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Subclinical Myocardial Necrosis and Cardiovascular Risk in Stable Patients Undergoing Elective Cardiac Evaluation

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Objective—The presence of subclinical myocardial necrosis as a prodrome to longer-term adverse cardiac event risk has been debated. The debate has focused predominantly within patients with acute coronary syndrome, and on issues of troponin assay variability and accuracy of detection, rather than on the clinical significance of the presence of subclinical myocardial necrosis (ie, “troponin leak”) within stable cardiac patients. Herein, we examine the relationship between different degrees of subclinical myocardial necrosis and long-term adverse clinical outcomes within a stable cardiac patient population with essentially normal renal function.

Methods and Results—Sequential consenting patients (N=3828; median creatinine clearance, 100 mL/min/1.73m²) undergoing elective diagnostic coronary angiography with cardiac troponin I (cTnI) levels below the diagnostic cut-off for defining myocardial infarction (<0.03 ng/mL) were evaluated. The relationship of subclinical myocardial necrosis with incident major adverse cardiovascular events (defined as any death, myocardial infarction, or stroke) over 3-year follow-up was examined. “Probable” (cTnI 0.001–0.008 ng/mL) and “definite” (cTnI 0.009–0.029 ng/mL) subclinical myocardial necrosis were observed frequently within the cohort (34% and 18%, respectively). A linear relationship was observed between the magnitude of subclinical myocardial necrosis and risk of 3-year incident major adverse cardiovascular events, particularly in those with cTnI 0.009 ng/mL or higher (hazard ratio, 3.00; 95% confidence interval, 2.4–3.8), even after adjustment for traditional risk factors, C-reactive protein, and creatinine clearance. The presence of subclinical myocardial necrosis was associated with elevations in acute phase proteins (C-reactive protein, ceruloplasmin; *P*<0.01 each) and reduction in systemic antioxidant enzyme activities (arylesterase; *P*<0.01) but showed no significant associations with multiple specific measures of oxidant stress, and showed borderline associations with myeloperoxidase, a marker of leukocyte activation.

Conclusion—In stable cardiology patients, prodromal subclinical myocardial necrosis is associated with substantially higher long-term risk for major adverse cardiovascular events. The underlying mechanisms contributing to this minimal troponin leak phenomenon warrants further investigation. (*Arterioscler Thromb Vasc Biol.* 2010;30:634-640.)

Detection of circulating cardiac troponins is associated with the presence of ongoing myocardial necrosis and fulfills the contemporary definition of myocardial infarction in the presence of ischemic signs and symptoms.¹ The prognostic role of cardiac troponin levels (either T or I subtypes) is well-established across the spectrum of acute coronary syndromes, including within patients with renal insufficiency and end-stage kidney disease, and serves as a reflection of myocardial necrosis or stress.²

The presence of “subclinical myocardial necrosis” as a prodrome to longer-term adverse cardiac event risk has been debated (ie, “troponin leak”). Some nonischemic conditions such as renal insufficiency have also been associated with detection of circulating cardiac troponin

levels,^{2–4} particularly in asymptomatic patients with end-stage kidney diseases.^{5,6} In fact, several recent reports have even associated detectable (but nondiagnostic) cardiac troponin levels in stable noncardiac subjects in the community with heightened risk for future cardiovascular events.^{7–9} At present, it is unclear whether microvascular ischemic insults, or various oxidative and inflammatory mediators, contribute to myocyte injury or apoptosis and progressive myocyte loss, leading to graded release of troponin fragments into the circulation. Herein, we sought to examine the phenotype, prognostic significance, and underlying pathophysiologic mechanisms that may contribute to the development of subclinical myocardial necrosis in stable cardiac patients.

Subjects and Methods

Study Population

The Cleveland Clinic GeneBank study is a large, prospective, cohort study that established a well-characterized clinical repository with clinical data and longitudinal outcomes from consenting subjects undergoing elective diagnostic coronary angiography from 2001 to 2006. All GeneBank participants gave written informed consent approved by the Cleveland Clinic Institutional Review Board. All blood samples were collected at the time of cardiac catheterization procedure, at which time arterial sheath access has been obtained before diagnostic catheterization or treatment (including heparin). This analysis included a cohort of 3828 consecutive consenting subjects without clinical evidence of acute coronary syndrome at the time of enrollment and is confirmed by including only those with cardiac troponin I (cTnI) <0.03 ng/mL, no history of revascularization within 30 days before enrollment, and at least 3 years of adjudicated follow-up data.

We defined coronary artery disease as any clinical history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, or angiographic evidence of significant stenosis ($\geq 50\%$) in 1 or more major coronary arteries. Dyslipidemia was defined as low-density lipoprotein cholesterol >130 mg/dL, high-density lipoprotein cholesterol <50 mg/dL, or triglyceride >150 mg/dL. An estimate of creatinine clearance was calculated using the Cockcroft-Gault equation, because a majority of subjects had relatively preserved renal function. Adjudicated outcomes were prospectively ascertained over the ensuing 3 years for all subjects after enrollment. Major adverse clinical event (MACE) was defined as death, nonfatal myocardial infarction, or nonfatal stroke after enrollment.

Subclinical Myocardial Necrosis

Plasma levels of cardiac troponin I were measured using the *STAT* Troponin I assay (Abbott Laboratories) in a research-based immunoanalyzer that provides a 3-decimal-point readout from venous blood samples collected by EDTA tubes. This assay provides highly sensitive analytic measurement of cTnI with a reported limit of detection reaching 0.009 ng/mL in the literature, and a diagnostic cut-off of 0.03 ng/mL for myocardial infarction defined by the upper limit of normal (99th percentile cut-off with 10% coefficient of variation).^{10,11} We confirmed these results by performing >10 replicates of the zero calibrator and 5 replicates of a low-level sample (target value, 0.035 ng/mL). Mean and SD were determined for each, and limit of detection was determined at 0.008 ng/mL using the formula $\text{limit of detection} = 2 \times \text{SD}_{\text{low}} \times [\text{measured levels} \div (\text{Mean}_{\text{low}} - \text{Mean}_{\text{zero}})]$.¹² Based on the analytic characteristics of the cTnI assay, we defined subclinical myocardial necrosis as “definite” in the range of cTnI 0.009 to 0.029 ng/mL, “probable” in the range of cTnI 0.001 to 0.008 ng/mL, and “none” if cTnI <0.001 ng/mL.

Biomarkers of Inflammation, Leukocyte Activation, and Oxidative Stress

High-sensitivity C-reactive protein (hsCRP), ceruloplasmin, myeloperoxidase, and serum creatinine and fasting lipid profiles were all measured simultaneously with the cTnI assay using the same analysis platform. Total leukocyte counts were analyzed on the Advia 120 Hematology Analyzer (Siemens). Serum arylesterase activity level was determined using a modification of a spectrophotometry-based assay as previously described.¹³ Briefly, initial hydrolysis rates at 25°C of phenylacetate substrate (3.6 mmol/L) were determined at 270 nm in 1:50 diluted serum in reactions mixtures comprising 9 mmol/L Tris hydrochloride, pH 8.0, and 0.9 mmol/L calcium chloride. An extinction coefficient (at 270 nm) of $1310 \text{ mol/L}^{-1} \text{ cm}^{-1}$ was used for calculating units of arylesterase activity, which are expressed as the amount of phenyl acetate hydrolyzed in $\mu\text{M}/\text{min}/\text{mL}$ of serum. In a random subset of subjects ($n=123$), multiple distinct fatty acid oxidation products (hydroxyicosatetraenoic acids, hydroxyoctadecadienoic acids, and $\text{F}_{2\alpha}$ -isoprostanes) within plasma stored in the presence of antioxidant cocktail and under inert (argon) atmosphere were determined using

high-performance liquid chromatography with online stable isotope dilution electrospray ionization tandem mass spectrometry, as previously described.¹⁴

Statistical Analysis

The Student *t* test or Wilcoxon rank-sum test for continuous variables and χ^2 test for categorical variables were used to examine the difference between the groups. Because a substantial proportion of patients had nondetectable cTnI levels (up to 37% of study cohort, corresponding to the first tertile), the study population was divided according to approximate tertiles based on cTnI levels rounded to the nearest 0.001 ng/mL. Unadjusted trends (adjusted for age and gender only) for all-cause mortality rates as well as nonfatal myocardial infarction/stroke rates with increasing tertiles of cTnI were evaluated with the Cochran-Armitage test using a time-to-event approach. Adjustments were made for individual traditional cardiac risk factor, Framingham risk factors (including age, gender, cigarette smoking, low-density and high-density lipoprotein cholesterol, systolic blood pressure), plus diabetes mellitus, log-transformed hsCRP, and creatinine clearance to predict incident 3-year MACE risks. Logistic regression models were developed to calculate odds ratios (OR) associated with the lowest tertile compared with the upper tertiles (ie, nondetectable vs detectable cTnI). Kaplan–Meier analysis with Cox proportional hazards regression was used for time-to-event analysis to determine hazard ratio (HR) and 95% confidence intervals (95% CI) for MACE. Levels of cTnI were then adjusted for traditional coronary artery disease risk factors in a multivariable model including Framingham risk factors, log-transformed hsCRP, and creatinine clearance. We confirmed that both the proportionality hazards and linearity assumptions were met. All analyses were performed using SAS version 8.2 and R 2.8.0. $P < 0.05$ was considered statistically significant.

Results

Study Population

The following is the description of reasons for cardiac catheterization within the study cohort (subjects can have >1 reason per person): history of positive or indeterminate stress test (50%), evaluation for possible ischemic causes of symptoms (66%), preoperative evaluation (12%), and history of cardiomyopathy (3%). In our study cohort, 52% of subjects (1993/3828) had evidence of cTnI detectable below the upper limit of normal (<0.03 ng/mL), including 18% (702/3,828) with “definite” (cTnI 0.009–0.29 ng/mL) and 34% (1291/3828) with “probable” (cTnI 0.001–0.008 ng/mL) subclinical myocardial necrosis. Baseline characteristics of the study population are shown in Table 1, stratified according to presence or absence of subclinical myocardial necrosis.

Overall, 68% of subjects had $\geq 50\%$ stenosis at any coronary artery, 32% of subjects had previous revascularization, and 84% had no subsequent revascularization within 30 days after catheterization. There were no significant differences in the prevalence of none, probable, or definite subclinical myocardial necrosis between these subgroups. However, we observed a trend toward higher rates of any (probable or definite) subclinical myocardial dysfunction in those who underwent subsequent revascularization within 30 days of cardiac catheterization compared to those who did not (61% vs 51%), as well as those with $\geq 50\%$ stenosis at any coronary artery compared to those without (55% vs 47%). Only 17% had a history of heart failure and 22% had left ventricular ejection fraction $<50\%$. Patients with evidence of subclinical myocardial necrosis were more likely to be older, with more cardiovascular risk factors and underlying heart failure/impaired left ventricular ejection fraction, and with

Table 1. Subject Characteristics

	Subclinical Myocardial Necrosis		
	None (n=1835)	Probable (n=1291)	Definite (n=702)
cTnI range, ng/mL	<0.001	0.001–0.008	0.009–0.029
Demographics and clinical data			
Age, y	63±11	63±11	66±11
Male, %	66	65	69
History of diabetes mellitus, %	29	31	41*
History of hypertension, %	70	71	75
Cigarette smoking (former/current), %	65/11	64/9	66/10
Previous coronary artery disease, %	68	66	76*
History of heart failure, %	11	15	33*
LV ejection fraction <50%, %	13	23	42*
Maximal stenosis ≥50%, %	65	69	76*
Framingham ATP III risk score	13 (11–15)	14 (11–15)	14 (12–16)
Laboratory data			
LDL cholesterol, mg/dL	95 (78–117)	97 (80–118)	92 (76–112)
HDL cholesterol, mg/dL	34 (28–41)	34 (28–41)	32 (27–40)
Triglycerides, mg/dL	114 (84–162)	115 (84–166)	115 (83–156)
C-reactive protein, mg/L	1.99 (0.91–4.51)	2.11 (0.86–4.58)	2.68 (1.19–6.24)*
Creatinine clearance, mL/min/1.73m ²	100 (77–126)	101 (76–126)	85 (62–113)*
Medications			
Aspirin, %	74	73	70
Statin, %	60	58	54
ACE inhibitors, %	44	51	60*
Beta-blockers, %	59	63	63†
Nitrates, %	29	28	32

ACE indicates angiotensin-converting enzyme; ATP III, Adult Treatment Panel III guidelines; cTnI, cardiac troponin I; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricular.
 Values expressed in mean±SD or median (interquartile range).
 **P*<0.01.
 †*P*<0.05.

slightly lower renal function at baseline, although overall they were still within the normal range (Table 1).

Subclinical Myocardial Necrosis and Prognosis

Overall, patients with evidence of subclinical myocardial necrosis (either probable or definite) were associated with a 2.2-fold increased risk of 3-year incidence of MACE (HR, 2.18; 95% CI, 1.77–2.68; *P*<0.0001) when compared to those with no evidence of subclinical myocardial necrosis. When analyzed at the limit of detection cut-off (0.008 ng/mL), those with evidence of subclinical myocardial necrosis still conferred significantly higher risk of death (HR, 2.23; 95% CI, 1.81–2.74; *P*<0.01), nonfatal myocardial infarction, and stroke (HR, 2.23; 95% CI, 1.81–2.74; *P*<0.01), and MACE (HR, 2.27; 95% CI, 1.86–2.76; *P*<0.01) at 3-year follow-up. After adjusting for traditional risk factors including Framingham risk factors, hsCRP, and creatinine clearance, evidence of subclinical myocardial necrosis (probable or definite) within stable cardiac patients remained a significant increased risk of incident MACE at 3 years (HR, 1.77; 95% CI, 1.43–2.18; *P*<0.01). Such prognostic value remained significant in various subgroups stratified by age,

gender, presence of diabetes mellitus, or in those with creatinine clearance ≥60 mL/min (but not <60 mL/min). Also, the results were similar when analyzed only within the cohort of patients without any subsequent revascularization over the ensuing 3 years of follow-up (68% of cohort; Table 2). The results also extended to those with no history of heart failure (adjusted HR for MACE, 2.09; 95% CI, 1.51–2.90; *P*<0.01), those with preserved (ie, ≥50%) left ventricular ejection fraction (adjusted HR for MACE, 2.83; 95% CI, 1.80–4.45; *P*<0.01), and those with statin therapy at baseline (adjusted HR for MACE, 2.04; 95% CI, 1.43–2.92; *P*<0.01).

There was a noticeable risk increase in those with probable subclinical myocardial necrosis (cTnI between 0.001–0.008 ng/mL) vs those without any detectable cTnI (<0.001 ng/mL). This graded risk increase can be clearly illustrated in the Kaplan–Meier analysis shown in Figure 1. The HR for adverse cardiac events appear to increase in a nearly linear fashion above cTnI levels of 0.004 ng/mL (Figure 2). Table 2 demonstrates the relationship between cTnI ranges with 3-year unadjusted and adjusted risks for incidence of death, myocardial infarction, and stroke, as well as MACE at 3 years, stratified by definite or probable subclinical myocar-

Table 2. Adjusted HR for Major Adverse Cardiac Events at 3-Year Follow-Up

Subclinical Myocardial Necrosis, n	All Subjects			Medically Managed Coronary Disease		
	None (n=1835)	Probable (n=1291)	Definite (n=702)	None (n=894)	Probable (n=627)	Definite (n=374)
cTnI range, (ng/mL)	<0.001	0.001–0.008	0.009–0.029	<0.001	0.001–0.008	0.009–0.029
All-cause death						
N of events	66	96	101	43	69	72
Unadjusted HR	1.0	2.06 (1.51–2.82)*	4.09 (3.00–5.58)*	1.0	2.32 (1.58–3.39)*	4.28 (2.93–6.23)*
Age-adjusted/gender-adjusted HR	1.0	1.86 (1.36–2.55)*	3.36 (2.45–4.61)*	1.0	2.06 (1.41–3.03)*	3.56 (2.42–5.24)*
Risk factor-adjusted HR	1.0	1.70 (1.24–2.34)*	2.43 (1.73–3.41)*	1.0	1.96 (1.33–2.87)*	2.90 (1.94–4.35)*
Nonfatal MI/stroke						
N of events	62	60	41	40	34	21
Unadjusted HR	1.0	1.76 (1.38–2.25)*	2.92 (2.27–3.76)*	1.0	1.78 (1.32–2.41)*	2.79 (2.06–3.80)*
Age-adjusted/gender-adjusted HR	1.0	1.64 (1.28–2.10)*	2.54 (1.96–3.28)*	1.0	1.62 (1.20–2.19)*	2.40 (1.75–3.28)*
Risk factor-adjusted HR	1.0	1.54 (1.20–1.98)*	1.98 (1.51–2.61)*	1.0	1.56 (1.15–2.12)*	2.04 (1.47–2.84)*
Death/nonfatal MI/stroke						
N of events	128	156	142	83	103	93
Unadjusted HR	1.0	1.74 (1.38–2.20)*	3.00 (2.36–3.81)*	1.0	1.80 (1.35–2.40)*	2.88 (2.14–3.87)*
Age-adjusted/gender-adjusted HR	1.0	1.62 (1.28–2.05)*	2.60 (2.04–3.32)*	1.0	1.63 (1.22–2.19)*	2.48 (1.84–3.36)*
Risk factor-adjusted HR	1.0	1.54 (1.21–1.95)*	2.09 (1.61–2.71)*	1.0	1.58 (1.18–2.12)*	2.12 (1.54–2.93)*

MI indicates myocardial infarction.

Values presented as HR and 95% CI in brackets.

Medically managed coronary disease is defined as those with significantly obstructive (>50% stenosis at any vessel) coronary disease but without revascularization within 30 days after catheterization.

* $P < 0.01$ and † $P < 0.05$ (compared with “none” category): (1) unadjusted; (2) adjusted for age and gender; and (3) adjusted for individual Framingham risk factors, including age, gender, low-density lipoprotein, high-density lipoprotein cholesterol, systolic blood pressure, smoking, diabetes, creatinine clearance, and high-sensitivity C-reactive protein.

dial necrosis. Remarkably, the presence of subclinical myocardial necrosis provided superior prognostic value for adverse long-term outcomes compared to other established cardiac risk factors (Figure 3).

In an effort to better-understand biological processes associated with development of subclinical myocardial necrosis, we examined the relationship between detectable cTnI levels and markers of inflammation, leukocyte activation, and systemic oxidative stress (Table 3). In subjects with subclinical myocardial necrosis, we observed graded and significantly higher levels in acute phase reactants, such as hsCRP and

ceruloplasmin, consistent with heightened systemic inflammation. In contrast, there did not appear to be an association between levels of cTnI and leukocyte count or plasma myeloperoxidase levels, an index of leukocyte activation. Nevertheless, patients with definite subclinical myocardial necrosis had significantly higher plasma myeloperoxidase levels compared to those with no subclinical myocardial necrosis ($P = 0.04$) or those with probable subclinical myocardial necrosis ($P = 0.03$). Interestingly, quantification of multiple distinct fatty acid oxidation products within plasma failed to show any evidence of enhanced

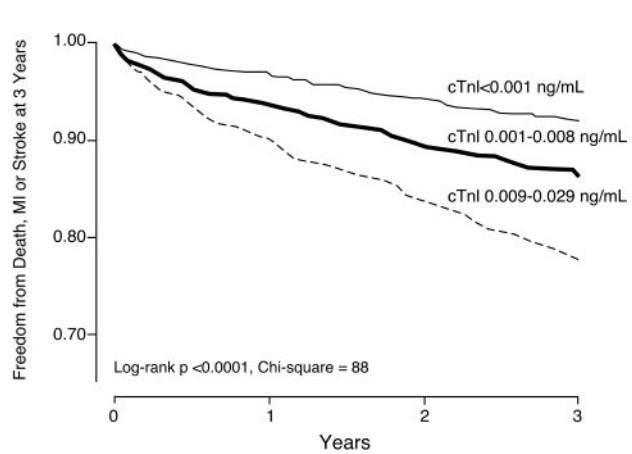


Figure 1. Kaplan-Meier analysis for 3-year major adverse clinical events, stratified according to definite (cTnI=0.009–0.029 ng/mL), probable (cTnI=0.001–0.008 ng/mL), and no (cTnI<0.001 ng/mL) subclinical myocardial necrosis.

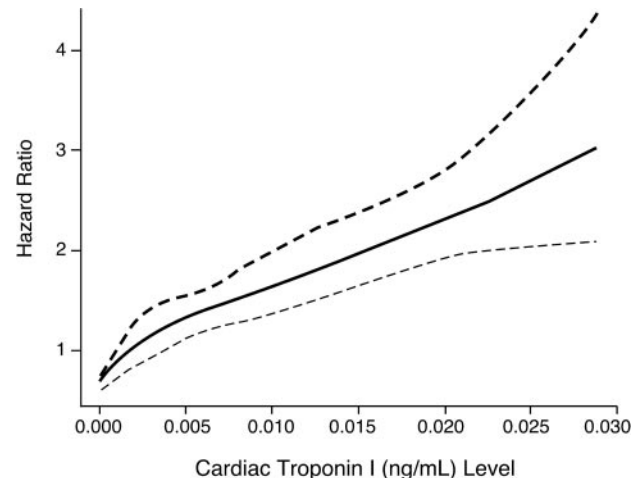


Figure 2. Cubic spline curve of HR for major adverse clinical events (death, nonfatal myocardial infarction, and stroke) at 3 years with cTnI levels.

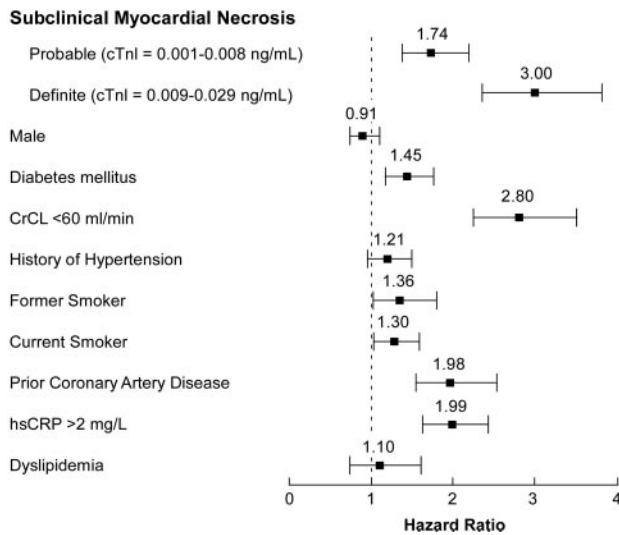


Figure 3. Forest plot of risk prediction for major adverse clinical events at 3 years according to cTnI cohorts in comparison with standard cardiovascular risk factors in multivariable Cox proportional hazard analysis. Major adverse clinical events defined as death, nonfatal myocardial infarction, and stroke over the ensuing 3 years of follow-up. CrCL indicates creatinine clearance.

oxidant stress among subjects with vs without evidence of subclinical myocardial necrosis (Table 3). However, serum levels of arylesterase activity, a cardioprotective antioxidant activity catalyzed by the high-density lipoprotein associated protein paraoxonase-1,⁷ were significantly ($P<0.01$) lower in

those with evidence of subclinical myocardial necrosis (Table 3). When tested for interaction with baseline statin therapy, only hsCRP demonstrated significant interaction ($P=0.014$) and arylesterase activity with borderline significance ($P=0.05$). Cardiac troponin I levels and all other inflammatory and oxidative biomarkers demonstrated no significant interaction with statin use.

Discussion

Myocardial necrosis is considered the primary consequence of ischemia during acute coronary syndromes and provides not only robust prognostic value but also clinical indication for therapeutic interventions. Over the years, improved recognition of assay limitations, as well as detection of a subset of patients with a “troponin leak” below the standard diagnostic range, has emerged. Discussions regarding the clinical utility of cardiac troponins in plasma have focused on analytic precision and accuracy, and revised definitions of 99th percentile cut-off values among apparently healthy subjects have been proposed as the upper limit of normal for diagnostic purposes.¹⁵ The prognostic utility of detecting a “troponin leak” below this cut-off has been debated and, correspondingly, whether pathophysiologic mechanisms are at play leading to detection of a “subclinical cardiac troponin leak” has remained unclear. Moreover, the clinical necessity of improved sensitivity cardiac troponin assays has also been intensively debated because few studies have examined the clinical prognostic value, particularly among stable patients, or with use of a central reference laboratory and a high-sensitivity assay capable of monitoring levels below the

Table 3. Inflammatory and Oxidative Biomarkers in Subgroup Stratified by Presence of Subclinical Myocardial Necrosis

Biomarkers	Subclinical Myocardial Necrosis			P
	None (n=1835)	Probable (n=1291)	Definite (n=702)	
Acute-phase reactants				
C-reactive protein	1.71 (0.84–3.81)	2.11 (0.86–4.59)	2.68 (1.19–6.25)	<0.01
Ceruloplasmin	22 (19.0–25.6)	23.2 (20.3–27.4)	24.2 (20.8–28.1)	<0.01
Leukocyte activation				
Total leukocyte count	6.1 (5.0–7.4)	6.0 (5.0–7.2)	6.2 (5.0–7.5)	0.78
Myeloperoxidase	98 (68–193)	102 (71–186)	113 (78–221)	0.07
Antioxidative pathways				
Arylesterase activity	105.2 (89.6–123.4)	103 (87.8–120.5)	95.5 (79.7–113.8)	<0.01
Oxidative fatty acids				
	(n=33)	(n=61)	(n=29)	
5-HETE	14.7 (9.6–20.3)	12.8 (8.8–20.1)	15.3 (12.0–21.0)	0.91
8-HETE	2.2 (1.6–3.0)	2.1 (1.3–3.1)	2.5 (1.7–3.4)	0.53
9-HETE	29.2 (18–42.9)	24.6 (16.5–42.6)	33.0 (22.0–43.9)	0.28
11-HETE	5.05 (4.05–5.94)	4.57 (3.47–6.29)	4.80 (3.38–6.20)	0.37
12-HETE	6.62 (5.07–8.50)	5.77 (3.94–9.05)	6.18 (4.97–8.20)	0.79
15-HETE	22.2 (14.5–27.4)	18.1 (11.6–26.7)	19.9 (10.8–25.5)	0.36
9-HODE	29.3 (22.9–35.1)	27.3 (16.9–38.6)	28.6 (16.8–37.7)	0.74
13-HODE	31.5 (23–38.8)	28.2 (18.7–43.8)	28.5 (17.8–40.5)	0.48
8-isoPGF _{2α}	7.9 (2.0–18.5)	6.1 (1.7–15.6)	7.4 (2.7–15.8)	0.46

8-iso PGF_{2α} indicates 8-isoprostane prostaglandin F_{2α}; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; all expressed in pM/mL.

*All levels expressed in median (interquartile range).

P by ANOVA.

classic clinical threshold for diagnosis of an ischemic event. Our observations provide strong support for the concept that any detectable cTnI level heralds enhanced long-term adverse cardiovascular event risk. We also demonstrated that within the cohort with detectable subclinical myocardial necrosis, there were heightened inflammatory and reduced antioxidative processes, even though there was only borderline evidence of significant leukocyte activation and no evidence of enhanced systemic indices of oxidant stress. The findings of heightened cardiovascular risk at levels equal or greater to traditional cardiovascular risk factors are consistent with recent report using a high-sensitivity cardiac troponin T assay¹⁶ and support the notion that detection of any cTnI within plasma warrants more globally aggressive risk reduction efforts and closer long-term monitoring because this represents a high-risk group of patients. It also suggests that therapeutic efforts at reducing cTnI leak and incident development of future adverse cardiovascular events in this group should also be considered.

Our data significantly extend the clinical information available about detection of subclinical myocardial necrosis in multiple aspects. Recent registry data have suggested the prognostic value of detectable levels of cardiac troponin (thus far determined qualitatively as above the “upper limit of normal”) in patients, such as those presenting with acute decompensated heart failure for detecting in-hospital mortality,¹⁷ or in those undergoing percutaneous coronary intervention for detecting up to 1-year mortality risk.¹⁸ Instead, our study focused entirely on individuals with cTnI levels below the upper limit of normal and on a large number of subjects without any subsequent coronary revascularization. The large, single-center cohort experience of stable subjects undergoing cardiac evaluation allows careful description of a study cohort regarding their selection, stability, and degree of atherosclerotic burden. The advantage of analyzing cTnI levels with the same assay in a core reference laboratory using a research-based immunoanalyzer allows the opportunity to quantify long-term risk beyond a dichotomous categorization of “positive” vs “negative” level (ie, above vs below the limit of detection) grossly utilized in published literature on this topic. A clear dose–response relationship for increased risk and relatively tight CI was observed within our cohort (Figure 2). The availability of traditional clinical and biochemical risk markers for multivariable adjustments also strengthen the support of the independent added clinical significance of detecting the presence of subclinical myocardial necrosis.

One of the more remarkable observations in the present study is how pervasive “troponin leak” is within a stable cardiology patient population. Almost half of the cohort had detectable cTnI levels, among which 65% were within the “probable” range (0.001–0.008 ng/mL). Of interest, only approximately one-third (37%) of patients with measurable plasma cTnI levels also demonstrated significant ($\geq 50\%$ stenosis in ≥ 1 major vessels) obstructive coronary artery disease on coronary angiography, suggesting the other two-thirds of subjects are at increased risk; however, this would not be observed using traditional risk stratification methods or invasive testing that relies on significant luminal narrowing (ie, most imaging methods). Whereas evidence of macrovascular ischemia as a contributor to the observed troponin

leak seems remote, the presence of vulnerable plaque with microvascular ischemic insults or microembolisms may be more prevalent than previously appreciated and serve as a contributory mechanism to the development of subclinical myocardial necrosis. The concomitant presence of heightened inflammatory and reduced antioxidative processes among subjects with subclinical myocardial necrosis suggests the potential for some common underlying pathophysiologic processes at play. Interestingly, our results did not identify differences in systemic levels of leukocytes or elevations in multiple distinct oxidized fatty acid species, and identified only borderline associations with myeloperoxidase levels, a marker of leukocyte activation. These results may suggest that the pathophysiologic process leading to subclinical myocardial necrosis may be regional or partially independent of leukocyte-mediated progression of disrupting vulnerable plaques, as seen in macrovascular ischemia.

The prognostic value in the “borderline” range of cTnI may vary widely depending on which assay is being used.¹⁹ There are several potential interferences that may influence circulating levels of cTnI, including the presence of heterophile antibodies or other noncardiac confounders such as renal insufficiency.^{10,20,21} The prevalence of 18% with definite subclinical myocardial necrosis (0.009–0.29 ng/mL range) in our study population is comparable with some but not all previous reports in the literature (up to 22% in an elderly population with $>0.01 \mu\text{g/L}$ by Beckman Coulter Access AccuTnI assay in a European cohort²²). In particular, detectable cardiac troponin T levels in otherwise healthy elderly subjects was also associated with poorer long-term prognosis (even though only 4% of that specific population had detectable levels).⁷ Whether the detection of subclinical myocardial necrosis can allow therapeutic targeting of potential modifiable risk factors for reversal or delay of the progression of disease processes remains to be determined. Further investigations are warranted regarding the potential for aggressive preventive strategies based on identification of an at-risk population defined by the presence of subclinical myocardial necrosis.

Study Limitations

A strength of the present study is that the study cohort is an aggressively treated cardiology population in the modern era of high-potency statin therapy. However, this also leads to a clear selection bias in the study population because they were deemed to have enough clinical suspicion (either by positive or equivocal stress test results or clinical presentation) to warrant an elective diagnostic invasive coronary angiographic evaluation. The diagnostic range of cTnI levels used in this study overlaps with a majority of otherwise “healthy controls,”¹⁰ which argues to the broader potential generality of the present findings beyond the relatively higher-risk population examined in the present clinical setting. Such low ranges are not routinely reported in standard laboratory evaluations, and their accuracy and reproducibility have not been extensively evaluated even though their sensitivities have been comparable to other sensitive cardiac troponin assays.²³

Conclusion

In stable patients undergoing elective cardiac evaluation, detectable cTnI levels by high-sensitivity assay are com-

monly observed despite being well below the consensus-defined diagnostic range. The present studies indicate that subclinical myocardial necrosis is a relatively common pathophysiologic phenomenon in the cardiology patient setting, and that detection of any cTnI within plasma may provide independent risk prediction of near-term and long-term adverse clinical events.

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Disclosures

W.H.W. Tang received research grant support from Abbott Laboratories, Inc. S.L. Hazen reports being listed as co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics; having been paid as a consultant or speaker for AstraZeneca Pharmaceuticals LP, BG Medicine, Inc, Merck & Co, Inc, Pfizer Inc, PrognostiX, Inc, and Takeda; having received research funds from Abbott and PrognostiX Inc; and having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and PrognostiX Inc, Abbott Laboratories, Inc, Biosite Incorporated, Frantz Biomarkers, LLC, and Siemens. Y. Wu, S.J. Nicholls, D.M. Brennan, M. Pepoy, S. Mann, A. Pratt, and F. Van Lente have no potential conflicts to disclose with regard to this study.

W.H.W. Tang and S.L. Hazen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; they were responsible for the study concept and design, acquisition of data, and study supervision. W.H.W. Tang, Y. Wu, S.J. Nicholls, D.M. Brennan, M. Pepoy, S. Mann, A. Pratt, F. Van Lente, and S.L. Hazen were responsible for analysis and interpretation of data. Y. Wu and D.M. Brennan were responsible for statistical analysis. W.H.W. Tang drafted the manuscript. W.H.W. Tang, Y. Wu, S.J. Nicholls, F. Van Lente, and S.L. Hazen were responsible for critical revision of the manuscript for important intellectual content. No medical writers or editors were involved in the preparation of the manuscript.

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