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Original Research Article

A STUDY OF NOVEL CARDIAC BIOMARKERS FOR CARDIOVASCULAR DISEASE DETECTION

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ABSTRACT

Introduction: The effectiveness of biomarkers in predicting cardiovascular events in community-based populations has been inconsistent, often failing to provide additional information beyond standard risk factors. Many previously studied biomarkers lack cardiovascular specificity.

Materials and Methods: To evaluate the predictive value of three novel biomarkers induced by cardiovascular stress, soluble ST2, growth differentiation factor-15, and high-sensitivity troponin I, we measured them in 134 participants. Using multivariable-adjusted proportional hazards models, we assessed the individual and combined ability of these biomarkers to predict adverse outcomes. Additionally, we created a "multimarker" score comprising the three biomarkers along with B-type natriuretic peptide and high-sensitivity C-reactive protein.

Results: In multivariable-adjusted models, the three new biomarkers were associated with each endpoint except for coronary events. Individuals in the highest quartile of multimarker scores had a three-fold risk of death, a six-fold risk of heart failure, and a two-fold risk of cardiovascular events.

Conclusion: Various biomarkers of cardiovascular stress are detectable in ambulatory individuals and enhance the prognostic value of standard risk factors for predicting death, overall cardiovascular events, and heart failure.

Keywords: Cardiac biomarkers, growth differentiation factor-15, high-sensitivity troponin, soluble ST2.

INTRODUCTION

Predicting cardiovascular events in individuals with low to intermediate risk poses a significant challenge, despite the fact that they collectively contribute to the majority of such events within the population. While the idea of using circulating biomarkers to enhance risk prediction is appealing, previous research has not consistently demonstrated their added value beyond traditional risk factors [1–6]. Notably, the US Preventive Services Task Force recently concluded that cardiovascular biomarkers offer limited clinical benefit [7], underscoring the necessity for identifying more effective biomarkers in community-based settings.

Many previously studied biomarkers suffer from a lack of cardiovascular specificity. For instance, high-sensitivity C-reactive protein (hsCRP), extensively investigated in general populations, is produced by the liver and may indicate inflammation from various sources. In recent years, newer biomarkers such as soluble ST2 (sST2), growth differentiation factor-15 (GDF-15), and high-sensitivity troponins have emerged, each being expressed or released by cardiovascular tissue in response to mechanical or pathological stress [8–10]. Studies have demonstrated the prognostic value of these biomarkers in individuals with acute coronary syndromes and heart failure [11–15]. Furthermore, recent evidence suggests that these biomarkers may also provide prognostic insights for ambulatory individuals [16–19]. However, sST2 has not been explored in a community-based cohort, the biomarkers have not been evaluated collectively, and there is limited data regarding their association with specific outcomes such as heart failure.

Thus, our study aims to assess the individual and combined predictive capacity of sST2, GDF-15, and high-sensitivity troponin I (hsTnI) for cardiovascular outcomes. We hypothesize that a panel of these biomarkers could effectively identify individuals at elevated risk of future cardiovascular disease in the preclinical phase and contribute to enhancing existing risk-prediction algorithms.

MATERIALS AND METHODS

A total of 134 participants underwent a standardized evaluation, which encompassed a comprehensive medical history and a physical examination conducted by a physician. Diabetes mellitus was defined by either a fasting glucose level of ≥126 mg/dL or the use of insulin or other hypoglycemic medications. Participants were categorized as current cigarette smokers if they reported regular cigarette usage within the year preceding the examination.

Blood biomarkers were assayed from morning samples collected after an overnight fast, with participants in a supine position for 5 to 10 minutes prior to phlebotomy. Upon collection, blood samples were promptly centrifuged, and plasma and serum were stored at -70°C. No freeze-thaw cycles were permitted before the execution of the assays described below.

The concentration of soluble ST2 (sST2) was determined utilizing a high-sensitivity, second-generation enzyme-linked immunosorbent assay (ELISA) [20]. High-sensitivity C-reactive protein (hsCRP) and B-type natriuretic peptide (BNP) were quantified using previously established methods [5]. Growth differentiation factor-15 (GDF-15) levels were assessed using a precommercial, automated electrochemiluminescent immunoassay.

During the follow-up period, suspected cardiovascular events underwent rigorous scrutiny by a committee comprising three experienced investigators, who reviewed hospital records, physician office notes, and pathology reports. The definition of a major cardiovascular event, as detailed previously [5], encompassed recognized myocardial infarction, coronary insufficiency (prolonged angina with documented electrocardiographic changes), coronary

heart disease death, heart failure, and stroke. Major coronary events were further delineated as recognized myocardial infarction, coronary insufficiency, and coronary heart disease death. Events based solely on historical accounts (e.g., symptoms of intermittent claudication or transient ischemic attack, or typical chest pain lacking electrocardiographic evidence of ischemia or injury) were classified as nonmajor events and were not included in the primary endpoint or multivariable regression models, following precedents set in prior studies [2,21].

We analyzed the relationship between the biomarkers and the risk of all-cause mortality, heart failure, first major cardiovascular events, and first major coronary events using multivariable proportional hazards (Cox) models. The biomarker distributions were standardized to a mean of 0 and standard deviation of 1 for comparison of effect sizes. The proportionality assumption was confirmed by testing the interaction of the biomarkers with follow-up time. The combined predictive ability of the 5 biomarkers (sST2, GDF-15, hsTnI, hsCRP, and BNP) was assessed by creating a "multimarker" risk score. This score was determined based on the proportional hazards regression coefficients for each biomarker in the multivariable model for the respective outcome.

RESULTS

Table 1 provides a comprehensive overview of baseline parameters in the study population, highlighting gender-specific differences in cardiovascular risk factors and biomarker levels. There was no significant difference between the parameters. BNP, GDF-15, hsCRP, hsTnI levels were slightly higher in women than men.

Table 1: Baseline parameters in study patients

Parameter	Men (n=61)	Women (n=73)
Age, in years	58±9	60±11

BMI, kg/m²	29.1±4.7	26.8±5.3
Diabetes mellitus, %	15	11
Cigarette smoking, %	17	14
Systolic blood pressure, mm Hg	129±16	128±19
Use of antihypertensive, %	32	26
Total cholesterol, mg/dL	205±38	219±37
HDL cholesterol, mg/dL	45±11	60±14
ECG LVH, %	2	0.3
Prevalent major CVD, %	9	4
Significant murmur, %	4	3
Biomarkers, median (IQR)		
BNP, pg/mL	7.1 (13.5)	10.6 (16.8)
GDF-15, ng/L	1078 (605)	1035 (502)
hsCRP, mg/L	2.05 (3.11)	2.67 (5.04)
hsTnI, pg/mL	1.77 (1.72)	1.09 (1.06)
Soluble ST2, ng/mL	24.8 (10.5)	19.6 (8.3)

Table 2 outlines the association of novel cardiac biomarkers with various cardiovascular diseases. sST2 exhibited a notable association with death (HR: 1.36, $p < 0.001$), cardiac failure (HR: 1.49, $p < 0.001$), and major cardiovascular events (HR: 1.27, $p < 0.001$), while GDF-15 and hsTnI also show significant associations with these outcomes. Combinations involving sST2, GDF-15, hsTnI, and BNP display statistically significant results. This table provides valuable insights into the association of novel cardiac biomarkers with different cardiovascular diseases, highlighting their potential utility in risk prediction and management.

Table 3 presents data on the multimarker score and its predictive value for future cardiovascular events, including death, cardiac failure, and major cardiovascular events. A higher multimarker score is significantly associated with an increased risk of death (HR: 1.78, $p < 0.001$), cardiac failure (HR: 1.81, $p < 0.002$), and major cardiovascular events (HR: 1.45, $p < 0.001$). The addition of the multimarker score improves the discriminatory ability for all outcomes, as evidenced by higher c-statistics and statistically significant p-values. Furthermore, integrated discrimination improvement and net reclassification improvement shows statistically significant improvements when the multimarker score is added to the model. Table 3

underscore the predictive utility of the multimarker score for future cardiovascular events, offering potential improvements in risk prediction beyond traditional clinical models.

Table 2: Association of Novel Cardiac Biomarkers with Cardiovascular Diseases

Parameter	Multivariable-Adjusted HR per SD, P			
	Death	**Cardiac Failure**	**Major Cardiovascular Event**	**Coronary heart diseases**
Individual Biomarkers				
sST2	1.36 (1.24–1.52) <0.001	1.49 (1.27–1.74) <0.001	1.27 (1.14–1.43) <0.001	1.12 (0.96–1.31) 0.41
GDF-15	1.70 (1.55–1.85) <0.001	1.56 (1.33–1.82) <0.001	1.30 (1.16–1.44) <0.001	1.14 (0.97–1.35) 0.31
hsTnI	1.20 (1.11–1.30) <0.001	1.32 (1.18–1.48) <0.001	1.22 (1.11–1.33) <0.001	1.05 (0.90–1.21) 0.87
Combinations of Biomarkers				
sST2	1.16 (1.06–1.28) 0.02	1.33 (1.12–1.58) 0.006	1.19 (1.06–1.34) 0.03	1.07 (0.91–1.26) 0.74
GDF-15	1.56 (1.41–1.71) <0.001	1.27 (1.07–1.51) 0.03	1.17 (1.04–1.32) 0.048	1.10 (0.91–1.31) 0.64
hsTnI	1.10 (1.01–1.20) 0.19	1.24 (1.08–1.40) 0.01	1.17 (1.06–1.28) 0.01	1.02 (0.87–1.18) 0.94
BNP	1.17 (1.06–1.28) 0.02	1.33 (1.14–1.56) 0.002	1.19 (1.07–1.31) 0.02	1.11 (0.95–1.30) 0.44
hsCRP	1.22 (1.11–1.34) 0.001	1.21 (1.01–1.45) 0.11	1.15 (1.02–1.30) 0.10	1.25 (1.06–1.47) 0.03

Table 3: Multimarker Score and Future cardiovascular Event prediction

Parameter	Death	Cardiac Failure	Major Cardiovascular Event

Score, per 1-unit increment	1.78 (1.66–1.96)	1.81 (1.58–2.13)	1.45 (1.25–1.68)
P Value	< 0.001	< 0.002	< 0.001
By quartile of score			
1st Quartile	Referent	Referent	Referent
2nd Quartile	1.28 (0.85–1.97)	1.49 (0.62–3.97)	1.03 (0.71–1.57)
3rd Quartile	1.53 (1.10–2.25)	2.25 (1.02–5.17)	1.14 (0.81–1.67)
4th Quartile	3.18 (2.27–4.66)	6.43 (3.12–14.21)	1.89 (1.35–2.75)
P for trend	< 0.001	< 0.001	< 0.002
c Statistics			
Best-fit clinical model	0.789	0.843	0.782
Best-fit clinical model Multimarker score	0.807	0.868	0.795
P Value	0.002	0.004	< 0.001
Net Reclassification Improvement (> 0) vs best-fit clinical model	0.44 (0.33–0.56)	0.41 (0.23–0.59)	0.23 (0.10–0.36)
P Value	< 0.001	0.001	< 0.001
Integrated Discrimination Improvement	0.06 (0.05–0.08)	0.05 (0.03–0.07)	0.03 (0.02–0.04)
P Value	< 0.002	< 0.001	< 0.001

DISCUSSION

In the general population, several biomarkers indicative of cardiovascular stress offer predictive insights beyond traditional risk factors. Our findings demonstrate that sST2, GDF-15, and hsTnI can forecast risk in addition to established biomarkers like hsCRP [14, 22–26]. sST2, an emerging biomarker, has shown promise in predicting adverse outcomes and mortality among individuals with established heart failure [14, 22–26]. Our study extends this by revealing that elevated levels of circulating sST2 can be detected in apparently healthy individuals, preceding adverse outcomes. Experimental evidence suggests that circulating sST2 serves as a sensitive indicator of cardiac stress, with myocardial ST2 gene expression upregulated in response to myocyte stretch akin to BNP [27]. Clinical investigations in heart failure patients have associated elevated sST2 levels with greater decompensation, abnormal cardiac function, and poorer long-term prognosis.

Similarly, GDF-15 concentrations were strongly linked to the risk of mortality and heart failure [12,28]. GDF-15, a member of the transforming growth factor Beta cytokine superfamily, exhibits increased expression in response to cardiovascular inflammation and tissue injury. Ischemia, mechanical stress, neurohormones, and proinflammatory cytokines stimulate GDF-15 expression in cardiac myocytes. Elevated GDF-15 levels in patients with acute coronary syndrome or chronic heart failure correlate with disease severity and mortality risk [29,30].

Our study also explored an innovative "ultrasensitive" troponin I assay capable of detecting troponin concentrations significantly lower than other highly sensitive assays. Troponins, structural proteins crucial for cardiomyocyte contraction and relaxation, are commonly measured to diagnose acute myocardial infarction. Elevated troponin levels in established heart failure patients, absent myocardial infarction, strongly predict prognosis [15, 31-33].

Interestingly, while these biomarkers were strongly associated with heart failure and mortality, none showed significant associations with coronary heart disease events (myocardial infarction or unstable angina) after adjusting for conventional risk factors. This suggests that their predictive value lies more in their correlation with myocardial stress rather than vascular stress or inflammation. Combining sST2, GDF-15, and hsTnI in risk models retained associations with heart failure or mortality, indicating that each biomarker captures distinct aspects of pathophysiology with relatively low correlation, providing complementary information [34].

These findings underscore the potential of these biomarkers in identifying individuals at risk of cardiovascular events and suggest avenues for future clinical investigations to validate their utility further.

CONCLUSION

Concentrations of sST2, GDF-15, and hsTnI predict the future risk of death, heart failure, and overall cardiovascular events, even in the context of robust clinical risk models. Addition of these biomarkers improves discrimination and leads to potentially relevant changes in risk

classification. Our findings highlight the prognostic value of newer biomarkers of underlying cardiovascular stress and injury in apparently healthy individuals.

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