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Highly sensitive cardiac troponin-I is a biomarker for occult coronary plaque burden and cardiovascular events in patients with rheumatoid arthritis

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ABSTRACT

Objectives. Patients with Rheumatoid Arthritis (RA) display greater occult coronary atherosclerosis burden and experience higher cardiovascular morbidity and mortality compared to controls. We here explored whether proinflammatory cytokines and highly-sensitive cardiac troponin-I (hs-cTnI)- a biomarker of myocardial injury- correlated with plaque burden and cardiovascular events (CVE) in RA.

Methods. We evaluated 150 patients with 64-slice coronary computed tomography angiography (CCTA). Coronary artery calcium (CAC), number of segments with plaque (SIS), stenotic severity (SSS), and plaque burden (PBS) were assessed. Lesions were described as non-calcified, mixed, or fully calcified. Blood levels of hs-cTnI and proinflammatory cytokines were assessed during CCTA. Subjects were followed over 60±26 months for CVE, both ischemic [cardiac death, non-fatal myocardial infarction (MI), stroke, peripheral arterial ischemia] and non-ischemic [new onset heart failure hospitalization].

Results. Plasma hs-cTnI correlated with all coronary plaque outcomes ($p<0.01$). Elevated hs-cTnI (≥1.5 pg/ml) further associated with significant calcification, extensive atherosclerosis, obstructive plaque, and any advanced mixed or calcified plaques after adjustments for cardiac risk factors or D'Agostino Framingham scores (all $p<0.05$). Eleven patients suffered CVE (1.54/100PY); eight ischemic and three non-ischemic. Elevated hs-cTnI predicted all CVE risk independently of demographics, cardiac risk factors, and prednisone use ($p=0.03$). Conversely, low hs-cTnI presaged lower risk for both extensive atherosclerosis ($p<0.05$) and incident CVE ($p=0.037$).

Conclusion. Plasma hs-cTnI independently associated with occult coronary plaque burden, composition, and long-term incident CVE in patients with RA. Low hs-cTnI forecasted lower risk for both extensive atherosclerosis as well as CVE; hs-cTnI may therefore optimize cardiovascular risk stratification in RA.

Keywords: occult coronary atherosclerosis, cardiovascular events, highly-sensitive cardiac troponin I, Rheumatoid arthritis

Introduction

Individuals with RA experience a higher rate of CVE compared to controls [1]. This may be explained by greater prevalence, severity, burden, and different composition of occult coronary lesions in RA compared to age and gender-matched controls [2]. Residual disease activity may further associate with more advanced, complex, prone to rupture coronary plaques [2]. Proinflammatory cytokines such as tumor necrosis factor-a (TNFa), Interleukin-6 (IL-6), and interleukin-17 (IL-17) reflect clinical activity and structural damage in RA and are higher in the blood of RA patients compared to controls [3]; the same cytokines have been identified in atherosclerotic plaque [4-7], and correlated with subclinical atherosclerosis independently of cardiac risk factors [8], coronary plaque complexity [9], plaque destabilization and CVE in subjects without autoimmune disease [10-12]. Nevertheless, the relationship between these cytokines and occult coronary plaque burden and composition in RA are unknown. Higher plaque load or vulnerability may be further reflected in elevations of biomarkers specific for myocardial injury [13]; indeed, cardiac troponin elevations measured by highly sensitive assays- and below thresholds used to diagnose acute coronary syndromes- were associated with higher CAC scores in a population-based study [13]. Additionally, both hs-cTnT and hs-cTnI predicted higher risk of fatal and non-fatal coronary heart disease (CHD), heart failure hospitalization, and overall mortality in the general population [13-16].

In a recent report, hs-cTnI was higher in RA patients compared to controls, independently of cardiovascular risk factors and inflammation [17]. Nevertheless, its association with subclinical coronary artery disease burden or its ability to predict future

CVE in RA are unknown. We here hypothesized that hs-cTnI and various proinflammatory cytokines may correlate with the presence, burden, and composition of occult coronary plaque in patients with RA evaluated with CCTA. We further postulated that hs-cTnI at the time of CTA might predict incident CVE on long-term follow-up (60±26 months).

Methods

Patient Recruitment

One hundred and fifty RA patients from a single center were enrolled and prospectively evaluated on a first come first served basis [2]. The study was approved by the local Institutional Review Board, all subjects signed informed consent, and the research was carried out in compliance with the Helsinki declaration. Inclusion criteria comprised ages ≥18 years, fulfillment of 2010 classification criteria for RA, and no symptoms or history of cardiovascular disease such as myocardial infarction (MI), revascularization, heart failure, transient ischemic attack (TIA), stroke, or peripheral arterial disease (PAD). Patients with concomitant autoimmune syndromes, malignancy ~~under treatment~~ within <5 years, chronic or active infection, weight >325 pounds (147.7 kg), hypotension [~~(~~systolic b~~B~~lood pressure (SBP) <90mmHg or diastolic blood pressure (DBP) <60mmHg]~~)~~ or hypertension (SBP >170mmHg or DBP >110 mmHg), uncontrolled tachycardia, irregular rhythm, iodine allergy, or glomerular filtration rate (GFR)< 60 ml/min were excluded.

Hypertension was defined as SBP ≥140mmHg or DBP ≥90mmHg or antihypertensive use. Diabetes mellitus (DM) encompassed HgbA1c>6.5%, or hypoglycemic medication

use. Hyperlipidemia constituted fasting cholesterol >200 mg/dl, or LDL>130 mg/dl. Current smoking entailed cigarette consumption within 30 days from screening. Positive family history was defined as coronary artery disease (CAD) in first-degree relatives younger than 55 for males or 65 for females. The Framingham 2008 D'Agostino modified general cardiovascular risk score (FRS-DA) was calculated for all study participants [18]. Disease duration, serologic status, radiographs and treatments were captured. RA activity was evaluated by a 28-joint count and c-reactive protein (DAS28-CRP).

Laboratory evaluations

Blood for regular chemistries, fasting lipids, erythrocyte sedimentation rate (ESR) and high sensitivity C-reactive protein (hs-CRP) was collected on the day of CCTA and evaluated at the local laboratory. Additionally, blood was collected in EDTA tubes, immediately processed and plasma was frozen at -80^{o}C until it was assayed. Hs-cTnI was measured at Singulex Inc. (Alameda, CA) by technicians blinded to the clinical data using a micro-particle immunoassay and single-molecule counting [19]. TNFa, IL-6, IL-17A and F, and vascular endothelial growth factor (VEGF) were also assessed using laboratory developed tests at Singulex based on single molecule counting [19].

Multi-Detector Computed Tomography Angiography (CTA)

Scans were performed with a 64-multidetector row Lightspeed VCT scanner (GE Healthcare) between 3/2010-3/2011, and images analyzed as previously described by a single, blinded interpreter (BMJ) [20]. CAC was quantified by the Agatston method [21].

Coronary arteries were evaluated on contrast-enhanced scans using a standardized 15-segment model [22]. Stenosis severity was scored from 0 to 4 based on grade of luminal restriction; 1 represented 1-29% stenosis, 2: 30-49%, 3: 50-69%, and 4: ≥70% stenosis. The area of each plaque visualized in at least 2 adjacent slices (slice thickness 0.625 mm) was determined on all affected slices. Plaque burden was graded from 0-3, defined as none (0), mild (1), moderate (2), and severe (3), based on the number of adjacent slices containing plaque. Lesions rendering >50% stenosis were considered obstructive. Plaque composition was defined as non-calcified (NCP), mixed (MP), or calcified (CP) as elsewhere discussed [23]. Subjects received 3 individual quantitative scores [23]; segment involvement score (SIS) represented the total number of segments with plaque (0-15); stenosis severity score (SSS) reported the cumulative stenosis grade conferred by plaque over all evaluable segments (0-60); plaque burden score (PBS) described the cumulative plaque size over all evaluable segments (0-45). Reproducibility of scoring measurements for our center has been previously reported [23].

Incident Cardiovascular Events (CVE)

All patients were followed for incident CVE over a period of 60±26 months. Those included both ischemic [cardiac death, non-fatal myocardial infarction (MI), stroke, TIA, PAD] as well as non-ischemic ones [new onset heart failure hospitalization]. Event adjudication was elaborated by the treating cardiologist, neurologist, or vascular surgeon respectively and based on standard definitions [24-26].

Analysis

Continuous variables were expressed as medians with inter-quartile ranges (IQR) and categorical ones as numbers with percentages. Spearman-Rho correlation coefficients evaluated preliminary associations between biomarkers and plaque outcomes. Medians between CVE groups were compared using the Mann-Whitney U-test, counts by the X^2 test. Further analyses were restricted to biomarkers with significant differences. Plaque outcomes were binarized based on median; those were >0 vs. 0 for CAC, >1 vs. ≤1 for SIS, >1 vs. ≤1 for SSS, and >2 vs. ≤2 for PBS. To evaluate plaque composition, the presence of MP or CP vs. the absence of both was used as an outcome. Similarly, hs-cTnI was binarized as "high" (>1.5pg/ml) vs. "low" (≤1.5pg/ml). Logistic regression models were constructed to evaluate associations between hs-cTnI and individual plaque parameters; models were adjusted either for age and gender (model 1), or additionally for hypertension, diabetes, hyperlipidemia, statin use, smoking, body mass index (BMI), and prednisone use (model 2), or for the patients' FRS-DA score (model 3). Similar logistic regression models were devised to predict individual or composite high-risk plaque outcomes; individual ones included CAC>100 vs. ≤100, SIS>5 vs. ≤5, SSS>5 vs. ≤5, and presence of obstructive plaque vs. not. Composite outcomes entailed presence of SSS>5 or CAC>100 vs. neither, and SIS>5 or SSS>5 or obstructive plaque vs. none. Results were reported as odds ratios with 95% confidence intervals. For composite and plaque composition outcomes, sensitivity, specificity, negative, and positive predictive values were determined with standard formulas. Cox proportional hazards regression analysis evaluated CVE risk (HR) associated with high cTnI (>1.5pg/ml) in raw and several adjusted models (model 1, 2, and 3) as previously described. Kaplan-Meier curves were compared by the log-rank method. Diagnostic

accuracy of CVE risk assessment for FRS-DA alone, FRS-DA+ hs-cTnI, and FRS-DA+ hs-cTnI+ high-risk composite outcome was evaluated as area under the receiver operator characteristic curves (AuROC) and compared with the DeLong method. Integrated discrimination improvement (IDI) between these constructs was computed, and improvement in prediction accuracy was evaluated considering p-value <0.05 as significant. Data was analyzed with SAS v9.4 or R v3.4.1.

Results

Patient demographics appear in table 1. Subjects were predominantly female, with established, seropositive, erosive, and well-controlled disease. RA parameters and traditional cardiac risk factors were not significantly different in patients incurring CVE; by contrast, FRS-DA, coronary atherosclerosis burden, including high-risk plaque parameters, and higher levels of hs-cTnI were significantly higher (all p<0.05).

Correlations of cytokines and hs-cTnI with occult coronary atherosclerosis

TNFa, IL-6, IL-17A, IL-17F, and VEGF showed no correlations with any coronary plaque parameters or hs-cTnI (online table 1); neither did ESR, CRP, TJC, SJC, or DAS28-CRP (not shown). Hs-cTnI was detectable in all patients- 1.5 (1.1-2.6) pg/ml; patients with any plaque had higher levels compared to those without [1.8 (1.1-2.6) pg/ml vs. 1.3 (0.9-1.8) pg/ml, p=0.02]. Moreover, hs-cTnI was correlated with all occult coronary plaque outcomes (all p <0.01). CAC, SIS, SSS, and PBS substantially increased from the lowest to the highest hs-cTnI tertile (p for trend of 0.006, 0.005, 0.01, and 0.009 respectively). Similarly, high-risk plaque outcomes such as CAC>100, SIS>5, SSS>5,

obstructive plaque, composite outcome, and presence of any advanced MP/CP lesions were considerably enriched across higher hs-cTnI tertiles (figure 1).

Hs-cTnI independently correlates with plaque burden and composition

Hs-cTnI levels associated with all plaque outcomes (table 2). After controlling for age and gender (model 1), additional adjustments for hypertension, diabetes, hyperlipidemia, statin use, smoking, BMI, and prednisone use (model 2), or FRS-DA score (model 3), hs-cTnI remained predictive of CAC, SSS and PBS. Furthermore, it associated with presence of more advanced mixed or calcified plaques whereas it showed no correlation with earlier, non-calcified lesions. Importantly, hs-cTnI further correlated with high-risk outcomes such as obstructive plaque, SSS>5, CAC≥100, or composite end points (table 2); significance persisted for several, even after adjustments for cardiac risk factors or FRS-DA scores.

Conversely, subjects with low hs-cTnI (<1.5 pg/ml) were less likely to have extensive coronary atherosclerosis; specifically, they displayed 81% lower risk of having SSS>5 or CAC≥100 and 70% less risk of obstructive plaque, SIS>5, or SSS>5 after controlling for FRS-DA score; area under the curve (AUC) improved from 0.79 (0.63-0.95) to 0.85 (0.72-0.98), $p<0.05$ (not shown). Out of all patients, 27 (18%) had CAC>100 or SSS>5 and 22 (15%) had obstructive plaque or SIS>5 or SSS>5. Compared to all patients, only 8% with low hs-cTnI displayed those respective plaque outcomes (online table 2); of patients with both low hs-cTnI and low FRS-DA scores, only 4% had extensive atherosclerosis compared to 11% of those with just low FRS-DA.

Elevated Hs-cTnI associates with long-term CVE in RA

Eleven patients suffered CVE during 60±26 months of follow-up (1.54/100PY): 8 were ischemic, including 1 cardiac death, 3 NSTEMI (non-ST elevation myocardial infarctions), 2 strokes, and 2 PAD events requiring revascularizations; the 3 non-ischemic events were new onset, hospitalized, heart failure. Hs-cTnI was higher in patients with CVE vs. those without [2.6 (2.1-4.4) vs. 1.5 (1.0-2.4) pg/ml, p=0.006). Elevated hs-cTnI predicted risk of incident CVE (Figure 2A, p=0.03), independently of demographics and traditional cardiac risk factors (Table 3). Importantly, patients with low hs-cTnI were 82% less likely to suffer CVE.

Hs-cTnI enhances cardiovascular event risk prediction when added to cardiac risk scores

The prognostic accuracy of FRS-DA alone vs. FRS-DA+ hs-cTnI and FRS-DA+ hs-cTnI+ high-risk plaque for CVE, based on AUC of the respective ROC curves, is depicted on Figure 2B. Addition of hs-cTnI information to FRS-DA score yielded higher prognostic accuracy (0.8431 vs. 0.7283, p=0.10); further addition of high-risk plaque information from CCTA [obstructive plaque or SIS>5 or SSS>5] resulted in significant enhancement of predictive accuracy over the FRS-DA (0.9165 vs. 0.7283, p=0.015) and an improvement trend over the FRS-DA+ hs-cTnI (0.9165 vs. 0.8431, p=0.21). Since AUC change in response to a new marker included to a model is often sensitive to only very large independent effects of that marker, we further calculated integrated discrimination improvement (IDI) to assess additional discrimination offered by inclusion of information from hs-cTnI and high-risk plaque in CVE prediction. Indeed, addition of hs-cTnI to FRS-DA significantly improved precision in CVE risk prediction vs. FRS-DA

alone [Table 4, IDI=0.0435 (0.0023-0.0847), p=0.038]; further addition of high-risk plaque information significantly enhanced accuracy of CVE-risk prediction over FRS-DA+ hs-cTnI [IDI=0.0818 (0.0032-0.1605), p=0.042].

DISCUSSION

Patients with RA incur a higher rate of CVE compared to individuals without autoimmune disease [1]. Therefore, periodic cardiovascular risk stratification according to national guidelines is an integral part of the care of RA patients [27]. However, general risk calculators do not sufficiently capture the incremental risk in patients with RA [28-30].

All stages of the atherogenic process appear enhanced in RA; endothelial dysfunction, increased arterial stiffness, plaque formation, and finally CVE [31]. Distinct biomarkers may reflect different stages of this pathway; from inflammation [hsCRP, IL-6] to plaque instability [Myeloperoxidase, Matrix Metalloproteinases], thrombosis [fibrinogen], myocardial stress [NT-pro-BNP], and myocardial necrosis [hs-cTn]. Individual associations of CRP, sensitive hs-cTn and NT-proBNP with CVE in general patients have been extensively described [32]. In RA, CRP may reflect uncontrolled systemic inflammation, rather than being a surrogate for the extent of vascular involvement [31]. NT-proBNP independently predicted mortality in one study of 182 RA patients [33].

Our study shows for the first time that hs-cTnI- a specific structural myocardial biomarker- may optimize long-term cardiovascular risk prediction in RA. Blood concentrations of cardiac troponin I and T subunits are elevated in the context of myocardial injury ~~(34)~~[34]. H~~Recent development of h~~igh-sensitivity assays measure

cTnI concentrations at levels much lower than~~allowing detection at limits at least 10-fold lower compared to~~ conventional assays with excellent precision at ≤10% coefficient of variation, both at and below the assay's 99th percentile value; this added sensitivity allows reliable ~~troponin subunit~~ estimation in almost 100% of~~apparently~~ healthy individuals and identification of subclinical myocardial injury ~~(35)~~ [35]. Elevated hs-cTnI was associated with incident long-term CV events in patients with RA, when controlled for traditional cardiac risk factors. This is consistent with reports in population-based studies that subthreshold elevations of either hs-cTnT or hs-cTnI predicted higher risk of CVE, heart failure hospitalization, and mortality [13-16]. By contrast, RA patients with low hs-cTnI were 82% less likely to suffer a CV event. This approximates the estimated 88% lower risk of CV death in a nested case-control study in general patients with low hs-cTnI measured with the same assay. Moreover, we demonstrated that hs-cTnI measurements significantly improved discrimination of long-term incident CVE risk over composite cardiac risk scores alone. A combination of CRP, NT-proBNP, and sensitive cTnI optimized the 10-year CV event risk prediction in two general European populations [36]; however, those have not yet been evaluated in RA. In our study, IL-6 was numerically higher in patients incurring CV events; nevertheless, a model of high IL-6 combined with hs-cTnI did not optimize event prediction over hs-cTnI alone (not shown). More multi-biomarker groupings will likely emerge in the future; however the optimal prognostic combinations will have to be defined.

Our second novel finding was the association of hs-cTnI with coronary plaque presence, burden and composition in patients with RA, as measured by CCTA. This non-invasive imaging modality has significantly enhanced prediction of incident CVE beyond clinical

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risk scores as well as CAC in general patients without known CVD [37,38]. In a prospective study, 69% of subjects with obstructive lesions suffered events at 52 months compared to 28% of those with non-obstructive lesions and 0% of those without plaque; similarly, 75% with SIS>5 and 80% with SSS>5 suffered CVE compared to 23% with SIS≤5 and 15% with SSS≤5 [39]. Hs-cTnI was considerably higher in patients with any plaque vs. those without; furthermore, it significantly increased across higher plaque burden scores. This is consistent with a prior report in general patients showing progressively higher cTnT in those with mild, moderate, and multi-vessel coronary artery disease on CCTA [40]. Hs-cTnI was strongly correlated with all quantitative plaque outcomes, including several high-risk ones (obstructive plaque, SSS>5, CAC>100, and composites thereof) after adjustments for traditional risk factors and cardiovascular scores. Moreover, it independently predicted the presence of any advanced- mixed or calcified- coronary plaque whereas it showed no correlation with earlier, non-calcified plaques.

In our study, hs-cTnI significantly improved discrimination of long-term incident CVE risk over cardiac risk scores alone. Additional information on presence of high-risk plaque outcomes from CCTA further optimized CVE risk discrimination compared to cardiac risk scores and hs-cTnI together. These observations provide the theoretical framework and a testable hypothesis for a two-step algorithm to optimize CVE risk prediction in RA: As part of the cardiac risk stratification, physicians could measure plasma hs-cTnI; if high (>1.5pg/ml), it may foreshadow significant hazard for high-risk plaque burden, vulnerability, or future CVE above and beyond cardiac risk scores. In that context, further non-invasive evaluation of coronary atherosclerosis with CCTA may refine

primary prevention recommendations based on presence and burden of coronary plaque. By contrast, if hs-cTnI is low (≤1.5 pg/ml), risk of significant coronary atherosclerosis and CVE is substantially decreased; therefore physicians may narrow their recommendations to address potential actionable clinical risk factors and in accordance with cardiac scores.

In our study, hs-cTnI was measured at the time of CCTA- when no chest pain was present; in fact, by design enrollees had no symptoms or diagnosis of CVD upon study entry. Hence, elevated hs-cTnI levels likely reflect latent myocyte damage. Higher hs-cTnI in general patients has been associated with unstable plaque features on CCTA [41], reflecting intermittent, chronic and clinically silent plaque remodeling and/ or rupture with subsequent microembolization, leading to unrecognized myocardial infarctions (UMI) [42, 43]. Consistently with these reports, we showed that hs-cTnI in RA patients only correlated with higher complexity mixed or calcified plaques, independently of cardiac risk factors or Framingham scores, but not earlier, non-calcified lesions. Greater hs-cTnI associated with presence of UMI at baseline, as well as with new or larger UMI on MRI 5 years later, in a series of community-living volunteers without history of MI [44]. RA patients are far more likely to experience UMI even prior to their RA diagnosis [45]. Indeed, in a pilot MRI study, 39% of RA patients without symptomatic CVD had delayed enhancement suggesting myocardial inflammation or scarring and 11% had nodular subendocardial delayed enhancement indicating silent MI [46]. Latent troponin leak has further been reported as a result of impaired cell membrane integrity due to systemic inflammation [47]; however, we

observed no associations between hs-cTnI, inflammatory markers, or cytokines, making inflammation an unlikely driver- consistently with an earlier report [17].

Interestingly, we observed no association between proinflammatory cytokines, ESR, or CRP and burden of coronary atherosclerosis; this observation may be partially explained by the fact that 58% of our patients were in remission (DAS28-CRP<2.6) at the time of CCTA, while 75% overall had low disease activity (DAS28-CRP<3.2), and 60% were under chronic anti-TNF medication exposure. Concordantly, in the vast majority IL-6, IL-17A and IL-17F levels were well under the 99% threshold observed in normals and similar to- or lower than- those reported by studies in treated RA patients using identical measurement assays [48, 49].

Our study has certain limitations; causal relationships between hs-cTnI levels and plaque burden or composition may not be inferred, due to their cross-sectional evaluation. Moreover, since our patients were well controlled and the levels of proinflammatory cytokines studied were generally low and reflective of that state, we may have underestimated the association of inflammation with both hs-cTnI and plaque burden. Our broader study design- of which the current report is a part- was powered to evaluate quantitative and qualitative plaque differences between 150 RA patients and an equal number of age and gender matched patients without autoimmune disease. Although evaluations of biomarkers and their associations with plaque presence, burden and composition in RA patients were pre-specified as exploratory analyses, they were not specifically powered for. Our findings would, therefore, have to be tested in larger, specifically powered studies and our proposed two-step algorithm for optimization of CVE risk prediction prospectively validated within that context. CVE appear numerically

low in our study (11 patients or 6.1%), which may have deflated overall significance rates- despite sizeable area differences- in AUC curves between FRS-DA alone and FRS-DA + hs-cTnI as well as between FRS-DA + hs-cTnI and FRS-DA + hs-cTnI + high-risk CCTA. This was certainly contributed to by our study design, which pre-specified recruitment of subjects without symptoms or prior diagnosis of CVD. Despite that, our observed event rate amounted to 1.5/100PY, which is similar to studies specifically enriching for CV risk [50], and considered overall high for populations of well controlled patients, chronically exposed to biologic agents.

Conclusion

We show for the first time that hs-cTnI associates with presence, burden, and composition of coronary artery atherosclerosis in RA patients without symptoms or prior diagnosis of cardiovascular disease- above and beyond traditional risk factors, cardiovascular scores, or inflammation. Hs-cTnI further associates with long-term risk of incident CVE beyond demographics and traditional cardiac risk factors, and improves discrimination for such risk prediction beyond that rendered by cardiac risk scores. It may provide a mechanistic explanation for the greater morbidity and mortality RA patients incur, and may serve as an adjunct predictive biomarker in refining cardiovascular risk determination in RA.

Key Messages

1. hs-cTnI correlates with presence, burden, and composition of occult coronary plaque in RA.

2. hs-cTnI correlates with long-term cardiac events in RA, after adjustment for cardiac risk factors.

3. Hs-cTnI may serve as a predictive biomarker in refining cardiovascular risk assessment in RA.

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Conflict of Interest Statement

This study was supported by an American Heart Association grant to Dr Karpouzas. The authors have no conflict of interest to declare.

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Legends for Tables and Figures

Table 1: Baseline patient characteristics. Values represent Median (IQR) or number (%). CVE: cardiovascular events, RF: Rheumatoid factor, ACPA: anti-cyclic citrullinated peptide antibodies, DAS28-CRP: disease activity score, ESR: erythrocyte sedimentation rate, CRP: c-reactive protein, DMARD: disease modifying anti- rheumatic drugs, TNFi: tumor necrosis factor-a inhibitors, CAD: coronary artery disease, hs-cTnI: highly sensitive cardiac troponin-I, TNF-a: tumor necrosis factor-a, IL-6: interleukin-6, IL-17A and F: interleukin-17A and F, VEGF: vascular endothelial growth factor, ¶ available in 146 patients, †p<0.1, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 2: Prediction of occult coronary plaque burden and composition by cTnI. *cTnI and coronary plaque outcomes (CAC, SIS, SSS, PBS) binarized based on median; 1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score; CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, PBS: plaque burden score, NCP: non-calcified plaque, MP: mixed plaque, CP: calcified plaque.

Table 3: Elevated hs-cTnI (>1.5pg/ml) predicts risk of Cardiovascular events. 1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score.

Table 4: Average improvement in precision of cardiovascular event risk prediction by integrating hs-cTnI and high-risk CTA. IDI: integrated discrimination improvement, FRS-DA: D'Agostino Framingham risk score, CCTA: Coronary computed Tomography Angiography. hs-cTnI: highly-sensitive cardiac troponin-I, High-risk CCTA: obstructive plaque or SIS>5 or SSS>5.

Figure 1: Several high-risk coronary plaque burden outcomes are significantly enriched across higher tertiles of hs-cTnI. CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, composite score: obstructive plaque or SIS>5 or SSS>5; MP: mixed plaque; CP: calcified plaque. hs-cTnI tertile ranges were ≤ 1.2 pg/mL, 1.2- 2.1 pg/mL, and ≥ 2.1 pg/mL. P-value for trend determined by Jonckheere-Terpstra test.

Figure 2: (A) Elevated hs-cTnI predicts long-term cardiovascular events in RA. **(B)** Addition of hs-cTnI information to the FRS-DA composite score increased prognostic accuracy (AUC=0.8431 vs. 0.7283, p=0.1). Further addition of high-risk plaque information from CCTA [obstructive plaque or SIS>5 or SSS>5] resulted in significant enhancement of predictive accuracy over the FRS-DA (0.9165 vs. 0.7283, p=0.015) and an improvement trend over the FRS-DA+ hs-cTnI (0.9165 vs. 0.8431, p=0.21). FRS-DA:

D'Agostino Framingham modified cardiovascular risk score. CCTA: coronary computed tomography angiography.

Supplementary Table S1: Correlations between inflammatory and structural biomarkers and coronary plaque outcomes¶. cTnI: cardiac troponin-I, IL-6: interleukin-6, IL-17A: interleukin-17A, IL-17F: interleukin-17F, VEGF: vascular endothelial growth factor, CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, PBS: plaque burden score; ¶ Values represent Spearman correlation coefficients; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supplementary Table S2: Functional performance characteristics of cTnI* for high coronary plaque burden or composition. *cTnI binarized based on median (1.5 pg/ml); SSS: segment stenosis score, SIS: segment involvement score, CAC: coronary artery calcium, MP: mixed plaque, CP: calcified plaque, PPV: positive predictive value, NPV: negative predictive value.

Highly sensitive cardiac troponin-I is a biomarker for occult coronary plaque burden and cardiovascular events in patients with rheumatoid arthritis

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ABSTRACT

Objectives. Patients with Rheumatoid Arthritis (RA) display greater occult coronary atherosclerosis burden and experience higher cardiovascular morbidity and mortality compared to controls. We here explored whether proinflammatory cytokines and highly-sensitive cardiac troponin-I (hs-cTnI)- a biomarker of myocardial injury- correlated with plaque burden and cardiovascular events (CVE) in RA.

Methods. We evaluated 150 patients with 64-slice coronary computed tomography angiography (CCTA). Coronary artery calcium (CAC), number of segments with plaque (SIS), stenotic severity (SSS), and plaque burden (PBS) were assessed. Lesions were described as non-calcified, mixed, or fully calcified. Blood levels of hs-cTnI and proinflammatory cytokines were assessed during CCTA. Subjects were followed over 60±26 months for CVE, both ischemic [cardiac death, non-fatal myocardial infarction (MI), stroke, peripheral arterial ischemia] and non-ischemic [new onset heart failure hospitalization].

Results. Plasma hs-cTnI correlated with all coronary plaque outcomes ($p<0.01$). Elevated hs-cTnI (≥1.5 pg/ml) further associated with significant calcification, extensive atherosclerosis, obstructive plaque, and any advanced mixed or calcified plaques after adjustments for cardiac risk factors or D'Agostino Framingham scores (all $p<0.05$). Eleven patients suffered CVE (1.54/100PY); eight ischemic and three non-ischemic. Elevated hs-cTnI predicted all CVE risk independently of demographics, cardiac risk factors, and prednisone use ($p=0.03$). Conversely, low hs-cTnI presaged lower risk for both extensive atherosclerosis ($p<0.05$) and incident CVE ($p=0.037$).

Conclusion. Plasma hs-cTnI independently associated with occult coronary plaque burden, composition, and long-term incident CVE in patients with RA. Low hs-cTnI forecasted lower risk for both extensive atherosclerosis as well as CVE; hs-cTnI may therefore optimize cardiovascular risk stratification in RA.

Keywords: occult coronary atherosclerosis, cardiovascular events, highly-sensitive cardiac troponin I, Rheumatoid arthritis

Introduction

Individuals with RA experience a higher rate of CVE compared to controls [1]. This may be explained by greater prevalence, severity, burden, and different composition of occult coronary lesions in RA compared to age and gender-matched controls [2]. Residual disease activity may further associate with more advanced, complex, prone to rupture coronary plaques [2]. Proinflammatory cytokines such as tumor necrosis factor-a (TNFa), Interleukin-6 (IL-6), and interleukin-17 (IL-17) reflect clinical activity and structural damage in RA and are higher in the blood of RA patients compared to controls [3]; the same cytokines have been identified in atherosclerotic plaque [4-7], and correlated with subclinical atherosclerosis independently of cardiac risk factors [8], coronary plaque complexity [9], plaque destabilization and CVE in subjects without autoimmune disease [10-12]. Nevertheless, the relationship between these cytokines and occult coronary plaque burden and composition in RA are unknown. Higher plaque load or vulnerability may be further reflected in elevations of biomarkers specific for myocardial injury [13]; indeed, cardiac troponin elevations measured by highly sensitive assays- and below thresholds used to diagnose acute coronary syndromes- were associated with higher CAC scores in a population-based study [13]. Additionally, both hs-cTnT and hs-cTnI predicted higher risk of fatal and non-fatal coronary heart disease (CHD), heart failure hospitalization, and overall mortality in the general population [13-16].

In a recent report, hs-cTnI was higher in RA patients compared to controls, independently of cardiovascular risk factors and inflammation [17]. Nevertheless, its association with subclinical coronary artery disease burden or its ability to predict future

CVE in RA are unknown. We here hypothesized that hs-cTnI and various proinflammatory cytokines may correlate with the presence, burden, and composition of occult coronary plaque in patients with RA evaluated with CCTA. We further postulated that hs-cTnI at the time of CTA might predict incident CVE on long-term follow-up (60±26 months).

Methods

Patient Recruitment

One hundred and fifty RA patients from a single center were enrolled and prospectively evaluated on a first come first served basis [2]. The study was approved by the local Institutional Review Board, all subjects signed informed consent, and the research was carried out in compliance with the Helsinki declaration. Inclusion criteria comprised ages ≥18 years, fulfillment of 2010 classification criteria for RA, and no symptoms or history of cardiovascular disease such as myocardial infarction (MI), revascularization, heart failure, transient ischemic attack (TIA), stroke, or peripheral arterial disease (PAD). Patients with concomitant autoimmune syndromes, malignancy within <5 years, chronic or active infection, weight >325 pounds (147.7 kg), hypotension [systolic blood pressure (SBP) <90mmHg or diastolic blood pressure (DBP) <60mmHg] or hypertension (SBP >170mmHg or DBP >110 mmHg), uncontrolled tachycardia, irregular rhythm, iodine allergy, or glomerular filtration rate (GFR)< 60 ml/min were excluded. Hypertension was defined as SBP ≥140mmHg or DBP ≥90mmHg or antihypertensive use. Diabetes mellitus (DM) encompassed HgbA1c>6.5%, or hypoglycemic medication use. Hyperlipidemia constituted fasting cholesterol >200 mg/dl, or LDL>130 mg/dl.

Current smoking entailed cigarette consumption within 30 days from screening. Positive family history was defined as coronary artery disease (CAD) in first-degree relatives younger than 55 for males or 65 for females. The Framingham 2008 D'Agostino modified general cardiovascular risk score (FRS-DA) was calculated for all study participants [18]. Disease duration, serologic status, radiographs and treatments were captured. RA activity was evaluated by a 28-joint count and c-reactive protein (DAS28-CRP).

Laboratory evaluations

Blood for regular chemistries, fasting lipids, erythrocyte sedimentation rate (ESR) and high sensitivity C-reactive protein (hs-CRP) was collected on the day of CCTA and evaluated at the local laboratory. Additionally, blood was collected in EDTA tubes, immediately processed and plasma was frozen at -80^{o}C until it was assayed. Hs-cTnI was measured at Singulex Inc. (Alameda, CA) by technicians blinded to the clinical data using a micro-particle immunoassay and single-molecule counting [19]. TNFa, IL-6, IL-17A and F, and vascular endothelial growth factor (VEGF) were also assessed using laboratory developed tests at Singulex based on single molecule counting [19].

Multi-Detector Computed Tomography Angiography (CTA)

Scans were performed with a 64-multidetector row Lightspeed VCT scanner (GE Healthcare) between 3/2010-3/2011, and images analyzed as previously described by a single, blinded interpreter (BMJ) [20]. CAC was quantified by the Agatston method [21]. Coronary arteries were evaluated on contrast-enhanced scans using a standardized 15-

segment model [22]. Stenosis severity was scored from 0 to 4 based on grade of luminal restriction; 1 represented 1-29% stenosis, 2: 30-49%, 3: 50-69%, and 4: ≥70% stenosis. The area of each plaque visualized in at least 2 adjacent slices (slice thickness 0.625 mm) was determined on all affected slices. Plaque burden was graded from 0-3, defined as none (0), mild (1), moderate (2), and severe (3), based on the number of adjacent slices containing plaque. Lesions rendering >50% stenosis were considered obstructive. Plaque composition was defined as non-calcified (NCP), mixed (MP), or calcified (CP) as elsewhere discussed [23]. Subjects received 3 individual quantitative scores [23]; segment involvement score (SIS) represented the total number of segments with plaque (0-15); stenosis severity score (SSS) reported the cumulative stenosis grade conferred by plaque over all evaluable segments (0-60); plaque burden score (PBS) described the cumulative plaque size over all evaluable segments (0-45). Reproducibility of scoring measurements for our center has been previously reported [23].

Incident Cardiovascular Events (CVE)

All patients were followed for incident CVE over a period of 60±26 months. Those included both ischemic [cardiac death, non-fatal myocardial infarction (MI), stroke, TIA, PAD] as well as non-ischemic ones [new onset heart failure hospitalization]. Event adjudication was elaborated by the treating cardiologist, neurologist, or vascular surgeon respectively and based on standard definitions [24-26].

Analysis

Continuous variables were expressed as medians with inter-quartile ranges (IQR) and categorical ones as numbers with percentages. Spearman-Rho correlation coefficients evaluated preliminary associations between biomarkers and plaque outcomes. Medians between CVE groups were compared using the Mann-Whitney U-test, counts by the X^2 test. Further analyses were restricted to biomarkers with significant differences. Plaque outcomes were binarized based on median; those were >0 vs. 0 for CAC, >1 vs. ≤1 for SIS, >1 vs. ≤1 for SSS, and >2 vs. ≤2 for PBS. To evaluate plaque composition, the presence of MP or CP vs. the absence of both was used as an outcome. Similarly, hs-cTnI was binarized as "high" (>1.5pg/ml) vs. "low" (≤1.5pg/ml). Logistic regression models were constructed to evaluate associations between hs-cTnI and individual plaque parameters; models were adjusted either for age and gender (model 1), or additionally for hypertension, diabetes, hyperlipidemia, statin use, smoking, body mass index (BMI), and prednisone use (model 2), or for the patients' FRS-DA score (model 3). Similar logistic regression models were devised to predict individual or composite high-risk plaque outcomes; individual ones included CAC>100 vs. ≤100, SIS>5 vs. ≤5, SSS>5 vs. ≤5, and presence of obstructive plaque vs. not. Composite outcomes entailed presence of SSS>5 or CAC>100 vs. neither, and SIS>5 or SSS>5 or obstructive plaque vs. none. Results were reported as odds ratios with 95% confidence intervals. For composite and plaque composition outcomes, sensitivity, specificity, negative, and positive predictive values were determined with standard formulas. Cox proportional hazards regression analysis evaluated CVE risk (HR) associated with high cTnI (>1.5pg/ml) in raw and several adjusted models (model 1, 2, and 3) as previously described. Kaplan-Meier curves were compared by the log-rank method. Diagnostic

accuracy of CVE risk assessment for FRS-DA alone, FRS-DA+ hs-cTnI, and FRS-DA+ hs-cTnI+ high-risk composite outcome was evaluated as area under the receiver operator characteristic curves (AuROC) and compared with the DeLong method. Integrated discrimination improvement (IDI) between these constructs was computed, and improvement in prediction accuracy was evaluated considering p-value <0.05 as significant. Data was analyzed with SAS v9.4 or R v3.4.1.

Results

Patient demographics appear in table 1. Subjects were predominantly female, with established, seropositive, erosive, and well-controlled disease. RA parameters and traditional cardiac risk factors were not significantly different in patients incurring CVE; by contrast, FRS-DA, coronary atherosclerosis burden, including high-risk plaque parameters, and higher levels of hs-cTnI were significantly higher (all p<0.05).

Correlations of cytokines and hs-cTnI with occult coronary atherosclerosis

TNFa, IL-6, IL-17A, IL-17F, and VEGF showed no correlations with any coronary plaque parameters or hs-cTnI (online table 1); neither did ESR, CRP, TJC, SJC, or DAS28-CRP (not shown). Hs-cTnI was detectable in all patients- 1.5 (1.1-2.6) pg/ml; patients with any plaque had higher levels compared to those without [1.8 (1.1-2.6) pg/ml vs. 1.3 (0.9-1.8) pg/ml, p=0.02]. Moreover, hs-cTnI was correlated with all occult coronary plaque outcomes (all p <0.01). CAC, SIS, SSS, and PBS substantially increased from the lowest to the highest hs-cTnI tertile (p for trend of 0.006, 0.005, 0.01, and 0.009 respectively). Similarly, high-risk plaque outcomes such as CAC>100, SIS>5, SSS>5,

obstructive plaque, composite outcome, and presence of any advanced MP/CP lesions were considerably enriched across higher hs-cTnI tertiles (figure 1).

Hs-cTnI independently correlates with plaque burden and composition

Hs-cTnI levels associated with all plaque outcomes (table 2). After controlling for age and gender (model 1), additional adjustments for hypertension, diabetes, hyperlipidemia, statin use, smoking, BMI, and prednisone use (model 2), or FRS-DA score (model 3), hs-cTnI remained predictive of CAC, SSS and PBS. Furthermore, it associated with presence of more advanced mixed or calcified plaques whereas it showed no correlation with earlier, non-calcified lesions. Importantly, hs-cTnI further correlated with high-risk outcomes such as obstructive plaque, SSS>5, CAC≥100, or composite end points (table 2); significance persisted for several, even after adjustments for cardiac risk factors or FRS-DA scores.

Conversely, subjects with low hs-cTnI (<1.5 pg/ml) were less likely to have extensive coronary atherosclerosis; specifically, they displayed 81% lower risk of having SSS>5 or CAC≥100 and 70% less risk of obstructive plaque, SIS>5, or SSS>5 after controlling for FRS-DA score; area under the curve (AUC) improved from 0.79 (0.63-0.95) to 0.85 (0.72-0.98), $p<0.05$ (not shown). Out of all patients, 27 (18%) had CAC>100 or SSS>5 and 22 (15%) had obstructive plaque or SIS>5 or SSS>5. Compared to all patients, only 8% with low hs-cTnI displayed those respective plaque outcomes (online table 2); of patients with both low hs-cTnI and low FRS-DA scores, only 4% had extensive atherosclerosis compared to 11% of those with just low FRS-DA.

Elevated Hs-cTnI associates with long-term CVE in RA

Eleven patients suffered CVE during 60±26 months of follow-up (1.54/100PY): 8 were ischemic, including 1 cardiac death, 3 NSTEMI (non-ST elevation myocardial infarctions), 2 strokes, and 2 PAD events requiring revascularizations; the 3 non-ischemic events were new onset, hospitalized, heart failure. Hs-cTnI was higher in patients with CVE vs. those without [2.6 (2.1-4.4) vs. 1.5 (1.0-2.4) pg/ml, p=0.006). Elevated hs-cTnI predicted risk of incident CVE (Figure 2A, p=0.03), independently of demographics and traditional cardiac risk factors (Table 3). Importantly, patients with low hs-cTnI were 82% less likely to suffer CVE.

Hs-cTnI enhances cardiovascular event risk prediction when added to cardiac risk scores

The prognostic accuracy of FRS-DA alone vs. FRS-DA+ hs-cTnI and FRS-DA+ hs-cTnI+ high-risk plaque for CVE, based on AUC of the respective ROC curves, is depicted on Figure 2B. Addition of hs-cTnI information to FRS-DA score yielded higher prognostic accuracy (0.8431 vs. 0.7283, p=0.10); further addition of high-risk plaque information from CCTA [obstructive plaque or SIS>5 or SSS>5] resulted in significant enhancement of predictive accuracy over the FRS-DA (0.9165 vs. 0.7283, p=0.015) and an improvement trend over the FRS-DA+ hs-cTnI (0.9165 vs. 0.8431, p=0.21). Since AUC change in response to a new marker included to a model is often sensitive to only very large independent effects of that marker, we further calculated integrated discrimination improvement (IDI) to assess additional discrimination offered by inclusion of information from hs-cTnI and high-risk plaque in CVE prediction. Indeed, addition of hs-cTnI to FRS-DA significantly improved precision in CVE risk prediction vs. FRS-DA

alone [Table 4, IDI=0.0435 (0.0023-0.0847), p=0.038]; further addition of high-risk plaque information significantly enhanced accuracy of CVE-risk prediction over FRS-DA+ hs-cTnI [IDI=0.0818 (0.0032-0.1605), p=0.042].

DISCUSSION

Patients with RA incur a higher rate of CVE compared to individuals without autoimmune disease [1]. Therefore, periodic cardiovascular risk stratification according to national guidelines is an integral part of the care of RA patients [27]. However, general risk calculators do not sufficiently capture the incremental risk in patients with RA [28-30].

All stages of the atherogenic process appear enhanced in RA; endothelial dysfunction, increased arterial stiffness, plaque formation, and finally CVE [31]. Distinct biomarkers may reflect different stages of this pathway; from inflammation [hsCRP, IL-6] to plaque instability [Myeloperoxidase, Matrix Metalloproteinases], thrombosis [fibrinogen], myocardial stress [NT-pro-BNP], and myocardial necrosis [hs-cTn]. Individual associations of CRP, sensitive hs-cTn and NT-proBNP with CVE in general patients have been extensively described [32]. In RA, CRP may reflect uncontrolled systemic inflammation, rather than being a surrogate for the extent of vascular involvement [31]. NT-proBNP independently predicted mortality in one study of 182 RA patients [33]. Our study shows for the first time that hs-cTnI- a specific structural myocardial biomarker- may optimize long-term cardiovascular risk prediction in RA. Blood concentrations of cardiac troponin I and T subunits are elevated in the context of myocardial injury [34]. High-sensitivity assays measure cTnI concentrations at levels

much lower than conventional assays with excellent precision at ≤10% coefficient of variation, both at and below the assay's 99th percentile value; this added sensitivity allows reliable estimation in almost 100% of healthy individuals and identification of subclinical myocardial injury [35]. Elevated hs-cTnI was associated with incident long-term CV events in patients with RA, when controlled for traditional cardiac risk factors. This is consistent with reports in population-based studies that subthreshold elevations of either hs-cTnT or hs-cTnI predicted higher risk of CVE, heart failure hospitalization, and mortality [13-16]. By contrast, RA patients with low hs-cTnI were 82% less likely to suffer a CV event. This approximates the estimated 88% lower risk of CV death in a nested case-control study in general patients with low hs-cTnI measured with the same assay. Moreover, we demonstrated that hs-cTnI measurements significantly improved discrimination of long-term incident CVE risk over composite cardiac risk scores alone. A combination of CRP, NT-proBNP, and sensitive cTnI optimized the 10-year CV event risk prediction in two general European populations [36]; however, those have not yet been evaluated in RA. In our study, IL-6 was numerically higher in patients incurring CV events; nevertheless, a model of high IL-6 combined with hs-cTnI did not optimize event prediction over hs-cTnI alone (not shown). More multi-biomarker groupings will likely emerge in the future; however, the optimal prognostic combinations will have to be defined.

Our second novel finding was the association of hs-cTnI with coronary plaque presence, burden and composition in patients with RA, as measured by CCTA. This non-invasive imaging modality has significantly enhanced prediction of incident CVE beyond clinical risk scores as well as CAC in general patients without known CVD [37,38]. In a

prospective study, 69% of subjects with obstructive lesions suffered events at 52 months compared to 28% of those with non-obstructive lesions and 0% of those without plaque; similarly, 75% with SIS>5 and 80% with SSS>5 suffered CVE compared to 23% with SIS≤5 and 15% with SSS≤5 [39]. Hs-cTnI was considerably higher in patients with any plaque vs. those without; furthermore, it significantly increased across higher plaque burden scores. This is consistent with a prior report in general patients showing progressively higher cTnT in those with mild, moderate, and multi-vessel coronary artery disease on CCTA [40]. Hs-cTnI was strongly correlated with all quantitative plaque outcomes, including several high-risk ones (obstructive plaque, SSS>5, CAC>100, and composites thereof) after adjustments for traditional risk factors and cardiovascular scores. Moreover, it independently predicted the presence of any advanced- mixed or calcified- coronary plaque whereas it showed no correlation with earlier, non-calcified plaques.

In our study, hs-cTnI significantly improved discrimination of long-term incident CVE risk over cardiac risk scores alone. Additional information on presence of high-risk plaque outcomes from CCTA further optimized CVE risk discrimination compared to cardiac risk scores and hs-cTnI together. These observations provide the theoretical framework and a testable hypothesis for a two-step algorithm to optimize CVE risk prediction in RA: As part of the cardiac risk stratification, physicians could measure plasma hs-cTnI; if high (>1.5pg/ml), it may foreshadow significant hazard for high-risk plaque burden, vulnerability, or future CVE above and beyond cardiac risk scores. In that context, further non-invasive evaluation of coronary atherosclerosis with CCTA may refine primary prevention recommendations based on presence and burden of coronary

plaque. By contrast, if hs-cTnI is low (≤1.5 pg/ml), risk of significant coronary atherosclerosis and CVE is substantially decreased; therefore, physicians may narrow their recommendations to address potential actionable clinical risk factors and in accordance with cardiac scores.

In our study, hs-cTnI was measured at the time of CCTA- when no chest pain was present; in fact, by design enrollees had no symptoms or diagnosis of CVD upon study entry. Hence, elevated hs-cTnI levels likely reflect latent myocyte damage. Higher hs-cTnI in general patients has been associated with unstable plaque features on CCTA [41], reflecting intermittent, chronic and clinically silent plaque remodeling and/ or rupture with subsequent microembolization, leading to unrecognized myocardial infarctions (UMI) [42, 43]. Consistently with these reports, we showed that hs-cTnI in RA patients only correlated with higher complexity mixed or calcified plaques, independently of cardiac risk factors or Framingham scores, but not earlier, non-calcified lesions. Greater hs-cTnI associated with presence of UMI at baseline, as well as with new or larger UMI on MRI 5 years later, in a series of community-living volunteers without history of MI [44]. RA patients are far more likely to experience UMI even prior to their RA diagnosis [45]. Indeed, in a pilot MRI study, 39% of RA patients without symptomatic CVD had delayed enhancement suggesting myocardial inflammation or scarring and 11% had nodular subendocardial delayed enhancement indicating silent MI [46]. Latent troponin leak has further been reported as a result of impaired cell membrane integrity due to systemic inflammation [47]; however, we observed no associations between hs-cTnI, inflammatory markers, or cytokines, making inflammation an unlikely driver- consistently with an earlier report [17].

Interestingly, we observed no association between proinflammatory cytokines, ESR, or CRP and burden of coronary atherosclerosis; this observation may be partially explained by the fact that 58% of our patients were in remission (DAS28-CRP<2.6) at the time of CCTA, while 75% overall had low disease activity (DAS28-CRP<3.2), and 60% were under chronic anti-TNF medication exposure. Concordantly, in the vast majority IL-6, IL-17A and IL-17F levels were well under the 99% threshold observed in normals and similar to- or lower than- those reported by studies in treated RA patients using identical measurement assays [48, 49].

Our study has certain limitations; causal relationships between hs-cTnI levels and plaque burden or composition may not be inferred, due to their cross-sectional evaluation. Moreover, since our patients were well controlled and the levels of proinflammatory cytokines studied were generally low and reflective of that state, we may have underestimated the association of inflammation with both hs-cTnI and plaque burden. Our broader study design- of which the current report is a part- was powered to evaluate quantitative and qualitative plaque differences between 150 RA patients and an equal number of age and gender matched patients without autoimmune disease. Although evaluations of biomarkers and their associations with plaque presence, burden and composition in RA patients were pre-specified as exploratory analyses, they were not specifically powered for. Our findings would, therefore, have to be tested in larger, specifically powered studies and our proposed two-step algorithm for optimization of CVE risk prediction prospectively validated within that context. CVE appear numerically low in our study (11 patients or 6.1%), which may have deflated overall significance rates- despite sizeable area differences- in AUC curves between FRS-DA alone and

FRS-DA + hs-cTnI as well as between FRS-DA + hs-cTnI and FRS-DA + hs-cTnI + high-risk CCTA. This was certainly contributed to by our study design, which pre-specified recruitment of subjects without symptoms or prior diagnosis of CVD. Despite that, our observed event rate amounted to 1.5/100PY, which is similar to studies specifically enriching for CV risk [50], and considered overall high for populations of well controlled patients, chronically exposed to biologic agents.

Conclusion

We show for the first time that hs-cTnI associates with presence, burden, and composition of coronary artery atherosclerosis in RA patients without symptoms or prior diagnosis of cardiovascular disease- above and beyond traditional risk factors, cardiovascular scores, or inflammation. Hs-cTnI further associates with long-term risk of incident CVE beyond demographics and traditional cardiac risk factors, and improves discrimination for such risk prediction beyond that rendered by cardiac risk scores. It may provide a mechanistic explanation for the greater morbidity and mortality RA patients incur, and may serve as an adjunct predictive biomarker in refining cardiovascular risk determination in RA.

Key Messages

1. hs-cTnI correlates with presence, burden, and composition of occult coronary plaque in RA.

2. hs-cTnI correlates with long-term cardiac events in RA, after adjustment for cardiac risk factors.

3. Hs-cTnI may serve as a predictive biomarker in refining cardiovascular risk assessment in RA.

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Conflict of Interest Statement

This study was supported by an American Heart Association grant to Dr Karpouzas.

The authors have no conflict of interest to declare.

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Legends for Tables and Figures

Table 1: Baseline patient characteristics. Values represent Median (IQR) or number (%). CVE: cardiovascular events, RF: Rheumatoid factor, ACPA: anti-cyclic citrullinated peptide antibodies, DAS28-CRP: disease activity score, ESR: erythrocyte sedimentation rate, CRP: c-reactive protein, DMARD: disease modifying anti- rheumatic drugs, TNFi: tumor necrosis factor-a inhibitors, CAD: coronary artery disease, hs-cTnI: highly sensitive cardiac troponin-I, TNF-a: tumor necrosis factor-a, IL-6: interleukin-6, IL-17A and F: interleukin-17A and F, VEGF: vascular endothelial growth factor, ¶ available in 146 patients, †p<0.1, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 2: Prediction of occult coronary plaque burden and composition by cTnI. *cTnI and coronary plaque outcomes (CAC, SIS, SSS, PBS) binarized based on median; 1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score; CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, PBS: plaque burden score, NCP: non-calcified plaque, MP: mixed plaque, CP: calcified plaque.

Table 3: Elevated hs-cTnI (>1.5pg/ml) predicts risk of Cardiovascular events. 1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score.

Table 4: Average improvement in precision of cardiovascular event risk prediction by integrating hs-cTnI and high-risk CTA. IDI: integrated discrimination improvement, FRS-

DA: D'Agostino Framingham risk score, CCTA: Coronary computed Tomography Angiography. hs-cTnI: highly-sensitive cardiac troponin-I, High-risk CCTA: obstructive plaque or SIS>5 or SSS>5.

Figure 1: Several high-risk coronary plaque burden outcomes are significantly enriched across higher tertiles of hs-cTnI. CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, composite score: obstructive plaque or SIS>5 or SSS>5; MP: mixed plaque; CP: calcified plaque. hs-cTnI tertile ranges were ≤ 1.2 pg/mL, 1.2- 2.1 pg/mL, and ≥ 2.1 pg/mL. P-value for trend determined by Jonckheere-Terpstra test.

Figure 2: (A) Elevated hs-cTnI predicts long-term cardiovascular events in RA. **(B)** Addition of hs-cTnI information to the FRS-DA composite score increased prognostic accuracy (AUC=0.8431 vs. 0.7283, p=0.1). Further addition of high-risk plaque information from CCTA [obstructive plaque or SIS>5 or SSS>5] resulted in significant enhancement of predictive accuracy over the FRS-DA (0.9165 vs. 0.7283, p=0.015) and an improvement trend over the FRS-DA+ hs-cTnI (0.9165 vs. 0.8431, p=0.21). FRS-DA: D'Agostino Framingham modified cardiovascular risk score. CCTA: coronary computed tomography angiography.

Supplementary Table S1: Correlations between inflammatory and structural biomarkers and coronary plaque outcomes¶. cTnI: cardiac troponin-I, IL-6: interleukin-6, IL-17A: interleukin-17A, IL-17F: interleukin-17F, VEGF: vascular endothelial growth factor, CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment

stenosis Score, PBS: plaque burden score; ¶ Values represent Spearman correlation coefficients; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Supplementary Table S2: Functional performance characteristics of cTnI* for high coronary plaque burden or composition. *cTnI binarized based on median (1.5 pg/ml); SSS: segment stenosis score, SIS: segment involvement score, CAC: coronary artery calcium, MP: mixed plaque, CP: calcified plaque, PPV: positive predictive value, NPV: negative predictive value.

Table 1: Baseline patient characteristics

	All (n=150)	No CVE (n=139)	With CVE (n=11)
Age	54 (46-60)	54 (45-60)	59 (53-70)†
Males	18 (13%)	16 (12%)	2 (18%)
RF+	129 (86)	120 (86%)	9 (82%)
ACPA+	127 (85)	118 (85%)	9 (82%)
X-Ray Erosions	99 (66)	90 (65%)	9 (82%)
RA-duration (years)	9 (5-14)	9 (4-14)	12 (9-18)
Tender Joint Count	0 (0-2)	0 (0-2)	0 (0-1)
Swollen Joint Count	0.5 (0-3)	0 (0-3)	1 (0-5)
DAS28-CRP	2.30 (1.8-3.3)	2.32 (1.76-3.29)	2.39 (1.59-2.93)
hsCRP (mg/dl)	0.41 (0.20-0.96)	0.42 (0.21-0.88)	0.31 (0.12-0.76)
Prednisone	52 (35)	48 (35%)	4 (36%)
n-DMARDs- concurrent	2 (1-3)	2 (1-3)	2 (1-3)
TNFi-exposed	90 (60)	84 (60%)	6 (55%)
TNFi duration (years)	4.4 (2.4-6.0)	4.0 (2.2-6.0)	6.0 (4.2-6.9)
Diabetes Mellitus	26 (18%)	22 (16%)	4 (36%)†
Hypertension	64 (44%)	58 (43%)	8 (73%)†
Smoking- current	13 (9%)	12 (9%)	1 (9%)
Family History of CAD	6 (4%)	5 (3.6%)	1 (9%)
Hyperlipidemia	26 (17%)	25 (18%)	1 (9%)
Body Mass Index (kg/m^2)	28.1 (25.8-32.6)	28.4 (26-32.8)	25.9 (23-30.5)†
D'Agostino Framingham score	6.4 (3.0-11.7)	6.2 (2.7-11.2)	15.6 (4.9-20.0)*
Coronary artery calcium (CAC)	0 (0-19)	0 (0-10)	120 (0-361)***
Segment Involvement score (SIS)	1 (0-3)	1 (0-2)	5 (1-7)**

Segment Stenosis score (SSS)	1 (0-4)	1 (0-3)	9 (1-14)**
Plaque burden Score (PBS)	1.5 (0-3)	1 (0-3)	9 (1-12)**
Obstructive plaque (>50%)	18 (12)	12 (9%)	6 (55%)****
Non-calcified Plaque Score (NCP)	1 (0-2)	1 (0-2)	1 (0-5)
Mixed Plaque Score (MP)	0 (0-1)	0 (0-0)	3 (0-7)*
Calcified Plaque Score (CP)	0 (0-0)	0 (0-0)	1 (0-3)
cTnI (pg/ml)[¶]	1.5 (1.1-2.6)	1.5 (1-2.4)	2.6 (2.1-4.4)**
IL-17a (pg/ml)[¶]	1.3 (0.8-1.8)	1.3 (0.8-1.9)	1.2 (0.8-1.5)
IL-17F (pg/ml)[¶]	35.8 (22.1-66.8)	36.3 (22.1-62)	31 (22.1-97.4)
IL-6 (pg/ml)[¶]	2.9 (1.8-5.8)	2.8 (1.7-5.4)	3.7 (2.6-13.4) [†]
TNFa (pg/ml)[¶]	11.2 (7.8-24)	11.3 (7.7-24)	11.1 (7.9-30.4)
VEGF (pg/ml)[¶]	134 (61-221)	134 (60-221)	134 (77-179)

Values represent Median (IQR) or number (%). CVE: cardiovascular events, RF: Rheumatoid factor, ACPA: anti-cyclic citrullinated peptide antibodies, DAS28-CRP: disease activity score, ESR: erythrocyte sedimentation rate, CRP: c-reactive protein, DMARD: disease modifying anti- rheumatic drugs, TNFi: tumor necrosis factor-a inhibitors, CAD: coronary artery disease, hs-cTnI: highly sensitive cardiac troponin-I, TNF-a: tumor necrosis factor-a, IL-6: interleukin-6, IL-17A and F: interleukin-17A and F, VEGF: vascular endothelial growth factor, [¶] available in 146 patients, [†]p<0.1, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 2: Prediction of occult coronary plaque burden and composition by cTnI*

	Unadjusted OR (95% CI)	Model 1[1] OR (95% CI)	Model 2[2] OR (95% CI)	Model 3[3] OR (95% CI)
CAC*	*2.95 (1.5-6.0)*	*2.2 (1.0-4.7)*	2.3 (1.0-5.2)	*2.7 (1.3-5.8)*
CAC>100 vs.≤100	*4.2 (1.3-13.5)*	3.0 (0.9-10.5)	*5.7 (1.2-25.9)*	*5.0 (1.3-19)*
SIS*	*2.2 (1.1-4.2)*	1.7 (0.9-3.4)	1.7 (0.8-3.5)	1.9 (1.0-3.8)
SIS>5 vs. SIS≤5	2.5 (0.7-8.5)	1.8 (0.5-6.6)	2.6 (0.6-11.0)	2.6 (0.7-9.9)
SSS*	*2.4 (1.3-4.7)*	1.9 (1.0-3.9)	2.0 (1.0-4.1)	*2.2 (1.1-4.3)*
SSS>5 vs. SSS≤5	*3.0 (1.1-8.2)*	2.4 (0.8-7.3)	2.8 (0.9-8.8)	*3.0 (1.0-9.0)*
PBS*	*3.1 (1.5-6.3)*	*2.4 (1.1-5.1)*	*2.6 (1.1-5.9)*	*2.9 (1.3-6.2)*
Obstructive plaque	*3.9 (1.2-12.5)*	3.1 (0.9-10.7)	*4.0 (1.0-15.5)*	*4.0 (1.1-14.1)*
CAC>100 or SSS>5	*4.7 (1.8-12.4)*	2.3 (0.7-6.9)	*4.9 (1.6-15.5)*	*5.2 (1.8-15.8)*
SIS>5 or SSS>5 or obstructive plaque	*3.2 (1.2-8.8)*	2.6 (0.9-7.7)	2.9 (0.9-9.0)	*3.3 (1.1-9.7)*
NCP>0 vs. NCP=0	1.4 (0.7-2.7)	1.3 (0.6-2.5)	1.3 (0.6-2.7)	1.4 (0.7-2.7)
MP/CP>0 vs. MP/CP=0	*3.6 (1.8-7.5)*	*2.7 (1.2-6.0)*	*2.9 (1.2-6.7)*	*3.5 (1.6-7.6)*

*cTnI and coronary plaque outcomes (CAC, SIS, SSS, PBS) binarized based on median; 1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score; CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, PBS: plaque burden score, NCP: non-calcified plaque, MP: mixed plaque, CP: calcified plaque

Table 3. Elevated hs-cTnI (>1.5pg/ml) predicts risk of Cardiovascular events

Model	HR (Hazards Ratio)	CI (Confidence Interval)	p-value
Unadjusted	4.7	1.0-21.7	0.048
Model 1[1]	4.8	1.0-23.1	0.052
Model 2[2]	5.3	1.1-25.9	0.037
Model 3[3]	4.3	0.9-19.7	0.064

1: adjusted for age and gender; 2: Adjusted for age, gender, hypertension, diabetes, dyslipidemia, statin use, smoking, BMI, prednisone use; 3: adjusted for D'Agostino Framingham score.

Table 4. Average improvement in precision of cardiovascular event risk prediction by integrating hs-cTnI and high-risk CCTA

Comparison	IDI* (95% CI)	p-value
FRS-DA vs. FRS-DA+hs-cTnI	0.0435 (0.0023 - 0.0847)	0.038
FRS-DA+hs-cTnI vs. FRS-DA+hs-cTnI+high-risk CCTA	0.0818 (0.0032 - 0.1605)	0.042

IDI: integrated discrimination improvement, FRS-DA: D'Agostino Framingham risk score, CCTA: Coronary computed Tomography Angiography. hs-cTnI: highly-sensitive cardiac troponin-I, High-risk CCTA: obstructive plaque or SIS>5 or SSS>5

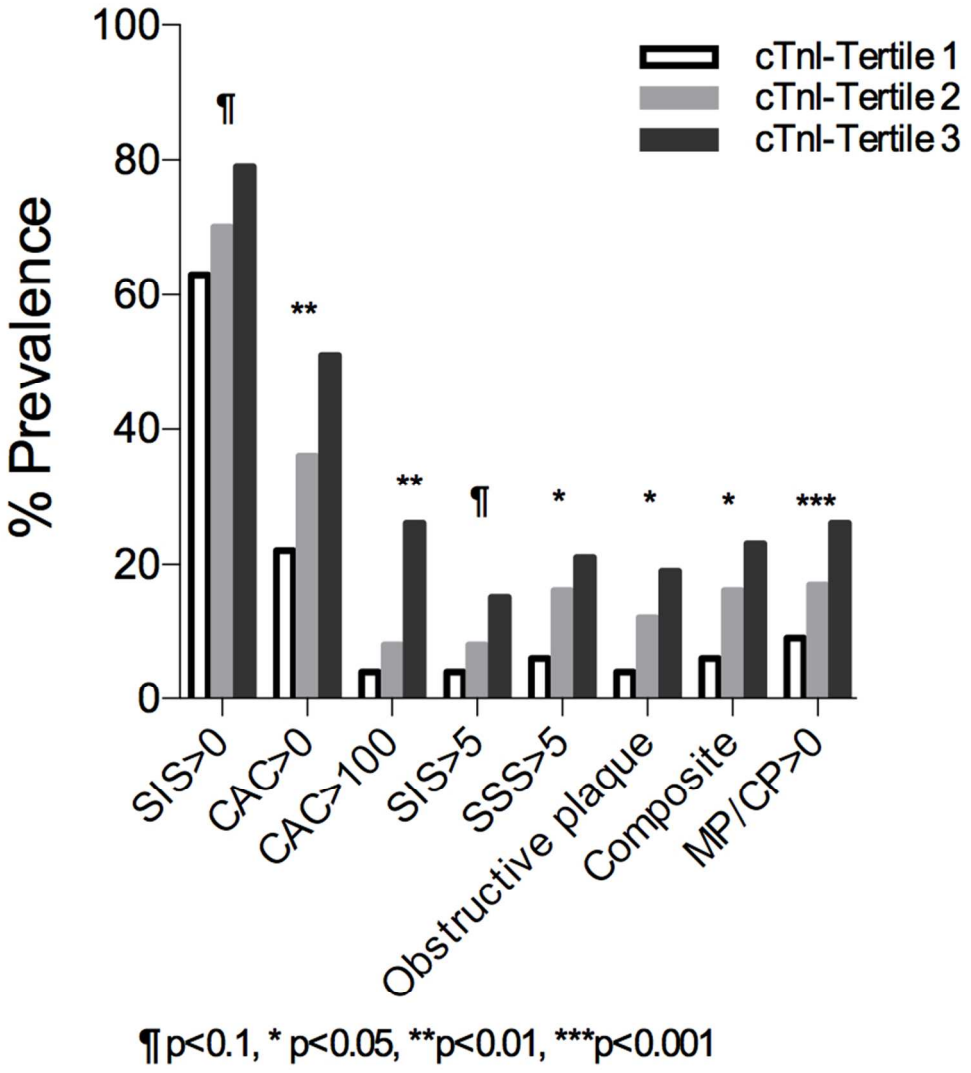

Figure 1: Several high-risk coronary plaque burden outcomes are significantly enriched across higher tertiles of hs-cTnI. CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, composite score: obstructive plaque or SIS>5 or SSS>5; MP: mixed plaque; CP: calcified plaque. hs-cTnI tertile ranges were ≤ 1.2 pg/mL, 1.2- 2.1 pg/mL, and ≥ 2.1 pg/mL. P-value for trend determined by Jonckheere-Terpstra test.

75x83mm (300 x 300 DPI)

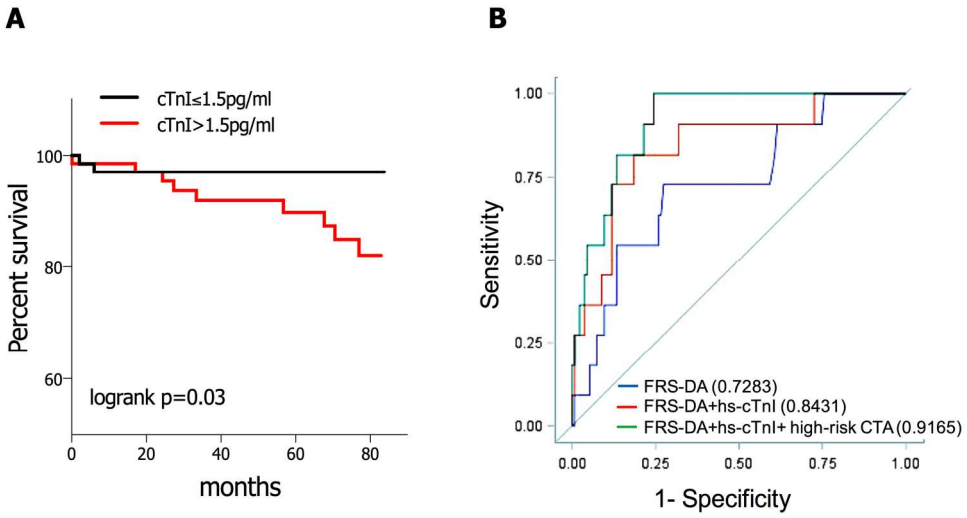

Figure 2: (A) Elevated hs-cTnI predicts long-term cardiovascular events in RA. (B) Addition of hs-cTnI information to the FRS-DA composite score increased prognostic accuracy (AUC=0.8431 vs. 0.7283, p=0.1). Further addition of high-risk plaque information from CCTA [obstructive plaque or SIS>5 or SSS>5] resulted in significant enhancement of predictive accuracy over the FRS-DA (0.9165 vs. 0.7283, p=0.015) and an improvement trend over the FRS-DA+ hs-cTnI (0.9165 vs. 0.8431, p=0.21). FRS-DA: D'Agostino Framingham modified cardiovascular risk score. CCTA: coronary computed tomography angiography.

178x96mm (300 x 300 DPI)

Online Table 1: Correlations between inflammatory and structural biomarkers and coronary plaque outcomes¶

	cTnI	IL-6	VEGF	TNF-a	IL-17A	IL-17F	CAC	SIS	SSS	PBS
cTnI	1.00	0.05	0.05	0.10	0.15	0.07	0.25**	0.20**	0.22**	0.23**
IL-6		1.00	0.09	0.21**	0.07	0.27**	0.05	0.07	0.06	0.09
VEGF			1.00	0.00	-0.11	-0.08	0.11	0.01	0.01	0.02
TNF-a				1.00	0.18*	0.10	0.01	-0.03	-0.01	-0.02
IL-17A					1.00	0.20*	-0.04	-0.05	-0.01	-0.01
IL-17F						1.00	-0.13	-0.12	-0.10	-0.08
CAC							1.00	0.71****	0.72****	0.75****
SIS								1.00	0.98****	0.98****
SSS									1.00	0.99****
PBS										1.00

cTnI: cardiac troponin-I, IL-6: interleukin-6, IL-17A: interleukin-17A, IL-17F: interleukin-17F, VEGF: vascular endothelial growth factor, CAC: coronary artery calcium; SIS: segment involvement score; SSS: segment stenosis Score, PBS: plaque burden score; ¶ Values represent Spearman correlation coefficients; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Online Table 2: Functional performance characteristics of cTnI* for high coronary plaque burden or composition

Outcome	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
All Patients				
SSS>5 or CAC>100	0.78 (0.62-0.93)	0.57 (0.48-0.66)	0.29 (0.19-0.40)	0.92 (0.86-0.98)
Obstructive plaque or SIS>5 or SSS>5	0.73 (0.54-0.91)	0.55 (0.46-0.64)	0.22 (0.13-0.32)	0.92 (0.86-0.98)
MP/CP>0	0.69 (0.51-0.87)	0.62 (0.51-0.73)	0.38 (0.24-0.52)	0.86 (0.77-0.95)
D'Agostino Framingham <10%				
SSS>5 or CAC>100	0.82 (0.59-1.00)	0.59 (0.49-0.69)	0.19 (0.08-0.30)	0.96 (0.92-1.00)
Obstructive plaque or SIS>5 or SSS>5	0.80 (0.55-1.00)	0.58 (0.48-0.68)	0.17 (0.06-0.28)	0.96 (0.92-1.00)
MP/CP>0	0.69 (0.57-0.82)	0.62 (0.52-0.72)	0.50 (0.38-0.62)	0.78 (0.69-0.88)

*cTnI binarized based on median (1.5 pg/ml); SSS: segment stenosis score, SIS: segment involvement score, CAC: coronary artery calcium, MP: mixed plaque, CP: calcified plaque, PPV: positive predictive value, NPV: negative predictive value.

Rheumatology RHE-17-1644: Highly sensitive cardiac troponin-I is a biomarker for occult coronary plaque burden and cardiovascular events in rheumatoid arthritis

Answers to reviewers' comments

We would like to thank the referees for their thoughtful and insightful review, remarks and comments. We have tried to address those to the best of our capability and have incorporated the corrections requested in the marked copy. We believe that those additions have enhanced our manuscript, and look forward to the opportunity of resubmitting an edited version. Please find below, comment by comment responses to the individual reviewer's queries.

Reviewer: 1

Comments to be transmitted to the Author
Accelerated atherosclerosis and cardiovascular (CV) disease have been associated with RA. There is a constant need for laboratory biomarkers. Numerous markers have been identified but there has been no gold standard. From this and other studies it seems that cardiac troponin may be one single, but major biomarker associated with coronary calcification and CV outcome. The study is well-designed, the results are sound.
Some minor issues

1. The Discussion is too short. Troponin results should be placed in context with other laboratory biomarkers. There are many. Why hs-cTnI would be better or worse? Specificity? Sensitivity? Also some groups suggest multi-biomarker approach by MDBA so it should be discussed whether a single biomarker would be as good as multi-biomarker assay

Assessing the utility of soluble biomarkers of CV risk in RA is limited by the time required to develop CVD after RA diagnosis and the relative scarcity of events in a single RA cohort. Most investigations report on a single cross-sectional measurement, usually of a surrogate CV end point, and as part of a retrospective analysis of a study where CV events were not the prespecified 1mary or 2dary end point. Lastly, it is unclear whether biomarkers of CV risk identified in the general population are poised to predict CVD risk in individual RA patients or they merely provide a surrogate measure of the systemic inflammatory load.
All stages of the atherogenic process appear enhanced in RA; endothelial dysfunction, increased arterial stiffness, plaque formation, coronary artery calcification, and finally CV events. However, it remains unclear whether the observed CV events arise through the same or different mechanisms from those in the general population. This latter argument in particular, further emboldens questions about the direct transferability or applicability of CV biomarkers -shown to add value in the general population- in patients with RA.
Distinct biomarkers may reflect different stages of the atherosclerotic pathway evolution at large; from inflammation [hsCRP, IL-6, TNFa, IL-1b, IL-18] to plaque instability [MPO, MMPs], to platelet activation [sCD40L], thrombosis and hemostasis [fibrinogen], to myocardial stress [NT-pro-BNP], to myocardial necrosis [hs-cTnI, hs-cTnT].

The risk of incident CV events in the general population is linear across a full range of CRP values, with CRP>20mg/L carrying the highest risk [Ridker PM et al. Circulation 2004;109:1955-9]. Additionally, the ESC states that hsCRP may be measured to redefine risk assessment in patients with unusual or moderate risk profiles (Class IIb) [Vlachopoulos C et al. Atherosclerosis 2015;241:507-532]. However, it is clear that CRP is unlikely to be causally related to vascular disease, since a meta-analysis of Mendelian randomization studies found that genes encoding for CRP were not associated with risk of coronary heart disease [BMJ 2011;342:d548]. Specifically in RA, incorporation of CRP towards CV risk determination, lead to underestimation of such risk, especially in women with high CRP [Crowson C et al. Am J Cardiol 2012;110:420-4, Arts EE et al. Ann Rheum Dis. 2015;74:668-74]. Nevertheless, baseline CRP was a predictor of CVD death in a study of 506 RA pts over a 10-year period [Goodson NJ et al. Arthritis Rheum 2005;52:2293-9]. Collectively, those observations indicate that CRP may provide prognostic information by indicating poor disease control and high levels of systemic inflammation, rather than being a surrogate for the extent of vascular involvement in RA. In our study, as elaborated in the manuscript text and tables discussion, baseline hs-CRP at the time of the CTA did not bear significant correlation with any coronary plaque outcomes, nor was it different in patients incurring CV events compared to those without.

A meta-analysis of Mendelian randomization studies of an IL-6 receptor variant (Asp358Ala) with effects consistent to IL-6R blockade in general patients reported a decreased risk of CHD per allele, supporting the causal role of the IL-6 pathway in CHD [Lancet 2012;379:1214-24]. Similarly, a 17-study meta-analysis in 5730 cases and 19,038 controls reported an adjusted OR of CHD of 1.83 (1.56-2.14) per SD increase in IL-6 values [Danesh G et al. PLoS Med. 2008;5(4):e78]. In our study IL-6 in isolation was not significantly associated with plaque outcomes (manuscript supplemental table); additionally, IL-6 combined with hs-cTnI did not optimize coronary plaque outcome prediction above and beyond that of hs-cTnI alone (not shown). Similarly, IL-6 levels were numerically (but not significantly) higher in individuals incurring incident CV events compared to those without. Additionally, high IL-6 based -upon median (>2.85pg/ml)- did not predict a higher risk of incident CV events compared to low IL-6 (please see figure 1A below). Moreover, while combination of high IL-6 (>2.85pg/ml) with high hs-cTnI (>1.5 pg/ml) significantly predicted CV events compared to low IL-6 and low hs-cTnI (Figure 1B), this was not superior from prediction rates of hs-cTnI alone (manuscript Figure 2A).

High IL-6 predicting CV events

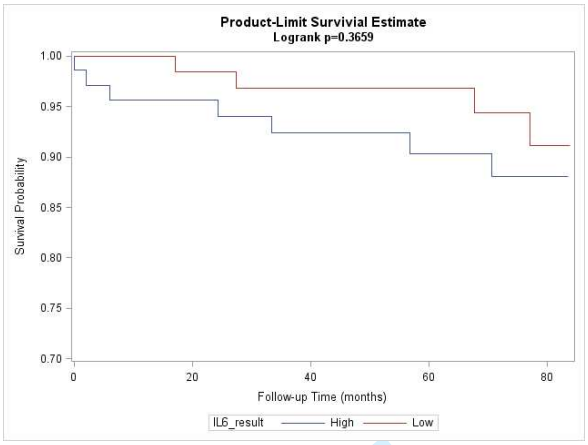

Figure 1A

High IL-6+High hs-cTnI predicting CV events

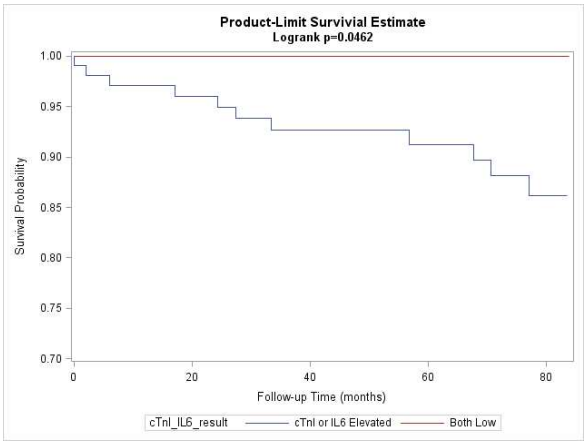

Figure 1B

Other proinflammatory cytokines such as IL-18 and TNF-a have exhibited associations with the risk of CHD in a recent large meta-analysis in general patients [Kaptoge S et al. Eur Heart J 2014;35:578-89] with relative risks of CHD per SD higher levels of 1.13 (95%CI 10.5-1.20) and 1.17 (1.09-1.25) respectively. These association have yet to be assessed reliably in Mendelian randomized studies. TNFa levels did not show associations with plaque outcomes or events in our study. Another Mendelian randomization study of the gene encoding IL-1 RA reported a per allele OR for CHD of 1.03 (95% CI 1.02-1.04) [Lancet Diabetes Endocrinol 2015;3:243-53]. In the same vein, in the recently published 10,000 patient CANTOS trial, Canakinumab -an IL-1b mAb- rendered a 15% reduction of non-fatal MI, nonfatal stroke or cardiovascular death [Ridker PM et al. NEJM 2017;377:1119-31]. The role if IL-1 pathway interception on CV risk prevention inn RA has not been interrogated.
NT-proBNP independently predicted mortality at 10 years in one study of 182 RA patients [Provan S et al. Ann Rheum Dis 2010;69:1946-50]. Our study is the first to report the association of hs-cTnI with CV events in RA.
In the general population a combination of biomarkers [CRP, NT-proBNP, and sensitive cTnI] optimized CVD risk prediction in European populations [Blankenberg S et al. Circulation 2010;121:2388-97, Melander O et al. JAMA 2009;302:49-57]. Such combinations have not yet been evaluated in RA. More multimarker combinations will likely emerge in the forthcoming years, however the optimal prognostic combinations will have to be defined. Multibiomarker approaches and their effect on optimization of CV risk prevention have not been investigated in RA.
As per your recommendation we have enriched the discussion section (pages 12 and 13) with some excerpts from the answer to this bullet (given space restrictions).

2. Is there any influence of smoking, ACPA and RF status on the results? Would cTn be associated with smoking or non-smoking, as well as seropositivity cs serinegativity? What is the value of cTn assessment is these subgroups?

As per your request, please find below a table displaying concentrations [median (IQR)] of hs-cTnI in patients based on RF, aCCP and smoking status. Results show that cTnI

is numerically higher for RF or aCCP positive individuals, but the difference is not significant at the sample size of this study. Similarly (not shown) patients with both RF and aCCP positivity had numerically higher cTnI, but the difference was also not significant compared to RF and/or aCCP negative individuals. Similar observations were made for smoking: smokers, had numerically higher hs-cTnI compared to non-smokers, however, again the difference was not statistically significant.

RF	N	Lower Quartile	Median	Upper Quartile	P-value
0	21	1	1.2	2.4	0.5662
1	125	1.1	1.5	2.6	
aCCP	**N**	**Lower Quartile**	**Median**	**Upper Quartile**	**P-value**
0	22	0.9	1.3	2.4	0.2995
1	124	1.1	1.6	2.6	
Smoking	**N**	**Lower Quartile**	**Median**	**Upper Quartile**	**P-value**
0	133	1	1.5	2.6	0.2895
1	13	1.4	2.1	2.2	

3. Authors fould no correlation between disease activity and CRP with cTnI. I guess this is about one-time CRP and DAS28. If DAS and CRP were determined repeatedly on a long-term basis (at least a few assessments), would that (area under curve) associate with cTnI?

We appreciate the reviewer's point; however, we did not have consistent and reliable collection of disease activity clinical and biomarker measurements prior to the study onset for the patients enrolled. Nevertheless, given the prospective nature of this study we have reliable and periodic collections of all disease metrics as well as traditional cardiac risk factor disposition and treatments for each subsequent clinic visit since baseline ealuation onwards. We are currently concluding re-evaluation of the same patient cohort with a repeat CTA and repeat hs-cTnI 5-6 years after their baseline evaluation. It was predetermined as a preplanned exploratory analysis in our original study design to construct AUC for in-between CTA scan disease activity and evaluate its effect on both hs-cTnI as well as plaque progression upon the follow-up evaluation.

4. Just a few weeks ago results of the CANTOS trial were published showing that Il-1 blockade by canakinumab reduces the risk of myocardial infarction. This underlines the role of IL-1 in CV disease. Authors assessed TNF-alpha, IL-6 and IL-17 but not IL-1.

We fully appreciate the reviewer's point. As mentioned in the manuscript, our cytokine biomarker evaluations where conducted through collaborators at Singulex, Alameda-CA, USA, using the Erenna platform. At the time of our study design and biomarker

batch evaluations, IL-1b quantification through that platform was not available, and therefore not pursued. However, both serum and plasma from the baseline as well as follow-up evaluations still exist; additionally, IL-1b quantification based on Erenna immunoassay is currently available and therefore will be pursued on the follow up report on both plaque progression as well as CV events on tis cohort.

Reviewer: 2

Comments to be transmitted to the Author
Review of RHEU-17-1644
In the present study the authors evaluate the highly sensitive cardiac troponin-I as a biomarker of cardiovascular risk in rheumatoid arthritis.
This is an interesting manuscript given that the general risk calculators available in clinical practice it is not sufficient to capture the incremental risk in RA patients, as also the authors comment. And, it would have been interesting to include a control group in this prospective study.

There are only some aspects to be improved, which are discussed below:
- In methods:

***** on page 5:***

a/ The weight should also be provided in kilogram (in parentheses).

The weight in Kg has been added in parenthesis.

b/ In the definition of hypotension, should not diastolic blood pressure also be included?

Per your recommendation the definition of hypotension has been amended as SBP<90mmHg and DBP<60mmHg.

c/ Specify the first time: SBP, DBP and GFR.

Per your recommendation those acronyms have been defined.

***** on page 6:***

a/ Patients on lipid-lowering therapy (especially statins) should be included in the definition of hyperlipidemia.

We fully appreciate the reviewer's suggestion. Based on our definition of hyperlipidemia [Cholesterol>200mg/dl (>5.175mmol/L) or LDL>130 mg/dl (>3.36mmol/L)], 26/150 patients fulfill hyperlipidemia criteria. However, a total of 60 patients are under statin treatment (please see Table 1 below). Of those, 49 do not fulfill our definition of hyperlipidemia. Importantly, 25/49 (50%) patients who were under statin treatment without being hyperlipidemic, had no indication for statin Rx based on 2013 AHA guidelines; they were non-diabetic, they had low clinical risk by standard calculator,

were in good disease control (mean DAS28=2.35), all had LDL<115mg/dl (2.97mmol/L) and average LDL was 84.5 mg/dl (2.19mmol/L). Lipid profiles for those 25 patients were reviewed backwards from the study baseline visit as long as possible [mean of 5 years], again yielding no indication for statin treatment based on aforementioned AHA guidelines. Since almost half the statin exposed patients had no indication for statin treatment and had no hyperlipidemia, we thought it would be more appropriate to adjust for statin use along with hyperlipidemia in model 2 (along with traditional and non-traditional cardiac risk factors) for both plaque outcomes (table 2) and events (table 3) instead of including statin exposure in the definition of hyperlipidemia. Those results are shown highlighted in table 2 below. You can readily appreciate that additional adjustment for statin treatment in model 2 yields numerically (though not statistically) better results. Those results have been updated on the respective tables 2 and 3 in the manuscript as well under the methods section (marked copy).

In regard to the aforementioned 25 patients without obvious indications for statin treatment who were given this therapy, it is worth mentioning that primary care physicians and rheumatologists in our institution are very aware and sensitized to the enhanced cardiovascular risk RA patients incur. Given limited empirical evidence, current recommendations for CV risk prevention in RA are based on expert opinion, {Agca R et al. Heart. 2016 May 15;102(10):790-5}. However, due to differences in expert opinions and drawbacks of the currently proposed CV prevention strategies, none of them have been uniformly accepted {Agca R et al. Heart. 2016 May 15;102(10):790-5}, {Hollan I et al. Autoimmun Rev. 2015;14(10): 952-69}, {Crowson CS et al. Rheumatology (Oxford). 2017 Jul 1;56(7):1102-1110}. As a result, approaches vary from treating RA patients as the general population, to adding a certain number of years (e.g., 10-15 years) to their age, treating RA patients as diabetics, or using specific mathematical formulas to adjust for the RA-related risk {Hollan I et al. Autoimmun Rev. 2015;14(10): 952-69}. The European guidelines on cardiovascular disease prevention in clinical practice declare that: “as yet there is no indication for the preventive use of lipid-lowering drugs only on the basis of the presence of autoimmune diseases” (class recommendation A, level of evidence B), while the 2013 position paper of the International Atherosclerosis Society does not mention autoimmune diseases at all. On the other hand, the ACC/AHA 2013 guidelines recommend considering statin treatment, according to clinical judgement, in serious comorbidities such as rheumatologic and inflammatory disease in spite of the insufficient evidence {Circulation 2014;129:S1-S45}. As a result of these confounding guidances, physician practices fluctuate from potential undertreatment to significant overtreatment (as in the case of the 25 patients aforementioned).

Table 1					
Patient numbers	Hyperlipidemia definition (chl>200mg/dl, LDL>130mg/dl)	Statin treatment	Diabetes Mellitus	Risk class	Notes
150	(yes): 26	Yes: 11			
		No: 15	Yes: 0/15	6/15: moderate	All LDL<190, 6/15 with LDL>130
	(No): 124	Yes: 49	Yes: 17/49		
			No: 32/49	7/32: moderate	
				25/32: Low	Mean LDL=84.5mg/dl All with LDL<115 mg/dl. Mean DAS-28=2.35
		No: 75	Yes: 8/75		
			No: 67/75		

Table 2	Unadjusted OR (95% CI)	Model 1[1] OR (95% CI)	Model 2[2] OR (95% CI)	Model 2 + statins OR (95% CI)	Model 3[3] OR (95% CI)
CAC*	*2.95 (1.5-6.0)*	*2.2 (1.0-4.7)*	2.2 (1.0-5.0)	2.3 (1.0,5.2)	*2.7 (1.3-5.8)*
CAC>100 vs.≤100	*4.2 (1.3-13.5)*	3.0 (0.9-10.5)	*5.5 (1.2-24.4)*	*5.7(1.2,25.9)*	*5.0 (1.3-19)*
SIS*	*2.2 (1.1-4.2)*	1.7 (0.9-3.4)	1.7 (0.8-3.5)	1.7 (0.8,3.5)	1.9 (1.0-3.8)
SIS>5 vs. SIS≤5	2.5 (0.7-8.5)	1.8 (0.5-6.6)	2.6 (0.6-10.8)	2.6(0.6,11.0)	2.6 (0.7-9.9)
SSS*	*2.4 (1.3-4.7)*	1.9 (1.0-3.9)	2.0 (1.0-4.1)	2.0 (1.0,4.1)	*2.2 (1.1-4.3)*
SSS>5 vs. SSS≤5	*3.0 (1.1-8.2)*	2.4 (0.8-7.3)	2.7 (0.9-8.7)	2.8 (0.9-8.8)	*3.0 (1.0-9.0)*
PBS*	*3.1 (1.5-6.3)*	*2.4 (1.1-5.1)*	*2.5 (1.1-5.6)*	*2.6 (1.1,5.9)*	*2.9 (1.3-6.2)*
Obstructive plaque	*3.9 (1.2-12.5)*	3.1 (0.9-10.7)	3.6 (0.9-13.9)	4.0(1.0,15.5)	*4.0 (1.1-14.1)*
CAC>100 or SSS>5	*4.7 (1.8-12.4)*	2.3 (0.7-6.9)	*4.7 (1.5-15.0)*	4.9 (1.6,15.5)	*5.2 (1.8-15.8)*
SIS>5 or SSS>5 or obstructive plaque	*3.2 (1.2-8.8)*	2.6 (0.9-7.7)	2.9 (0.9-8.9)	2.9(0.9,9.0)	*3.3 (1.1-9.7)*
NCP>0 vs. NCP=0	1.4 (0.7-2.7)	1.3 (0.6-2.5)	1.3 (0.6-2.7)	1.3 (0.6,2.7)	1.4 (0.7-2.7)
MP/CP>0 vs. MP/CP=0	*3.6 (1.8-7.5)*	*2.7 (1.2-6.0)*	*2.9 (1.2-6.6)*	2.9 (1.2,6.7)	*3.5 (1.6-7.6)*
CV events	*4.7 (1.0,21.7)*	*4.8 (1.0,23.1)*	*5.4 (1.1,25.9)*	5.3 (1.1,25.9)	*4.3 (0.9,19.7)*

b/ CAD should be specified….and, please check all the acronyms of the manuscript and verify that they have been specified.

As per your recommendation CAD (coronary artery disease) and all other acronyms have been defined in the manuscript text (marked copy).

c/ It is assumed that CTA was performed with contrast, it should be added to the text (justification to exclude patients with iodine allergy).

Thank you for your comment. It has been added on page 7 line 4 (marked copy).

- In discussion:

***** on page 13:***
a/ check "cTnT".....should be hs-cTnT"? (the same in table 1).

Thank you for the suggestion. This is absolutely correct and has been updated both on the manuscript text (marked copy) as well as table 1.

b/ the sentence "By contrast, RA patients with low levels of hs-cTnI were 82%....". First: 82% should be checked if it is correct (in results, on page 10 it says 81%).

The statement in page 10 refers to lower risk of extensive non-obstructive or obstructive ***coronary plaque*** in patients with low hs-cTnI; hence: "subjects with low hs-cTnI (<1.5 pg/ml) were less likely to have extensive coronary atherosclerosis; specifically, they displayed 81% lower risk of having SSS>5 or CAC≥100 after controlling for FRS-DA score"
The statement in page 13 refers to lower risk ***of CV event*** in patients with low hs-cTnI; hence: "RA patients with low levels of hs-cTnI were 82% less likely to suffer a CV event".
Both statements are correct.

Second: The explanation should be expanded a little and clarify if this also happens in the general population.

In a nested case-control study [(Minnesota Heart Study)- with 211 cases and 253 controls matched for age, gender and year of study] in general patients hs-cTnI evaluated with the same assay as in our study [Erenna Immunoassay] adjusted odds ratio of death from CV disease, stroke or heart failure in patients with high cTnI was 8.53 (95% CI of 1.68-43) [Apple FS et al. Clinical Chemistry 2012;58:930-935]. This means that patients with low cTnI had 88% Lower risk of death compared to those with high hs-cTnI. This estimate, although referring to CV death rather than all CV events is very close to our observed of 81% lower risk of CV events in RA. Per your request this information has been added to the discussion in page 13 (marked copy).