ORIGINAL RESEARCH

Association of Novel Biomarkers of Cardiovascular Stress With Left Ventricular Hypertrophy and Dysfunction: Implications for Screening

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***Background*—**Currently available screening tools for left ventricular (LV) hypertrophy (LVH) and systolic dysfunction (LVSD) are either expensive (echocardiography) or perform suboptimally (B-type natriuretic peptide [BNP]). It is unknown whether newer biomarkers are associated with LVH and LVSD and can serve as screening tools.

***Methods and Results*—**We studied 2460 Framingham Study participants (mean age 58 years, 57% women) with measurements of biomarkers mirroring cardiac biomechanical stress (soluble ST-2 [ST2], growth differentiation factor-15 [GDF-15] and high-sensitivity troponin I [hsTnI]) and BNP. We defined LVH as LV mass/height^2 $\geq$the sex-specific 80th percentile and LVSD as mild/greater impairment of LV ejection fraction (LVEF) or a fractional shortening $<$0.29. Adjusting for standard risk factors in logistic models, BNP, GDF-15, and hsTnI were associated with the composite echocardiographic outcome (LVH or LVSD), odds ratios (OR) per SD increment in log-biomarker 1.29, 1.14, and 1.18 (95% CI: 1.15 to 1.44, 1.004 to 1.28, and 1.06 to 1.31), respectively. The C-statistic for the composite outcome increased from 0.765 with risk factors to 0.770 adding BNP, to 0.774 adding novel biomarkers. The continuous Net Reclassification Improvement was 0.212 (95% CI: 0.119 to 0.305, $P<0.0001$) after adding the novel biomarkers to risk factors plus BNP. BNP was associated with LVH and LVSD in multivariable models, whereas GDF-15 was associated with LVSD (OR 1.41, 95% CI: 1.16 to 1.70), and hsTnI with LVH (OR 1.22, 95% CI: 1.09 to 1.36). ST2 was not significantly associated with any outcome.

***Conclusions*—**Our community-based investigation suggests that cardiac stress biomarkers are associated with LVH and LVSD but may have limited clinical utility as screening tools. **(*J Am Heart Assoc*. 2013;2:e000399 doi: 10.1161/JAHA.113.000399)**

Key Words: biomarkers • echocardiography • heart failure • hypertrophy • screening

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The high lifetime risk of heart failure (HF) combined with the aging population has led to a dramatic rise in HF prevalence worldwide.[1,2] Up to half of HF patients have a preserved left ventricular (LV) ejection fraction (HFPEF), whereas the remainder has a reduced ejection fraction (HFREF). Both forms of HF are associated with substantial morbidity, mortality, and cost to the health care system.[3–5] Thus, preventing HF is critical, and attention has turned toward screening for HF risk by identifying "high-risk" individuals (Stage A in the American Heart Association [AHA]/American College of Cardiology [ACC] classification schema).[6,7] Myocardial damage leading to LV systolic dysfunction (LVSD) is the primary precursor to HFREF, and LV hypertrophy (LVH) due to hypertension is the major antecedent of HFPEF.[8,9] Therefore, LVH and LVSD represent key preclinical phenotypes (Stage B HF)[7] that can be screened for within the community. Indeed, HF meets several of the criteria for a "screenable" condition: its prevalence is high, its incidence is rising, and treatment is available for its preclinical stages (ie, LVSD, and LVH on the basis of hypertension).[10,11] However, the high prevalence of hypertension and relatively lower prevalence of myocardial infarction (MI) among older adults renders these 2 risk factors inadequate for

differentiating high-risk from low-risk individuals. Additionally, echocardiography is expensive and its routine use for screening the general population for HF risk is neither feasible nor likely to be cost effective.[10,11] Consequently, investigators have assessed the utility of biomarkers for screening for LVH and LVSD. Clinical risk scores used for predicting HF and existing biomarkers (such as circulating levels of the B-type natriuretic peptide [BNP]) are associated with LVH and LVSD, yet perform suboptimally for community-wide screening for HF risk.[12] The recent availability of novel biomarkers that mirror cardiac biomechanical stress, notably ST2, growth differentiation factor 15 (GDF-15), and high-sensitivity troponin I (hsTnI), has generated the possibility that a combination of circulating biomarkers may be associated with LVH and LVSD, and may serve as a screening tool (beyond clinical risk factors and BNP). People with LVH and LVSD, identified via such an initial biomarker screening strategy, could then undergo confirmatory echocardiography.

We hypothesized that higher circulating levels of ST2, GDF-15, and hsTnI are associated with a greater prevalence of LVH or LVSD, and that a combination of these biomarkers with BNP and clinical risk factors may have sufficiently robust performance characteristics to serve as a screening tool for LVH and LVSD in the community. We tested our hypotheses in the large community-based Framingham Heart Study sample.

Methods

Study Sample

The study design and methods of the Framingham Heart Study have been described in detail at http://www.framingham heartstudy.org.[13] We included Framingham Offspring participants who attended the sixth examination cycle (1995–1998) when routine echocardiography was performed. Of 3532 attendees, 1072 participants were excluded for the following reasons: non-available biomarker levels (n=49), inadequate echocardiographic information (n=872), serum creatinine levels ≥2 mg/dL (n=21), history of heart failure (n=35), or missing covariate information (n=95). The study protocols were approved by the Boston University Medical Center Institutional Review Board. All participants provided written informed consent.

Echocardiographic Measurements

All attendees underwent standardized 2D transthoracic echocardiography with Doppler color flow imaging. A sonographer or a cardiologist (experienced in echocardiography), blinded to clinical information and biomarker results, read all echocardiograms. The reproducibility of echocardiographic measurements was excellent.[14] Interobserver variability ranged from 0.9% to 5% for LVDD, from 2% to 2.9% for diastolic posterior wall thickness, from 3.6% to 6.5% for the interventricular septum in diastole, and from 0.8% to 4% for calculated LV mass. Corresponding figures for intraobserver variability ranged from 0.3% (LVDD) to 4% (interventricular septal thickness).[14] Digital M-mode measurements from ≥3 cardiac cycles were averaged to estimate LV internal dimensions in end-systole and end-diastole, and thicknesses of the interventricular septum and LV posterior wall at end-diastole (in accordance with the American Society of Echocardiography [ASE] guidelines).[15] Fractional shortening (FS) was calculated using LV internal dimensions at end-diastole and end-systole.

In all participants, visual assessment of LV global systolic function was performed in multiple views to estimate the LV ejection fraction (LVEF), which was categorized as normal (LVEF >0.55), borderline (LVEF 0.51 to 0.55), mildly reduced (LVEF 0.41 to 0.50), moderately diminished (LVEF 0.31 to 0.40), or severely impaired (LVEF ≤0.30). The accuracy of the aforementioned estimation of LVEF has been validated in prior reports.[16] Both the qualitative variable LVEF and the quantitative variable FS were used to define LV systolic dysfunction[12] (ie, the presence of either abnormal LVEF or abnormal FS) because they provide complementary information: the former may not be sensitive for detecting subtle alterations in LV systolic function whereas the latter focuses on the base of the heart (and may miss diminished LV contractility in other regions).

Biomarker Measurements

Blood samples were collected after an overnight fast[17] and stored at −70°C until assayed. Although N-terminal pro-atrial natriuretic peptide (NT-ANP) levels were also available at the sixth examination cycle, we focused on BNP in the present investigation given our previous report,[12] which demonstrated that BNP yielded better discrimination statistics for LVSD and LVH relative to NT-ANP. BNP was measured using the Shionogi assay,[12] ST2 using a second-generation, high-sensitivity enzyme-linked immunosorbent assay (Critical Diagnostics, detection limit 2 ng/mL), GDF-15 using an automated, pre-commercial electrochemilluminescent immunoassay (Roche Elecsys, detection limit of <10 ng/L), and hsTnI using an ultrasensitive immunoassay with a novel, single-molecule counting technology (Singulex, detection limit is 0.2 pg/mL, range 0.5 to 70 pg/mL). The interassay imprecisions for GDF-15 were 2.3% and 1.8% at GDF-15 concentrations of 1100 and 17 200 ng/L, respectively. The interassay coefficient of variation (CV) ranged from 8% to 10%. The lower QC control for hsTnI provided an average value of 4.71 pg/mL with an interassay CV of 10%. The higher QC control provided an average value of 19.05 pg/mL with an interassay CV of 8%. The intra- and interassay coefficients of variation for ST2 were <4%.

Statistical Analysis

We natural-logarithmically transformed all biomarkers to stabilize their distributions and reduce influence from extreme biomarker values. We also used this transformation to be consistent with our prior approach for some of these biomarkers.[12]

We defined 3 echocardiographic outcomes of interest[12]:

1. LVH_80, defined as a value of LVM (left ventricular mass)/height^2 ≥the sex-specific 80th percentile value.
2. Mild/greater LVSD (LVSD_MILDGR), defined as the presence of mild or greater degree of impairment of LVEF on 2-dimensional (2D) assessment of the echocardiogram, or an M-mode FS <0.29 (corresponding to an LVEF ≤0.50).
3. Moderate/severe LVSD (LVSD_MODSEV), defined as the presence of moderate or severe degree of impairment of LVEF on 2D assessment, or a FS <0.22 (corresponding to an LVEF ≤0.40).

Additionally, we performed analyses using the ASE cut points (LVM/height ≥127 g/m in men and ≥100 g/m in women)[15] to define LVH. To minimize multiple statistical testing, we defined a composite variable (presence of LVH_80 or mild/greater LVSD, corresponding to Stage B HF) and its components as our primary outcomes, while moderate/severe LVSD served as a secondary outcome (given its much lower prevalence). The composite outcome was chosen because it is desirable to identify a set of screening biomarkers that can identify both LVH and LVSD, which would then have the potential of preventing both HFPEF and HFREF.

Analysis of the composite echocardiographic outcome

We considered the following clinical risk factors previously associated with either component of the composite outcome: age, sex, body mass index (BMI), presence of MI, hypertension status (defined as systolic/diastolic blood pressure ≥140/90 mm Hg, respectively, or use of antihypertensive treatment), diabetes status, alcohol consumption, and the presence of atrial fibrillation (AF) or valve disease. We performed stepwise logistic regression analysis on these risk factors using P=0.1 as the retention criterion to develop an initial model. Accounting for selected clinical risk factors, we then performed stepwise logistic regression analysis on the biomarkers (BNP, GDF-15, ST2, and hsTnI) relating them to the presence of the composite outcome. We also examined performance characteristics of the biomarker combinations with BNP and with clinical risk factors to assess their incremental utility. We used 2 metrics to characterize performance characteristics of the novel biomarkers over BNP plus standard clinical risk factors: the increment in the C-statistic (area under the receiver operating characteristic curve [ROC]) upon adding the novel biomarkers, and the continuous Net Reclassification Improvement (NRI)[18]; NRI values <0.2 are considered weak, those ≈0.4 are considered intermediate, and values of ≈0.60 are considered strong.[19]

We applied the final models to assess the predictive ability of these novel biomarkers in the following clinical subgroups: ≥65 and <65 years; with and without hypertension; and with and without prior MI. We evaluated individuals with an MI, although they are likely to have an echocardiogram on a routine basis, so that we obtain an estimate of the performance of the biomarkers in the setting of prior myocardial damage.

Individual echocardiographic outcomes (LVH_80 and LVSD)

We repeated the stepwise procedure separately for all 3 outcomes and created a final set of risk factors combining the statistically significant variables from the 3 separate stepwise procedures. We then performed a stepwise regression analysis on the 4 biomarkers of interest for each outcome separately, forcing in the final set of risk factors identified above. We evaluated the NRI and the increment in the C-statistic. These analyses were repeated for the clinical subgroups. All analyses were performed using SAS software version 9.2.[20]

A 2-sided value of P<0.05 was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Our sample consisted of middle-aged to older participants, with a moderate prevalence of hypertension (Table 1). We observed weak correlations among the novel biomarkers (Table 2).

Predictive Utility of Novel Biomarkers for the Composite Echocardiographic Outcome

Of 2460 participants, 574 (23%) had either LVH_80 or mild/greater LVSD, of whom 298 were women. Age, BMI, presence of MI, hypertension status, diabetes status, presence of AF, and valve disease were associated with the composite outcome; these were used in all multivariable models. After forcing in the clinical risk factors, the composite outcome was associated with circulating concentrations of BNP, GDF-15, and hsTnI (P<0.05 for each, Table 3), but not ST-2. Compared with a model containing only risk factors (C=0.765), the discrimination of the model increased to 0.770 upon adding BNP, and to 0.774 with the further addition of GDF-15 and hsTnI (Table 4). The addition of these 2 novel biomarkers resulted in a statistically significant NRI (Table 4).

Table 5 shows the number of people with a composite outcome within each clinical subgroup. Plasma BNP was associated with the composite outcome in all subgroups

Table 1. Characteristics of Study Sample

Variable	Men (N=1063)	Women (N=1397)
Clinical characteristics		
Age, y	58±10	58±9
Systolic blood pressure, mm Hg	129±17	126±20
Diastolic blood pressure, mm Hg	77±9	74±9
Body mass index, kg/m^2	28.0±3.9	26.8±5.1
Hypertension, %	42.8	34.7
Hypertension treatment, %	29.9	21.7
Diabetes, %	12.9	7.7
Prevalent myocardial infarction, %	6.4	0.9
Valve disease, %	3.3	2.4
Prevalent atrial fibrillation, %	3.7	0.8
Biochemical features		
BNP, pg/mL	6.1 (4.0, 15.7)	9.6 (4.0, 19.4)
GDF-15, ng/L	1016 (792, 1336)	991 (799, 1253)
hsTnI, pg/mL	1.6 (1.03, 2.62)	1.12 (0.78, 1.86)
sST2, ng/mL	23.36 (19.12, 28.9)	18.38 (14.96, 22.73)
Echocardiographic characteristics		
LV mass (indexed to height), g/m^2 80th percentile 90th percentile	62.7±14.3 72.9 81.2	53.6±12.2 63.1 69.5
Fractional shortening	0.35±0.06	0.38±0.05
Mild/greater LV systolic dysfunction, %	9.7	2.8
Moderate-to-severe LV dysfunction, %	4.1	0.5

Values are mean±SD for age, systolic and diastolic blood pressure, body mass index, LV mass, and fractional shortening, or median (Q1, Q3) for BNP, GDF-15, hsTnI, and sST2. Fractional shortening, a measure of basal LV systolic function, was calculated as: FS (%)=[LVID at end-diastole−LVID at end-systole]/LVID at end-diastole. BNP indicates B-type natriuretic peptide; GDF, growth differentiation factor; hsTnI, high-sensitivity troponin I; LV, left ventricular; LVID, LV internal dimension; sST2, soluble ST2.

(Table 6). Additionally, GDF-15 was associated with the composite outcome among people without an MI and in those <65 years old, whereas hsTnI showed an association with the composite outcome among those with and without an MI; those <65 years old; and those with hypertension (Table 6). Although the increase in the C-statistic was not statistically significant with the addition of the 2 novel biomarkers (data not shown), we observed a statistically significant NRI for most subgroups (Table 7).

Table 2. Age- and Sex-Adjusted Pearson Correlation Coefficients Among the Novel Biomarkers With Each Other and With BNP

	Log ST2	Log hsTnI	Log BNP
Log GDF-15	0.20 (<0.0001)	0.12 (<0.0001)	0.11 (<0.0001)
Log ST2		0.08 (<0.0001)	0.05 (0.009)
Log hsTnI			0.13 (<0.0001)

Values are sample Pearson correlation coefficients (*P* value). BNP indicates B-type natriuretic peptide; GDF-15, growth differentiation factor-15; hsTnI, high-sensitivity troponin I.

Predictive Utility of Novel Biomarkers for the Individual Echocardiographic Outcomes

The number of participants with each individual outcome is shown in Table 5. The combination of the statistically significant clinical covariates from the separate stepwise regression analyses for each individual outcome included age, sex, BMI, presence of MI, hypertension status, diabetes status, presence of AF, and valve disease; these variables were used in all multivariable models for each outcome.

In the full sample, BNP was associated with LVSD and LVH, after adjusting for clinical risk factors. GDF-15 was associated with mild/greater LVSD, whereas hsTnI was associated with LVH_80 (Table 3). ST2 was not associated with any echocardiographic outcome. The C-statistic of the model did not significantly increase with the addition of the biomarkers to the risk factors plus BNP for any outcome, whereas the NRI was statistically significant for both LVH_80 and LVSD (Table 4), with the magnitude in the weak-to-intermediate range.

In subgroup analyses, BNP was associated with all outcomes among most subgroups, after adjusting for risk factors. Associations among GDF-15, hsTnI, and the outcomes varied across subgroups (Table 6). The addition of these 2 biomarkers did not result in significant increases in the C-statistic for the subgroups as compared to the use of risk factors plus BNP (data not shown); however, we observed statistically significant NRI for LVH_80 among all subgroups and for mild/greater LVSD among most subgroups (Table 7).

In analyses modeling echocardiographic traits as continuous variables, BNP was associated with LVM, LVDD, and LVDS; hsTnI with both LVM and LVWT; and GDF-15 with LVWT ($P<0.05$ for all).

Discussion

The use of single biomarkers (including BNP) for screening for LVH and LVSD in the general population has proven to be

Table 3. Multivariable-adjusted Association of Statistically Significant Biomarkers With Prevalence of Each Echocardiographic Outcome

	Composite (574/2460)†	LVH_80 (489/2460)†	LVSD_MILDGR (142/2460)†	LVSD_MODSEV (51/2460)†
BNP	1.29 (1.15 to 1.44)**	1.25 (1.11 to 1.41)**	1.43 (1.17 to 1.73)**	1.79 (1.30 to 2.47)**
GDF-15	1.14 (1.004 to 1.28)*	NS	1.41 (1.16 to 1.70)**	NS
hsTnI	1.18 (1.06 to 1.31)*	1.22 (1.09 to 1.36)**	NS	NS

Values represent odds ratios (95% confidence intervals) per 1 SD increment in log-biomarker. ST2 was not significantly associated with any outcome. BNP indicates B-type natriuretic peptide; GDF-15, growth differentiation factor-15; hsTnI, high-sensitivity troponin I; LVH, left ventricular hypertrophy; LVSD, left ventricular systolic dysfunction; LVSD_MILDGR, mild/greater LVSD; LVSD_MODSEV, moderate/severe LVSD; NS, no statistically significant association.
*P<0.05, **P<0.001.
†Number of outcomes/number at risk.

suboptimal in prior studies.[12] We recently showed that multiple biomarkers reflecting cardiac biomechanical stress in this cohort of individuals predicted incident HF (AHA/ACC Stage C/D).[17] Accordingly, we examined if these biomarkers were associated with LVH and LVSD, and could serve as potential screening tools (for AHA/ACC Stage B). To our knowledge, investigations evaluating multiple novel biomarkers mirroring cardiac stress for associations with and community screening for the presence of LVH and LVSD are sparse.[21] Previous studies[12] evaluating biomarkers for screening for LVH or LVSD focused on the increment in the C statistic as the sole criterion for assessing their utility, and did not investigate the NRI that provides complementary information. Therefore, we evaluated 3 novel biomarkers for their associations with LVH and LVSD and their use for screening purposes in a large community-based sample. We also evaluated their performance in high-risk subgroups to examine the premise that any strategy using these biomarkers could selectively target groups of individuals in whom the screening yield may be maximal, thereby rendering these biomarkers cost effective.

Principal Findings

Circulating concentrations of BNP, GDF-15, and hsTnI were associated with the composite outcome. BNP was associated with both LVH and LVSD, while GDF-15 and hsTnI were associated with LVSD and LVH, respectively. ST2 was not associated with any outcome. In secondary analyses, only BNP was associated with moderate/severe LVSD. The combination of GDF-15 and hsTnI minimally increased the model's predictive utility for the composite outcome, as quantified by the NRI, compared to a model with risk factors and BNP. Analyses of LVH and LVSD as separate outcomes showed similar patterns to that for the composite outcome. Analyses within clinical subgroups suggested an improvement in predictive utility for most subgroups, using the NRI; however, the clinical interpretation of a statistically significant NRI is not as clear.

Table 4. Incremental Utility of Biomarkers Over Clinical Risk Factors

	Composite	LVH_80	LVSD_MILDGR	LVSD_MODSEV
C-statistic				
Standard risk factors (1)	0.765 (0.742 to 0.788)	0.778 (0.755 to 0.801)	0.763 (0.714 to 0.812)	0.925 (0.892 to 0.958)
Standard risk factors+BNP (2)	0.770 (0.747 to 0.793)	0.783 (0.760 to 0.806)	0.769 (0.722 to 0.817)	0.931 (0.897 to 0.965)
Standard risk factors+biomarkers* (3)	0.774 (0.751 to 0.796)	0.786 (0.763 to 0.808)	0.782 (0.736 to 0.828)	N/A
P Value for difference (1 vs 3)	0.03	0.03	0.14	0.40
P Value for difference (2 vs 3)	0.13	0.20	0.15	N/A
NRI				
NRI (1 vs 3)	0.220 (0.127 to 0.313)	0.246 (0.148 to 0.345)	0.395 (0.227 to 0.562)	0.505 (0.237 to 0.773)
NRI (2 vs 3)	0.212 (0.119 to 0.305)	0.243 (0.145 to 0.340)	0.207 (0.037 to 0.376)	N/A
P Value for NRI (1 vs 3)	<0.0001	<0.0001	<0.0001	0.0004
P Value for NRI (2 vs 3)	<0.0001	<0.0001	0.02	N/A

BNP indicates B-type natriuretic peptide; LVH, left ventricular hypertrophy; LVSD, left ventricular systolic dysfunction; LVSD_MILDGR, mild/greater LVSD; LVSD_MODSEV, moderate/severe LVSD; N/A, non-applicable, since models (2) and (3) were identical for LVSD_MODSEV; NRI, net reclassification improvement.
*Model includes risk factors, BNP, and the biomarker that was significantly associated with each outcome.

Table 5. Numbers of Individuals With Outcomes in Clinical Subgroups of Participants

Subgroups/Echo Abnormality	Sample Size	Composite Outcome	Mild/Greater LVSD	Moderate/Severe LVSD	LVH Defined as Exceeding the Sex-specific 80th Percentile
Full sample	2460	574 (23.3)	142 (5.8)	51 (2.1)	489 (19.9)
With MI	79	55 (69.6)	41 (51.9)	25 (31.6)	34 (43.0)
Without MI	2379	517 (21.7)	99 (4.2)	24 (1.0)	454 (19.1)
Age ≥65 years	641	243 (37.9)	52 (8.1)	27 (4.2)	217 (33.9)
Age<65 years	1819	331 (18.2)	90 (4.9)	24 (1.3)	272 (15.0)
With HTN	940	340 (36.2)	75 (8.0)	34 (3.6)	303 (32.2)
Without HTN	1520	234 (15.4)	67 (4.4)	17 (1.1)	186 (12.2)

Values in parentheses are percentages. HTN indicates hypertension; LVH, left ventricular hypertrophy; LVSD, left ventricular systolic dysfunction; MI, myocardial infarction.

Comparison with the Published Literature

Several investigations have reported that circulating BNP is a suboptimal screening tool for LVH and LVSD.[12,22] Other novel biomarkers have been evaluated as screening tools, but none has been proven to detect HF precursors. For example, blood galectin-3, a marker of myocardial fibrosis, has been previously evaluated by our group but did not show an association with LVH or LVSD after adjustment for risk factors.[23] In contrast, investigators have studied midregional proadrenomedullin in relation to LVH and LV volumes and observed an association of higher levels with LVH and greater cardiac volumes.[23–25] We did not measure this biomarker. Given our recent data showing substantial HF risk prediction

Table 6. Association of Biomarkers With Prevalence of the Composite Echocardiographic Outcome Within Clinical Subgroups

Biomarker	Full Sample	MI Status		Age, y		HTN Status	
		With MI	Without MI	≥65	<65	With HTN	Without HTN
Composite outcome							
BNP	1.29** (1.15 to 1.44)	2.28* (1.22 to 4.25)	1.25** (1.12 to 1.41)	1.37** (1.14 to 1.64)	1.29** (1.12 to 1.49)	1.31** (1.13 to 1.52)	1.26* (1.05 to 1.50)
GDF-15	1.14* (1.004 to 1.28)	N/A	1.14* (1.01 to 1.30)	N/A	1.19* (1.03 to 1.37)	N/A	N/A
hsTnI	1.18* (1.06 to 1.31)	2.20* (1.11 to 4.37)	1.15* (1.03 to 1.29)	N/A	1.25** (1.09 to 1.42)	1.26* (1.08 to 1.47)	N/A
Mild/Greater LVSD							
BNP	1.43** (1.17 to 1.73)	2.17* (1.31 to 3.62)	1.31* (1.05 to 1.62)	1.43* (1.04 to 1.97)	1.41* (1.10 to 1.80)	1.57** (1.21 to 2.05)	N/A
GDF-15	1.41** (1.16 to 1.70)	N/A	1.55** (1.27 to 1.89)	N/A	1.40* (1.12 to 1.75)	1.47* (1.15 to 1.88)	N/A
Moderate/Severe LVSD							
BNP	1.79** (1.30 to 2.47)	1.66* (1.01 to 2.74)	1.85* (1.22 to 2.81)	1.66* (1.06 to 2.59)	2.12* (1.35 to 3.32)	2.01** (1.37 to 2.96)	N/A
LVH (exceeding the sex-specific 80th percentile of height-indexed LV mass)							
BNP	1.25** (1.11 to 1.41)	N/A	1.24** (1.09 to 1.40)	1.40** (1.17 to 1.68)	1.27* (1.08 to 1.49)	1.27* (1.09 to 1.48)	1.24* (1.02 to 1.50)
hsTnI	1.22** (1.09 to 1.36)	2.93* (1.48 to 5.80)	1.17* (1.04 to 1.32)	N/A	1.29** (1.12 to 1.48)	1.33** (1.13 to 1.56)	N/A

Values represent odds ratios (95% confidence intervals) per 1 SD increment in log-biomarker. ST2 was not associated with any outcome. BNP indicates B-type natriuretic peptide; GDF-15, growth differentiation factor-15; hsTnI, high-sensitivity troponin I; HTN, hypertension; LVSD, left ventricular systolic dysfunction; MI, myocardial infarction; N/A, no statistically significant association.
*P<0.05, **P<0.001.

Table 7. Incremental Utility of Biomarkers Over Clinical Risk Factors and BNP Within Clinical Subgroups

	Composite	LVH_80	LVSD_MILDGR
With MI			
NRI	0.580 (0.133, 1.028)	0.834 (0.429, 1.239)	0.276 (−0.161, 0.713)
P Value for NRI	0.018	0.0002	0.220
Without MI			
NRI	0.178 (0.081, 0.275)	0.225 (0.124, 0.326)	0.297 (0.096, 0.498)
P Value for NRI	0.0004	0.00002	0.004
≥65 years			
NRI	0.084 (−0.075, 0.243)	0.227 (0.066, 0.389)	0.260 (−0.022, 0.541)
P Value for NRI	0.305	0.006	0.073
<65 years			
NRI	0.274 (0.156, 0.392)	0.281 (0.154, 0.409)	0.202 (−0.01, 0.413)
P Value for NRI	0.00001	0.00002	0.062
With HTN			
NRI	0.246 (0.114, 0.378)	0.306 (0.172, 0.441)	0.270 (0.035, 0.505)
P Value for NRI	0.0003	0.00001	0.025
Without HTN			
NRI	0.128 (−0.011, 0.266)	0.173 (0.021, 0.324)	0.112 (−0.132, 0.356)
P Value for NRI	0.073	0.027	0.371

Values represent net reclassification improvement (NRI) and *P*-values for comparison between a model including risk factors plus BNP and a model including risk factors, BNP, and novel biomarkers. We did not observe any statistically significant change in the C-statistic between these models; therefore we did not report data on the C-statistic. BNP indicates B-type natriuretic peptide; HTN, hypertension; LVH, left ventricular hypertrophy; LVSD, left ventricular systolic dysfunction; LVSD_MILDGR, mild/greater LVSD.

from a multiple biomarker panel (consisting of GDF 15, ST2, and hsTnI) in this cohort,[17] we focused on their screening yield over risk factors and BNP.

Biological Pathways/Biomarkers and LV Dysfunction and LV Hypertrophy

All 3 cardiac stress biomarkers that were evaluated, along with BNP, have been associated prospectively with incident HF and with adverse outcomes in patients with overt HF.[26–36] There is substantial experimental evidence that also implicates these biomarkers in LV remodeling. Both ST2 and GDF 15 are anti-hypertrophic, with GDF-15 also being anti-apoptotic and having anti-inflammatory properties.[37–42] hsTnI is an intrinsic myocyte protein, whose levels are associated with myocyte turnover. Furthermore, all 4 biomarkers mirror myocyte stretch in basic studies.[40,43,44] It is conceivable that they may also reflect other forms of stress, including but not limited to inflammatory, oxidant, or bioenergetic stress. Therefore, it is not surprising that levels of these biomarkers so strongly predict HF onset in the community.[17] We demonstrated the association between GDF-15 and hsTnI with the composite outcome, and with LVSD and LVH, respectively. Of note, ST2 was not associated with any outcome. We speculate that the association of ST2 with incident heart failure may be because it reflects systemic inflammation or vascular stiffness or other pathways to heart failure other than cardiac remodeling.

Strengths and Limitations

The present study included a community-based sample from a large epidemiological study, used echocardiographic measurements with excellent reproducibility, and studied a panel of biomarkers for screening for LVSD and LVH, assessing also a composite outcome that would capture precursors of both HFPEF and HFREF. Several limitations of the present study merit consideration. The composite outcome we chose was most amenable to analyses with adequate statistical power and corresponds to Stage B heart failure. However, our analyses of the important outcome of moderate/severe LVSD (a trait for which definitive treatment strategies have been delineated in controlled trials and guidelines) were limited by the low prevalence of the condition. Additionally, we were not able to use the quantitative estimation of 2D LVEF to define LVSD, as it was not performed routinely during the Offspring Cohort 6th examination cycle. The generalizability of our findings is limited by the use of white, middle- to older-age

Table 8. Association of Biomarkers With Echocardiographic Outcomes Using ASE Criteria for Defining Echocardiographic LVH

	Composite	LVH_ASE
BNP	1.27 (1.14 to 1.42)	1.24 (1.10 to 1.39)
GDF-15	1.14 (1.01 to 1.28)	NS
hsTnI	1.16 (1.05 to 1.29)	1.20 (1.07 to 1.33)

Values represent odds ratios (95% confidence intervals) per 1 SD increment in log-biomarker. ST2 was not significantly associated with any outcome. ASE indicates American Society of Echocardiography; BNP, B-type natriuretic peptide; GDF-15, growth differentiation factor-15; hsTnI, high-sensitivity troponin I; LVH, left ventricular hypertrophy; NS no significant association.

participants of European ancestry. We used a composite echocardiographic outcome as the primary phenotype for analyses and reported all statistical tests conducted (even if nonsignificant) to address multiple statistical testing issues; results of secondary analyses may be considered hypothesis generating. Furthermore, the choice of the cut points for defining LVH was based on the sex-specific distribution of LV mass. However, using the ASE cut points produced essentially similar results (Tables 8 and 9). Additionally, we did not split our sample into test and validation samples. Accordingly, replication of our results in external cohorts would be desirable. Finally, some risk factors were modeled as categorical variables (reflecting common clinical practice). Furthermore, we did not include electrocardiographic findings as a risk factor for LVH because of prior investigations suggesting limited accuracy of the electrocardiogram for detecting LVH.[45] It is conceivable that modeling the risk factors as continuous variables and adjusting for electrocardiographic LVH would reduce the incremental predictive utility of the biomarkers.[46] We are aware of the inherent limitation of the continuous NRI that captures minor shifts in probabilities of outcomes and may be challenging to interpret in terms of clinical utility.[47] We chose it over the categorical NRI in the absence of clinically meaningful categories for the echocardiographic outcomes. Additionally, whereas sensitivity and specificity are the metrics often used to assess screening tests, it is challenging to estimate these indices when a combination of biomarkers is used for this purpose. Finally, the central premise of our investigation is that screening with biomarkers is likely to be less expensive than standard echocardiography (eg, BNP assays cost ≈$40 or less whereas an echocardiogram may be 10 times more expensive),[48] However, the advent of high-quality, hand-held echocardiography may reduce the gap in costs of biomarker assays versus that of echocardiography.[49]

Table 9. Incremental Utility of Biomarkers Over Standard Risk Factors Using ASE Criteria for Defining LVH

	Composite	LVH_ASE
Standard risk factors (1)	0.76 (0.734 to 0.780)	0.77 (0.743 to 0.789)
Standard risk factors+BNP (2)	0.76 (0.739 to 0.785)	0.77 (0.748 to 0.794)
Standard risk factors+ biomarkers* (3)	0.77 (0.743 to 0.788)	0.77 (0.751 to 0.796)
P for difference (1 vs 3)	0.03	0.03
P for difference (2 vs 3)	0.19	0.26

Values are C statistics (95% CI) and *P*-values. ASE indicates American Society of Echocardiography; BNP, B-type natriuretic peptide; LVH, left ventricular hypertrophy. *Model includes standard risk factors, BNP and the biomarker that was significantly associated with each outcome.

Conclusions

In our large community based-sample, GDF-15 and hsTnI were associated with the composite echocardiographic outcome, and with LVSD and LVH, respectively. These biomarkers also offered limited incremental predictive utility over clinical risk factors and BNP for identifying stage B HF. However, the clinical significance of the modest NRI values remains unclear, thereby rendering it challenging to advocate the use of these novel biomarkers for screening purposes in clinical practice. Additional studies of larger multi-ethnic cohorts are warranted to confirm our results and further investigations are needed to elucidate the clinical significance of modest NRI values (which may be statistically significant).

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