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Utility of novel serum biomarkers to predict subclinical atherosclerosis: A sub-analysis of the EISNER study

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HIGHLIGHTS

- Over 50% of intermediate risk patients without known atherosclerotic cardiovascular disease (ASCVD) had coronary artery calcium (CAC) at baseline.
- Baseline CAC was related to OPN, TROY, and TNFR1α in unadjusted analyses.
- Only osteopontin (OPN) independently predicted progression of CAC.

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ABSTRACT

Background and aims: Certain novel biomarkers may predict atherosclerotic cardiovascular disease (ASCVD) events; however, data on their relationship to coronary atherosclerosis and its progression as measured by coronary artery calcium (CAC) scanning is lacking. We evaluated the association between novel biomarkers and presence or progression of CAC.

Methods: The EISNER study was a prospective trial of patients without known ASCVD. Data on CAC and several biomarkers (hs-CRP, LTβR, osteopontin [OPN], RAGE, TNFR1α and TROY) were available at baseline and 4-year follow-up. Biomarkers were standardized and summed for a composite score. CAC progression was defined by the square-root (CAC_{SQRT}) method and rapid (top decile) progression. Adjusted regression models created a final prediction model for baseline CAC and CAC progression.

Results: 1207 subjects (mean age 58.4 ± 8 years, 53% male) were evaluated; 621 had a baseline CAC > 0, in whom 323 progressed by CAC_{SQRT}, and 121 rapidly progressed. Baseline CAC was associated only with OPN ($p = 0.03$), TROY ($p = 0.0058$) and TNFR1α ($p = 0.0039$) in unadjusted analyses. In adjusted analyses, only OPN was independently related to CAC progression using CAC_{SQRT} ($p = 0.04$).

Conclusions: OPN identifies progression of atherosclerosis in persons free of ASCVD at baseline and may be a useful predictive tool to guide ASCVD prevention management.

1. Introduction

Coronary atherosclerosis is responsible for over 600,000 coronary heart disease events in the United States each year [1,2]. Given these staggering statistics, augmenting traditional atherosclerotic cardiovascular disease (ASCVD) risk models with biomarkers and non-invasive imaging to predict subclinical atherosclerosis is paramount [3–5]. Recently, both coronary artery calcification (CAC) scores and biomarkers, most prominently high-sensitivity C-reactive protein (hs-CRP) have improved the efficacy of traditional risk calculators [6–8]. However, the comparison of CAC scores or CAC progression as a marker of subclinical atherosclerosis with multiple serum biomarkers remains largely unexplored.

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The Early-Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research (EISNER) study is a randomized prospective cohort trial of patients without known ASCVD. Over a four-year period, the EISNER study measured CAC scores and several biomarkers, including: C-Reactive Protein (CRP), Tumor Necrosis Factor Receptor 1 α (TNFR1α), TNFRSF expressed on mouse EmbrYo (TROY), Lymphotoxin Beta Receptor (LTβR) Osteopontin (OPN), and Receptor for Advanced Glycation End-product (RAGE).

These biomarkers signal the initial inflammatory and calcium deposition phases of atherosclerosis. Hs-CRP contributes to atherosclerosis [9,10] by activating and chemo-attracting monocytes, mediating coronary endothelial dysfunction, creating a pro-thrombotic state, increasing and amplifying cytokine release, and activating the complement system [11]. The TNFα Receptor Super Family, including LTβR, TROY, TNFR1α, mediate major inflammatory cytokines involved in the formation and destabilization of atherosclerotic plaques [12–16]. OPN is a cytokine upregulated in response to calcium deposition in atherosclerotic plaques [17,18]. RAGE transduces effects of multiple pro-inflammatory, pro-thrombotic and reactive oxygen species ligands and promotes vascular osteogenesis, likely contributing to exaggerated cellular damage [19–24].

We evaluated the relation between these novel serum biomarkers, individually and summed, with the presence and progression of CAC as a surrogate measure for atherosclerosis.

2. Materials and methods

2.1. Study population and data collection

The recruitment and selection process of the EISNER study been previously described in detail [25,26]. Briefly, 2137 subjects without known ASCVD with intermediate ASCVD risk factors were initially evaluated. Exclusion criteria included prior history of cardiac or cerebrovascular disease or chest pain, age ≥80 years, pregnancy, significant medical co-morbidity, and prior coronary catheterization or prior CAC scanning. Subjects were randomized to a “scan” group or “no scan” in a 2:1 ratio at baseline, underwent a CAC scan utilizing cardiac computed tomography, and where then followed for four years (at which time all subjects received a CAC scan). The research was approved by CSMC institutional review board and all subjects signed informed consent.

We utilized data from subjects who had both baseline and follow-up measures of CAC as well as blood samples for measurement of biomarkers (n = 1207). Baseline measurements were obtained for fasting total cholesterol, high density lipoprotein, triglycerides, fasting serum glucose; systolic and diastolic blood pressure measurements, and BMI was calculated by height and weight measurements. Additionally, biomarkers CRP, LTβR, OPN, RAGE, TNFR1α and TROY were drawn and processed with a Luminex Sandwich assay (Alere San Diego Inc, San Diego, CA). Lower and upper detectable levels for the biomarkers are as follows: CRP: 0.065–35μg/mL, LTβR: 0.028–53 ng/mL, OPN: 2–3000 ng/mL, RAGE: 0.019–100 ng/mL, TNFR1α: 0.025–90 ng/mL, TROY: 0.044–86.52861 ng/mL. Values below the low cutoff have been set to the low cutoff. Values above the high cutoff have been set to the high cut off.

2.2. CAC scanning and measurements

As previously described [26], CAC scanning was performed using electron-beam or multi-slice computed tomography (Imatron C-150 or GE eSpeed; GE Healthcare, Waukesha, Wisconsin), with little systematic difference between these modalities noted [27]. Acquisitions consisted of approximately 30–40 slices about 3 mm thick [28]. Each area of CAC was scored by an experienced technician blinded to patient characteristics, using semiautomatic software on a NetraMD workstation (ScImage, Los Altos, California). A segment was scored by detecting a lesion of a minimum of 3 contiguous pixels (voxel size 1.03 mm3) of peak density > 130 Hounsfield units. All CAC scores were verified by an experienced cardiologist. CAC scores were calculated using the method of Agatston et al. [29]. This was completed at baseline and at four year follow up.

We based our definition of progression of CAC on two models seen in recent literature: Continuous progression and the square root (SQRT) method. Continuous progression was defined as the rate of change computed from the log-transformed CAC at year 4 (CAC_{y4})– baseline log-transformed CAC at baseline (CAC_{bl}), and used as a continuous variable. The square root (SQRT) method was proposed by Budoff et al. (2010) which utilizes the formula $\sqrt{CAC_{y4}} - \sqrt{CAC_{bl}}$, where values greater than 2.5 were considered progressors, all others were non-progressors [30]. A third method, “Rapid” progression, was defined as the top decile of change in log-transformed CAC, also a binary variable.

2.3. Statistical analysis

All statistical analysis was done with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Baseline hs-CRP, LTβR, OPN, RAGE, TNFR1α and TROY were normalized using a log scale and standardized. A biomarker index was calculated based on each individual's standardized value into a z-score. For all patients at baseline and follow-up, we compared baseline laboratory values and cardiovascular risk factors using the Chi-square test of proportions and the Student's t-test for continuous variables (or Wilcoxon Signed Rank test for those non-normally distributed).

Risk-factor adjustment was performed for developing regression models with the following factors: age, gender, hyperlipidemia, hypertension, diabetes, family history of coronary artery disease (CAD) smoking history. Linear regression models with difference in log-transformed biomarkers and the cumulative z-score compared to baseline CAC and continuous progression were constructed with and without adjustment for risk factors. CAC progression was measured by multiple methods, as one method is not considered standard [30]. Thus, logistic regressions were also run to identify the biomarkers associated with progression of CAC based on the SQRT and rapid progression definitions with OR ± confidence intervals produced.

3. Results

General characteristics of our study sample are presented in Table 1 at baseline and 4-year follow up. There were 1207 subjects evaluated, of whom 644 (53.4%) were male, 913 (75.6%) were white. Over the four-year follow-up period, there was an increase in the prevalence of hypertension and diabetes and in BMI, though there was a decrease in the prevalence of hyperlipidemia. There were also significant increases in CAC scores as well as in all biomarkers, with the exception of OPN.

3.1. Relation of circulating biomarker levels to CAC

The biomarkers were evaluated by CAC at baseline (Table 2), separated into groups of $CAC_{bl} = 0$ (n = 586), CAC_{bl} between 0 and 100 (n = 374), and CAC_{bl} greater than 100 (n = 247). There were no significant differences between the biomarker averages (ANOVA) with the exception of OPN ($p = 0.03$), TROY ($p = 0.0058$) and TNFR1α ($p = 0.0039$). After log transformation and standardization, each biomarker was transformed to z-score, which were then summed to form a composite z-score.

3.2. Association of biomarkers with baseline CAC and CAC progression

Association of change in unadjusted log-transformed biomarkers with log-transformed CAC baseline (Table 3) revealed a significant positive association with LTβR, OPN, TROY, and TNFR1α. However, these results were significantly attenuated by adjustment for standard risk factors, after which none were associated with baseline CAC. Interestingly, hs-CRP was inversely related to CAC_{bl}, but failed to produce an association with CAC when measured continuously or categorically.

Table 1
Baseline characteristics of study subjects at baseline and follow-up.

Characteristic	Baseline	Follow-up	*p* value
	(N = 1207)	(N = 1207)	
	Mean ± SD or N (%)		
Age (years)	58.4 ± 8.3	62.5 ± 8.3	< 0.001
Gender			
Male	644 (53.4%)		
Female	563 (46.6%)		
Race			
White	913 (75.6%)		
Black	67 (5.6%)		
Hispanic	54 (4.5%)		
Asian/Pacific Islander	129 (10.7%)		
Other/Not collected	44 (3.6%)		
Hypertension	693 (57.4%)	814 (67.4%)	< 0.001
BMI (kg/m^2)	27.5 ± 5.3	27.7 ± 5.3	0.002
Current smoker	64 (5.3%)	44 (3.7%)	< 0.001
Family history of CAD	335 (27.8%)	413 (34.2%)	< 0.001
Diabetes	97 (8.0%)	136 (11.3%)	< 0.001
Hypercholesterolemia	830 (68.8%)	720 (59.7%)	< 0.001
CAC score	105.51 ± 294.94	157.28 ± 420.42	< 0.001
CAC score = 0	586 (48.6%)	498 (41.3%)	< 0.001
CAC score > 0 and < 100	374 (31%)	380 (31.5%)	< 0.001
CAC score ≥ 100	247 (20.5%)	329 (27.3%)	< 0.001
hs-CRP (μg/mL)	5.05 ± 7.72	6.33 ± 8.72	< 0.001
LTβR (ng/mL)	0.44 ± 1.53	0.5 ± 1.53	< 0.001
Osteopontin (ng/mL)	67.91 ± 93.63	69.66 ± 95.68	0.321
RAGE (ng/mL)	2.55 ± 1.2	2.49 ± 1.35	< 0.009
TROY (ng/mL)	0.52 ± 1.18	0.62 ± 0.79	< 0.001
TNFR1α (ng/mL)	1.47 ± 2.62	1.7 ± 2.67	< 0.001

BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LTβR, Lymphotoxin β receptor; RAGE, receptor for advanced glycation and end product; TROY, TNFRSF expressed On mouse embrYo; TNFR1α, Tumor Necrosis Factor receptor 1α, CAC, coronary artery calcium; CAD, coronary artery disease.

Table 2
Baseline biomarker measurements by baseline CAC score (n = 1207).

Biomarker	CAC Score			*p*-value
	0	> 0 and < 100	≥100	
	(n = 586)	(n = 374)	(n = 247)	–
CRP (μg/mL)	5.26 ± 8.04	4.87 ± 7.36	4.85 ± 7.48	0.7219
LTBR (ng/mL)	0.39 ± 0.19	0.54 ± 2.73	0.43 ± 0.24	0.0681
OPN (ng/mL)	62.83 ± 40.15	73.63 ± 156.24	71.31 ± 44.79	0.03
RAGE (ng/mL)	2.59 ± 1.2	2.5 ± 1.17	2.54 ± 1.25	0.3807
TROY (ng/mL)	0.46 ± 0.18	0.59 ± 2.09	0.53 ± 0.24	0.0058
TNFR1 α (ng/mL)	1.33 ± 0.52	1.66 ± 4.62	1.52 ± 0.7	0.0039

CRP, high-sensitivity C-reactive protein; LTβR, lymphotoxin β receptor; RAGE, receptor for advanced glycation and end product; TROY, TNFRSF expressed On mouse embrYo; TNFR1α, Tumor Necrosis Factor receptor 1α; OPN, osteopontin, CAC, coronary artery calcium.

The SQRT method and Rapid Progression are shown in Table 3. Notably, OPN is associated with CAC progression via the SQRT method ($p = 0.04$), while the other biomarkers were not. No biomarker was associated with rapid CAC progression (Fig. 1).

4. Discussion

Emerging evidence suggests the role of inflammation in atherosclerosis and progression to significant cardiovascular disease [31]. Identifying serum biomarkers that can predict microvascular and macrovascular disease progression may allow for improved risk-stratification and treatment to prevent future coronary events. Of the six biomarkers we studied, associated with coronary inflammation, only OPN was found to be independently associated with CAC progression ($p = 0.04$) by SQRT method, adjusting for traditional parameters. Hs-CRP was not found to be associated with CAC progression by both methodologies. OPN, a calcium-binding glycophosphoprotein linked to bone calcification, inflammation and vascular calcification, is elevated as the body detects calcium deposition leading to arterial stiffness and CAC [32,33]. Mazzone et al. demonstrated patients with CAD with both rapid coronary plaque progression and in-stent restenosis had the highest levels of OPN, suggesting that persistent elevation of OPN contributes to accelerated plaque calcification and may be utilized as a prognostic tool [33]. While previous studies indicate that OPN is found in patients with clinical ASCVD, our study is one of the first clinical evaluations assessing OPN as a marker of subclinical ASCVD. Of the six biomarkers and composite score tested, OPN independently predicted subclinical CAC progression. Recent data has suggested that patients with elevated CAC scores in patients with subclinical ASCVD benefit more from statin therapy [34]. Elevated OPN levels as a surrogate for CAC progression could identify populations at increased risk of early and progressive atherosclerosis, anti-inflammatory medical therapies such as statins or angiotensin II inhibitors may be initiated earlier, thus preventing future coronary events. Lorezen et al. described in a prospective, multi-center, double-blind study that both statin and angiotensin II inhibitors reduced OPN levels [35]. The benefits of these therapies are clearly well-established in reducing coronary artery disease progression; thus, reduction in OPN levels might be a useful surrogate to demonstrate a decline or cessation of coronary atherosclerosis progression in an intermediate risk population.

High-sensitivity C-reactive protein (hs-CRP) has been widely used as a risk-stratification biomarker for intermediate-risk individuals since the release of the large-scale JUPITER trial in 2008 [36]. It was estimated that a portion of the intermediate-risk participants risk status might change based on additional testing with hs-CRP levels. In contrast, other studies have not shown a similar association between hs-CRP and atherosclerotic progression [37,38]. Our study demonstrated that hs-CRP, when adjusted for all covariates, was not predictive of CAC progression. As prior studies have suggested, hs-CRP may play a role in predicting future cardiovascular events; however, it lacks specificity for progression of atherosclerosis or CAC.

RAGE, TNFR1α, LTβR, and TROY are inflammatory biomarkers that are useful for identifying chronic inflammatory diseases, and a subset of which were associated with our CAC baseline results; however, had limited utility in detecting CAC progression. TNFR1α and LTβR are both signaling molecules that regulate inflammation. RAGE is accelerated during a hyperglycemic state, and has been found to elevated and predictive of cardiovascular events in type 2 diabetics [39]. While these markers play a large role in the inflammatory milieu that leads to atherosclerosis, their role for risk-stratification for subclinical atherosclerosis remains unknown.

In this study, we demonstrate that OPN independently predicted CAC progression in a predominantly non-diabetic population with subclinical ASCVD. Diabetic patients are at risk of developing subclinical ASCVD as revealed by CAC, and as a result, this population has been studied extensively for biomarkers to predict development of ASCVD [40]. Osteopontin, as well as another bone calcification regulation biomarker, OPG, have both been shown to predict subclinical ASCVD in this group [7,32,41,42]. Studies have shown both to be upregulated in diabetic patients with atherosclerosis, with one study showing a four-fold increase in OPN compared to a two-fold increase in OPG [32,43]. Anand et al. showed that OPG was superior to hs-CRP and IL-6 in predicting CAC in diabetic patients with subclinical ASCVD [7]. In our study, hs-CRP also did not predict progression of CAC by any method tested. To our knowledge, OPG has not been previously shown to predict presence of CAC or CAC progression in a general patient

Table 3
Relation of biomarkers (risk-factor unadjusted and adjusted) to baseline and progression of coronary calcium.

Biomarkers (units)	Baseline CAC		CAC progression: continuous		CAC progression: SQRT method		CAC progression: rapid	
	Linear regression coefficient		Linear regression coefficient		Logistic regression odds ratio		Logistic regression odds ratio	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
CRP (μg/mL)	−0.09	−0.001	0.007	0.004	1.23	0.95	0.98	0.85
LTβR (ng/mL)	0.32*	−0.03	−0.03	−0.007	1.29	1.01	0.93	0.72
OPN (ng/mL)	0.31†	0.07	0.04	0.06	1.31	1.25*	1.18	1.00
RAGE (ng/mL)	−0.15	−0.04	0.02	0.05	1.23	1.18	1.1	1.12
TROY (ng/mL)	0.52†	0.01	−0.2	0.006	1.07	1.08	1.01	1.19
TNFR1α (ng/mL)	0.52†	−0.05	−0.06	−0.03	0.92	1.06	0.88	1.02
Z-score Sum	0.24*	0.10	0.009	0.03	1.12	1.09	0.99	0.99

*$p < 0.05$, †$p < 0.01$.
CRP, high-sensitivity C-reactive protein; LTβR, lymphotoxin β receptor; RAGE, receptor for advanced glycation and end product; TROY, TNFRSF expressed On mouse embrYo; TNFR1α, Tumor Necrosis Factor receptor 1α; OPN, osteopontin, CAC, coronary artery calcium.

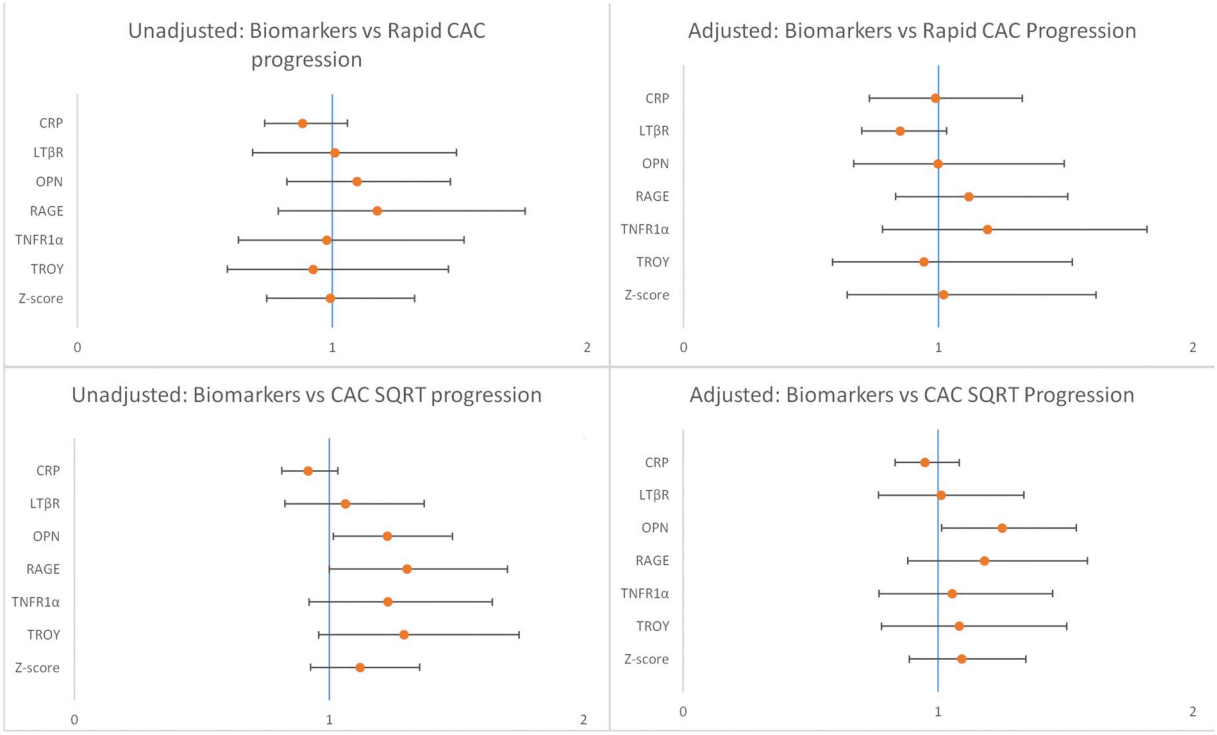

Fig. 1. (A and B) Risk factor unadjusted and adjusted (respectively) individual biomarkers and sum z-score OR with 95% confidence intervals demonstrating no clear significance for biomarkers, relating to CAC rapid progression. (C and D) Risk factor unadjusted and adjusted (respectively) biomarkers and sum z-score OR with 95% confidence intervals, demonstrating significance of OPN to predict CAC progression by SQRT method.
CRP, high-sensitivity C-reactive protein; LTβR, lymphotoxin β receptor; RAGE, receptor for advanced glycation and end product; TROY, TNFRSF expressed On mouse embrYo; TNFR1α, Tumor Necrosis Factor receptor 1α; OPN, osteopontin, CAC, coronary artery calcium.

population. OPN is predictive of ASCVD progression in intermediate risk diabetic populations [42] and as our study shows, this finding can be expanded to a more general patient population. Given the small number of head-to-head studies, a direct comparison between these two biomarkers is difficult. Further evaluation of OPN and OPG in patients with subclinical ASCVD is warranted to better characterize the relationship of these two important biomarkers.

The principal limitation to our findings was that the study period was 4-years and there are only two points in time when the biomarkers are obtained, thus data on long-term progression of CAC or adequate follow-up to examine prognostic implications of our biomarkers as related to future ASCVD events were not available. In addition, we did not have a sufficient number of persons with diabetes to specifically examine biomarker associations with CAC in those with diabetes separately. Additionally, the primary outcome was baseline CAC levels and CAC progression, thus conclusions about major adverse cardiovascular events (MACE) and mortality cannot be drawn. Finally, the subjects were largely upper-middle class with good access to healthcare, creating a somewhat homogenous population.

We demonstrate that osteopontin might be utilized as a tool for identifying early atherosclerosis in intermediate risk patients. As a marker of the activity of calcium deposition, it could identify patients who merit aggressive preventive therapy such as statin treatment. The relation of other serum biomarkers LTβR, RAGE, TNFR1α and TROY to CAC levels and progression is less consistent. Further investigation is needed to elucidate the relationship of these other biomarkers and CAC. OPN could be a useful screening tool for intermediate-risk patients in the outpatient setting to allow for earlier initiation or adjusting intensity of therapies, such as statins, aspirin, and angiotensin II inhibitors. Future prospective studies should be conducted to evaluate for long-term impact of OPN level surveillance, early therapy initiation, and its prediction of impact on both MACE and all-cause mortality.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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