at regression of atherosclerotic lesions could be the way to go. Several trials have demonstrated that high-dose statin therapy results in measurable regression of atherosclerotic lesions.31,32 It remains to be seen whether atherosclerotic regression would be sufficient to reduce the cellular stress indicated by circulating hs-cTnT levels.

Study Limitations
A study limitation may be the precision of assessment of coronary stenosis using CCTA. It is known that compared with conventional angiography, the accuracy of CCTA in the assessment of the extent of lesions is limited by spatial resolution constraints.33 However, a large number of patients were studied, and we used different methods for the assessment of CAD, providing more or less equivalent correlations with hs-cTnT levels. Also, we demonstrated a good interobserver agreement, with a $\kappa$ value of 0.79. Taken together, it is likely that the plaque burden, assessed with CCTA, is an adequate reflection of the actual plaque burden in the large patient cohort presented here. Another limitation is that participants were mainly White.

Conclusion
Coronary atherosclerosis in symptomatic patients without ACS is associated with quantifiable circulating levels of hs-cTnT, even in mild CAD.

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Disclosures
None.

References


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