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Plasma Proteomic Profile Predicts Survival in Heart Failure With Reduced Ejection Fraction

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ORIGINAL ARTICLE

Plasma Proteomic Profile Predicts Survival in Heart Failure With Reduced Ejection Fraction

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BACKGROUND: It remains unclear whether the plasma proteome adds value to established predictors in heart failure (HF) with reduced ejection fraction (HFrEF). We sought to derive and validate a plasma proteomic risk score (PRS) for survival in patients with HFrEF (HFrEF-PRS).

METHODS: Patients meeting Framingham criteria for HF with EF<50% were enrolled (N=1017) and plasma underwent SOMAscan profiling (4453 targets). Patients were randomly divided 2:1 into derivation and validation cohorts. The HFrEF-PRS was derived using Cox regression of all-cause mortality adjusted for clinical score and NT-proBNP (N-terminal pro-B-type natriuretic peptide), then was tested in the validation cohort. Risk stratification improvement was evaluated by C statistic, integrated discrimination index, continuous net reclassification index, and median improvement in risk score for 1-year and 3-year mortality.

RESULTS: Participants' mean age was 68 years, 48% identified as Black, 35% were female, and 296 deaths occurred. In derivation (n=681), 128 proteins associated with mortality, 8 comprising the optimized HFrEF-PRS. In validation (n=336) the HFrEF-PRS associated with mortality (hazard ratio, 2.27 [95% CI, 1.84–2.82], $P=6.3\times10^{-14}$), Kaplan-Meier curves differed significantly between HFrEF-PRS quartiles ($P=2.2\times10^{-6}$), and it remained significant after adjustment for clinical score and NT-proBNP (hazard ratio, 1.37 [95% CI, 1.05–1.79], $P=0.021$). The HFrEF-PRS improved metrics of risk stratification (C statistic change, 0.009, $P=0.612$; integrated discrimination index, 0.041, $P=0.010$; net reclassification index=0.391, $P=0.078$; median improvement in risk score=0.039, $P=0.016$) and associated with cardiovascular death and HF phenotypes (eg, 6-minute walk distance, EF change). Most HFrEF-PRS proteins had little known connection to HFrEF.

CONCLUSIONS: A plasma multiprotein score improved risk stratification in patients with HFrEF and identified novel candidates.

Key Words: heart failure ■ mortality ■ plasma ■ prognosis ■ risk

Heart failure (HF) is a morbid illness with variable rates of progression and considerable public health burden; roughly 6 million adults in the United States experience HF,[1] and its prevalence continues to rise, predicted to be 9 million by 2030.[2] Given this, disease monitoring and risk stratification are critical to optimal individual and population management. Current state risk stratification includes clinical risk scores and established protein biomarkers. Widely accepted and validated clinical risk scores include the Seattle HF model score[3] and the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) score.[4] The MAGGIC score utilizes commonly available input data, was recently reconfirmed using 51 043 patients from the Swedish Heart Failure Registry,[5] and has consistent performance regardless of race.[6] Regarding biomarkers, the most established and utilized biomarkers in HF are the natriuretic peptides,[7,8] which are now recommended for risk stratification by both European and American consensus guidelines.[9–11]

Despite these techniques, one of the continuing challenges in HF care and treatment is trying to better recognize the underlying substantial variability in HF prognosis/progression.[12] Modern-Omics and multiplexing

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Nonstandard Abbreviations and Acronyms

EF	ejection fraction
HF	heart failure
HFrEF	heart failure with reduced ejection fraction
MAGGIC	Meta-Analysis Global Group In Chronic heart failure
NMB	neuromedin B
NT-proBNP	N-Terminal pro-B-Type Natriuretic Peptide
PRS	proteomic risk score
SOMAmers	slow off-rate modified aptamers

technologies may enable further improvement of our risk stratification strategies and potentially a better understanding of the HF disease process. Recent examples showing some promise include metabolomics, genomics, RNA profiling, and others.[13–15] Until recently, proteomics had not been as thoroughly explored as some of these other techniques partly due to comparatively low throughput. However, emerging proteomic technologies have recently matured and newer high-throughput techniques now allow larger scale proteomic characterization.[16,17] One of these newer technological innovations, enhanced aptamer-based assays,[18] has enabled a massively expanded candidate approach that borders on true proteomics in scale; thousands of protein-derived factors can be efficiently assayed simultaneously in a small biologic sample. This technique has been explored extensively in coronary disease and atherosclerosis, importantly adding to existing risk stratification.[19,20] The largest such array to date by SomaLogic (SomascanV4) captures nearly 5000 proteins and was recently applied to 11 different health indicators[21] and utilized in patients with HF,[22] providing further proof of concept, but has not yet been fully investigated for risk stratification in heart failure with reduced ejection fraction (HFrEF) patients to our knowledge.

In this study, we aimed to explore this new large-scale protein array using an established HF patient registry to discern if the plasma proteome could meaningfully predict the risk of death or HF worsening and add to best conventional risk stratification, including clinical score and natriuretic peptides. If indeed the circulating proteome can incrementally improve risk prediction, it could add a new tool to help manage patients with HF and contribute to discovery of novel HF markers and pathways.

METHODS

A full-length description of the methods is available as part in the Data Supplement. The overall statistical pipeline is summarized in Figure 1. This research study was approved by the Henry Ford Hospital institutional review board, and all participants provided written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

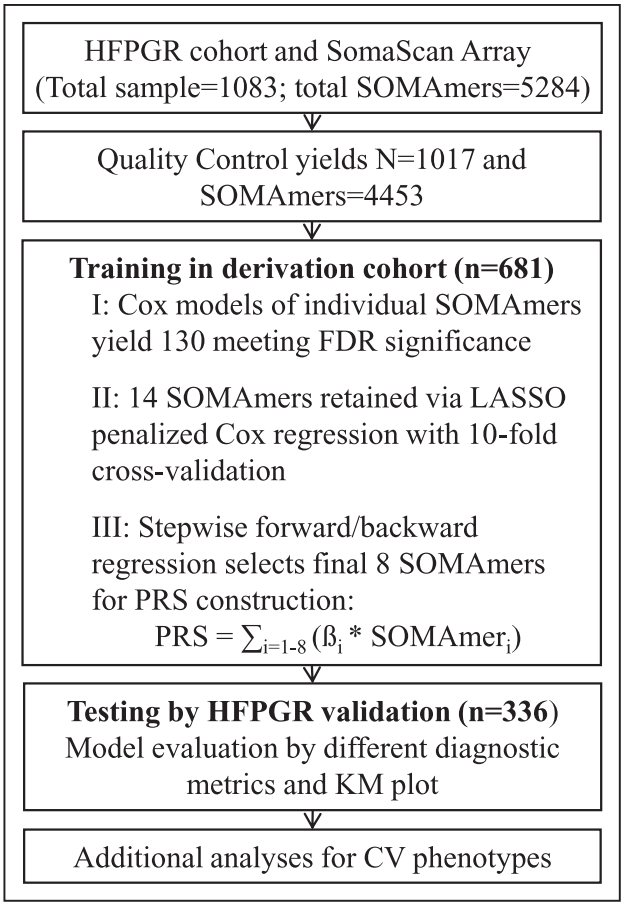

Figure 1. Flow chart of protein polygenic score analysis in Henry Ford Heart Failure Pharmacogenomic Registry (HFPGR) cohort.
Beta$_i$ indicates coefficient of ith SOMAmer (i=1–8) in multivariable Cox regression; CumSum, cumulative summation; CV, cardiovascular; FDR, false discovery rate; KM, Kaplan-Meier; LASSO, least absolute shrinkage and selection operator; PRS, proteomic risk score; SOMAmer, slow off-rate modified aptamer; and SOMAmer$_i$, log-transformed expression abundance of ith SOMAmer from SOMA array.

RESULTS

The baseline patient characteristics for the total cohort (N=1017), derivation (n=681), and validation (n=361) subgroups are summarized in Table 1. The mean age of the study participants was 67.9 years, females comprised 35.2% of the cohort, and 47.5% were self-identified as Black. Mean (±deviation) ejection fraction (EF) was 34.8±10.9%, MAGGIC score was 19.2±7.9, and NT-proBNP (N-terminal pro-B-type natriuretic peptide) level was 352.3 ±375.7 mol/L. The primary outcome was all-cause mortality and there were 296 (29.1%) deaths after an average follow-up of 1327±686 days (median of 1308

Table 1. Patient Baseline Characteristics Overall and By Cohort

Variable	Overall (N=1017)	Derivation (n=681)	Validation (n=336)	*P* value*
MAGGIC (mean±SD)	19.2±7.9	19.1±8.0	19.4±7.8	0.660
NT-proBNP (mean±SD), mol/L	352.3±375.7	355.8±387.5	345.2±351.1	0.662
Follow-up days (mean±SD)	1327±686	1332±682	1317±694	0.750
Death, N (%)	296 (29.1)	194 (28.5)	102 (30.46)	0.586
CV Death, N (%)	217 (21.3)	145 (21.3)	72 (21.4)	1.000
Race				
Black, N (%)	483 (47.5)	320 (47.0)	163 (48.5)	0.344
European American, N (%)	507 (49.9)	343 (50.4)	164 (48.8)	
Others, N (%)	27 (2.7)	18 (2.6)	9 (2.7)	
Ischemic cause, N (%)	446 (43.9)	302 (44.3)	144 (42.9)	0.702
Creatinine (mean±SD), μmol/L	109.7±65.6	110.6±68.1	107.8±60.2	0.513
Diabetes, N (%)	424 (41.7)	279 (41.0)	145 (43.2)	0.550
COPD, N (%)	222 (21.8)	141 (20.7)	81 (24.1)	0.248
Hypertension, N (%)	906 (89.1)	610 (89.6)	296 (88.1)	0.546
CAD, N (%)	589 (57.9)	398 (58.4)	191 (56.8)	0.676
AFib, N (%)	279 (27.4)	185 (27.1)	94 (25.7)	0.843
6MWD (mean±SD), feet	1038±345	1048±341	1019±351	0.257
KCQQ total symptom score (mean±SD)	77.4±24.9	77.4±25.2	77.4±24.3	0.995
EF at enrollment (mean±SD)	34.8±10.9	34.9±11.1	34.7±10.6	0.849
EF at follow-up (mean±SD)†	39.5±14.6	39.1±14.9	40.1±14.0	0.339
Age in years (mean±SD)	67.9±11.7	67.9±11.5	67.8±12.1	0.952
Female sex, N (%)	358 (35.2)	245 (36.0)	113 (33.6)	0.505
BMI (mean±SD)	31.0±7.3	31.0±7.1	31.0±7.8	0.931
SBP (mean±SD)	129.4±23.0	129.3±22.8	129.6±23.4	0.866
NYHA class				
Class I, N (%)	614 (60.4)	414 (60.8)	200 (54.6)	0.804
Class II, N (%)	166 (16.3)	114 (16.7)	52 (14.2)	
Class III, N (%)	107 (10.5)	68 (10.0)	39 (10.7)	
Class IV, N (%)	130 (12.8)	85 (12.5)	45 (12.3)	
Current smoker, N (%)	122 (12.0)	98 (14.4)	24 (7.1)	0.001
HF duration >=18 mo, N (%)	766 (75.3)	507 (74.4)	259 (77.1)	0.401
Beta blocker, N (%)	696 (68.4)	469 (68.9)	227 (67.6)	0.726
ACE/ARB, N (%)	659 (64.8)	440 (64.6)	219 (65.2)	0.914

6MWD indicates 6-minute walk distance; ACE/ARB, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers; AFib, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; EF, ejection fraction; HF, heart failure; KCQQ, Kansas City Cardiomyopathy Questionnaire; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; and SBP, systolic blood pressure.

*Variables were compared between derivation group and validation group; *P* values were from independent *t* test for continuous variables, and χ^2 test for categorical variables.

†EF at follow-up (after enrollment) was collected from the latest EF measurement for each patient, with 790 patients having nonmissing data.

days). Tests of difference (*t* test or χ^2 test) between derivation and validation indicated the 2 subgroups were well randomized with almost all the variables tested were nonsignificantly different except for current smoking status.

A total of 4453 slow off-rate modified aptamers had high-quality analyzable output from the SOMAscan. We first performed a descriptive analysis in the entire cohort (N=1017). The unadjusted overall association of plasma proteins with survival time was high, illustrated by Quantile-Quantile plot showing markedly more than expected associations (Figure IA in the Data Supplement). To try to exclude survival associations based on known clinical risk factors, we also examined overall single-protein associations adjusted for MAGGIC and NT-proBNP. Although this reduced the slope of the resulting Quantile-Quantile plot, it still indicated a higher than expected number of proteins associated with survival time (Figure IB in the Data Supplement).

Protein Risk Score Derivation and Testing

To assess the predictive power of the circulating proteome, particularly incremental to current risk stratification for HFrEF, we attempted to build and validate an optimized HFrEF-proteomic risk score (PRS) using a multistaged analysis plan that included adjustment for conventional risk modeling and a distinct validation cohort (Figure 1). Individual marker association analyses in the derivation cohort yielded 130 slow off-rate modified aptamers targeting 128 unique proteins that were significantly associated with mortality at a level of false discovery rate <0.05 (Table I in the Data Supplement). Among these, NMB (neuromedin B) was the protein most strongly associated with survival time (hazard ratio [HR], 2.59 [95% CI, 1.85–3.61], $P=2.25\times10^{-8}$), although it was not ultimately retained in score optimization. The marker most highly associated individually that was ultimately retained in the score was butrylcholinesterase (aka pseudocholinesterase, encoded by the gene *BCHE*) with HR of 0.23 (95% CI, 0.14–0.39, $P=6.61\times10^{-8}$). After entering all individually significant markers into LASSO-penalized Cox regression with adjustment for MAGGIC score and NT-proBNP and then using stepwise forward and backward selection in the derivation cohort, 8 proteins were ultimately retained. The coefficients in the single-protein Cox model and final multiprotein Cox model are shown in Table 2. Testing of proportional hazards assumption showed it was not statistically significant for any of those 8 proteins in the single-protein model (false discovery rate >0.05; Table I in the Data Supplement) or for the global test of the multiprotein model ($P=0.128$), hence supported our assumption of proportional hazards in the Cox regression analyses. These proteins and their coefficients were used to build the PRS, which was then tested in validation cohort. In the validation cohort Cox model, the PRS was strongly associated with survival (HR 2.27, $P=6.36\times10^{-14}$), and similar results were seen in Kaplan-Meier analysis of HFrEF-PRS quartiles (Figure 2, log-rank $P=2.2\times10^{-6}$). The observed protein abundances within PRS quartiles and corresponding normal ranges are shown in Table 3.

Risk Stratification Improvement Using the HFrEF-PRS

To quantify the incremental predictive value for all-cause mortality of the HFrEF-PRS, we first compared a base model with MAGGIC and NT-proBNP to a full model including these predictors plus the HFrEF-PRS using only the validation cohort. The results for PRS alone, the base model, and the full model for both the derivation and validation groups are shown in Table 4. In the full model the PRS remained statistically significant (HR, 1.37, $P=0.021$) despite adjustment for the established base predictors and increased the model pseudo R^2 value (0.284 versus 0.272). To get at true predictive improvement we compared the C statistic for each predictor (PRS, MAGGIC, NT-proBNP) and their combinations (Figure 3), as well as examining other metrics of predictive performance. The C statistic for the HFrEF-PRS alone was similar to the 2 other established HF predictors in validation cohort (0.815 versus 0.822 or 0.801 for 1-year survival; 0.724 versus 0.753 or 0.765 for 3-year survival). In terms of improving prediction of mortality, we examined C statistic change, integrated discrimination index, continuous net reclassification index, and the median improvement in risk score within the validation group. Table 5 shows these metrics evaluating the difference between the base and full models. For 1-year survival, integrated discrimination index (0.041) and median improvement in risk score (0.039) were significantly improved (both $P<0.05$), whereas continuous net reclassification index (0.392) trended towards improvement

Table 2. Cox Model Output for the 8 Proteins of the HFrEF-PRS in Derivation Group (n=681)

SOMA ID	Target protein	Gene symbol	Single-protein association*			Multiprotein association†		
			HR	95% CI	*P* value	HR	95% CI	*P* value
15514-26	Pseudocholinesterase	*BCHE*	0.23	0.14–0.39	6.61×10^{-8}	0.46	0.25–0.85	1.36×10^{-2}
4930-21	Stanniocalcin-1	*STC1*	4.35	2.55–7.45	7.80×10^{-8}	2.50	1.42–4.42	1.61×10^{-3}
3396-54	Renin	*REN*	1.59	1.32–1.9	5.73×10^{-7}	1.52	1.24–1.85	4.50×10^{-5}
2677-1	ERBB1	*EGFR*	0.14	0.06–0.30	7.02×10^{-7}	0.13	0.05–0.35	5.40×10^{-5}
10818-36	ASM	*SMPD1*	2.44	1.64–3.63	1.12×10^{-5}	1.66	1.08–2.56	2.09×10^{-2}
7891-45	UGT 1A6	*UGT1A6*	2.72	1.73–4.26	1.34×10^{-5}	1.96	1.17–3.3	1.11×10^{-2}
3352-80	Carbonic anhydrase 6	*CA6*	0.63	0.5–0.79	9.83×10^{-5}	0.74	0.57–0.95	1.94×10^{-2}
13589-10	APBB3	*APBB3*	0.42	0.25–0.71	1.07×10^{-3}	0.55	0.34–0.89	1.56×10^{-2}

APBB3 indicates amyloid beta A4 precursor protein-binding family B member 3; ASM, sphingomyelin phosphodiesterase; ERBB1, epidermal growth factor receptor 1; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PRS, proteomic risk score; and UGT 1A6, UDP-glucuronosyltransferase 1-6.

*Cox model was tested for each individual protein with adjustment of MAGGIC and NT-proBNP level.

†Cox model was tested for all proteins together with adjustment of MAGGIC and NT-proBNP level.

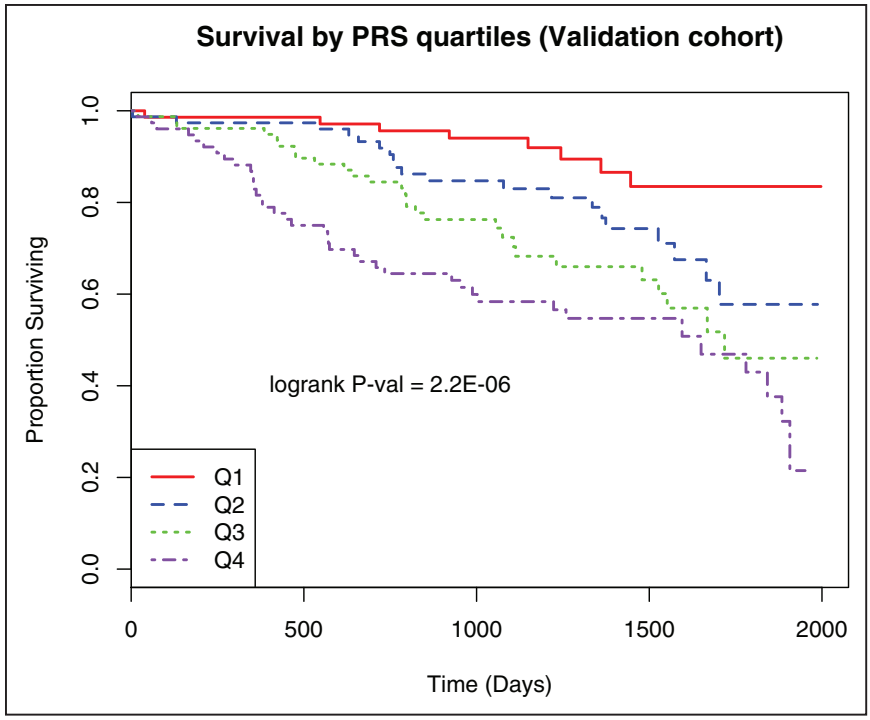

Figure 2. Kaplan-Meier survival curves of the validation cohort (n=336) separated by heart failure with reduced ejection fraction–proteomic risk score (PRS) quartiles.

but did not quite meet statistical significance (P=0.078). In contrast, the C statistic change (0.009) was not significant. When examining 3-year survival prediction, the PRS did not perform as well with only integrated discrimination index (0.022) being significantly improved (P=0.018).

Relationship of the HFrEF-PRS to Cardiovascular Phenotypes and Pathway Analysis

To assess the relationship of the HFrEF-PRS specifically to cardiovascular or HF phenotypes we assessed many secondary end points. First, we tested the association of PRS with cardiovascular death (Table 4). In unadjusted Cox models, the HFrEF-PRS was strongly associated with the risk of cardiovascular death with HR of 2.16 (P=5.67×10^{-9}) in the validation group. In models adjusted for both MAGGIC and NT-proBNP, the HFrEF-PRS was no longer statistically significant but trended towards hazard (HR, 1.2, P=0.2). We also examined several HF-relevant phenotypes, many of which were strongly related to the HFrEF-PRS, summarized in Table 6. The HFrEF-PRS was associated with the presence of HF preconditions, such as coronary artery disease (HR, 1.4) and type 2 diabetes mellitus (HR, 1.43), and was also

Table 3. Observed Median Abundances in Highest and Lowest Quartiles of HFrEF-PRS and Reference Ranges for the 8 Proteins Contributing to the Score

		Derivation cohort		Validation cohort		Reference Range		
SOMA ID	Protein target	PRS_Q1	PRS_Q4	PRS_Q1	PRS_Q4	5th%	Median	95th%
15514-26	Pseudocholinesterase	7473	5105	7343	4914	5597	7899	11 634
4930-21	Stanniocalcin-1	1442	2028	1437	2025	968	1608	3005
3396-54	Renin	14 683	41 550	12 098	39 454	5431	15 350	50 133
2677-1	ERBB1	13 743	9058	14 288	9289	11 970	17 793	25 181
10818-36	ASM	1042	1153	1077	1139	755	1385	2722
7891-45	UGT 1A6	512	589	551	551	402	686	1061
3352-80	Carbonic anhydrase 6	4603	2282	5034	2143	1829	4861	12 550
13589-10	APBB3	1624	1467	1589	1510	1125	1519	4038

All abundance values are raw RFU reflecting the intensity on the array. PRS_Q1 and PRS_Q4 denotes HFrEF patient groups stratified by first and fourth quartile of PRS score, respectively. Reference is from an external cohort of healthy individuals underwent the same SOMA array. APBB3 indicates amyloid beta A4 precursor protein-binding family B member 3; ASM, sphingomyelin phosphodiesterase; ERBB1, epidermal growth factor receptor 1; HFrEF, heart failure with reduced ejection fraction; PRS, proteomic risk score; RFU, relative fluorescence units; and UGT 1A6, UDP-glucuronosyltransferase 1-6.

Table 4. Model Fitting for PRS in Derivation and Validation Group

Groups	Model	Variables*	All-cause mortality				Cardiovascular mortality			
			HR	95% CI	*P* value	R^2	HR	95% CI	*P* value	R^2
Derivation	PRS	PRS	3.21	2.78–3.70	$<2.0\times10^{-16}$	0.302	2.89	2.43–3.43	$<2.0\times10^{-16}$	0.180
	Base model	MAGGIC	1.08	1.06–1.11	1.34×10^{-14}	0.241	1.08	1.06–1.11	5.07×10^{-1}	0.179
		NT-proBNP	1.59	1.33–1.90	3.28×10^{-7}	0.241	1.64	1.33–2.01	3.07×10^{-6}	
	Full model	MAGGIC	1.06	1.04–0.09	5.95×10^{-8}	0.360	1.06	1.03–1.09	8.85×10^{-6}	0.245
		NT-proBNP	1.23	1.02–1.47	2.65×10^{-2}	0.360	1.38	1.12–1.70	2.58×10^{-3}	
		PRS	2.53	2.15–2.99	$<2.0\times10^{-16}$	0.360	2.19	1.80–2.67	8.72×10^{-15}	
Validation	PRS	PRS	2.27	1.84–2.82	6.36×10^{-14}	0.162	2.16	1.67–2.80	5.67×10^{-9}	0.109
	Base model	MAGGIC	1.07	0.04–1.10	1.31×10^{-6}	0.272	1.07	1.03–1.11	1.41×10^{-4}	0.226
		NT-proBNP	1.97	1.55–2.51	3.12×10^{-8}	0.272	2.14	1.60–2.85	2.30×10^{-7}	
	Full model	MAGGIC	1.06	1.03–1.09	2.79×10^{-4}	0.284	1.06	1.02–1.10	1.98×10^{-3}	0.230
		NT-proBNP	1.81	1.41–2.32	3.59×10^{-6}	0.284	2.02	1.49–2.72	4.97×10^{-6}	
		PRS	1.37	1.05–1.79	2.10×10^{-2}	0.284	1.22	0.89–1.69	2.23×10^{-1}	

The increment shown for the PRS one SD of the score. The increment of MAGGIC score is per point. The increment for NT-proBNP is per unit change of log-transformed BNP (around 2.72-fold change of raw NT-proBNP value in mol/L). R^2 is pseudo-R^2 for cox regression, showing the improvement in likelihood between the fitted model and a model without predictor variables (null model). BNP indicates B-type natriuretic peptide; HR, hazard ratio; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and PRS, proteomic risk score.

*NT-proBNP was log-transformed; PRS was standardized to have mean=0 and SD=1, so the HR for PRS represented one SD difference.

associated with worse quality of life, shorter 6-minute walk distance, and greater decrement in EF over time (−2.3%, $P=1.49\times10^{-5}$).

To investigate the possible biologic mechanisms underlying the proteins of interest, we undertook annotation of the 8 proteins comprising the PRS (Table II in the Data Supplement) and pathway analysis of the 128 individually significant proteins (Table III in the Data Supplement). Examining the individual HFrEF-PRS proteins, some of them are known contributors to HF or cardiovascular diseases (eg, Renin), whereas most appear somewhat novel to the setting of HFrEF. In gene-set analysis of individually significant proteins, there were several statistically significant Gene Ontology gene-sets enriched, but these were all very general cellular components instead of canonical pathways, the most significant being endoplasmic reticulum lumen, extracellular space, and extracellular region.

DISCUSSION

In this study, we focused on the plasma proteome's association with overall survival in patients with HFrEF and took a staged (derivation and separate validation) approach, successfully building a PRS comprised of 8 markers. Our results, one of the first to our knowledge in HF using the expanded (≈5000 proteins) SOMA array and the largest HF cohort to be studied using any SomaScan,[23] indicates that the circulating proteome in patients with HFrEF shows substantial differences as the risk of death increases and that PRS improved prediction on top of current standard of risk stratification. The PRS improved some metrics of 1-year prediction but contributed less at 3 years, possibly reflecting that the dynamic nature of the proteome may make its measurement more suited to nearer-term versus longer-term predictions. Although model C statistic changes were not significant, other risk assessment metrics (integrated discrimination index, continuous net reclassification index, and median improvement in risk score) were significantly improved and the Kaplan-Meier curves separate significantly. The base model with MAGGIC score and NT-proBNP achieves a high C statistic of 0.838 for 1-year survival, making further increments a challenge. Moreover, C statistics have been criticized in the setting of biomarker evaluation,[24] where there may be greater utility in other metrics.[25] Regardless, our data offer proof of concept that the plasma proteome as measured via the enhanced SOMA array independently indicates risk of death in HFrEF and as a standalone has performance comparable to MAGGIC or NT-proBNP. Moreover, the PRS likely identifies patients at risk for worsening heart failure since it is also associated with cardiovascular death, lower functional status, and worsening future EF. This underscores the potential physiological and clinical value of this approach.

Additional work is needed to operationalize this or other PRS for HF. Clinical scores and biomarkers are used today to give patients and physicians an idea of risk which is critical for setting reasonable expectations and for appropriately pairing intervention aggressiveness to risk level. However, for an individual patient, the estimates always have wide confidence intervals and so further refinement of risk prediction may be helpful. Another aspect is that having a complex clinical score and multiple individual markers is cumbersome to utilize and perhaps an integrated profile (as a single test) could be desirable. Our HFrEF-PRS could be further pursued

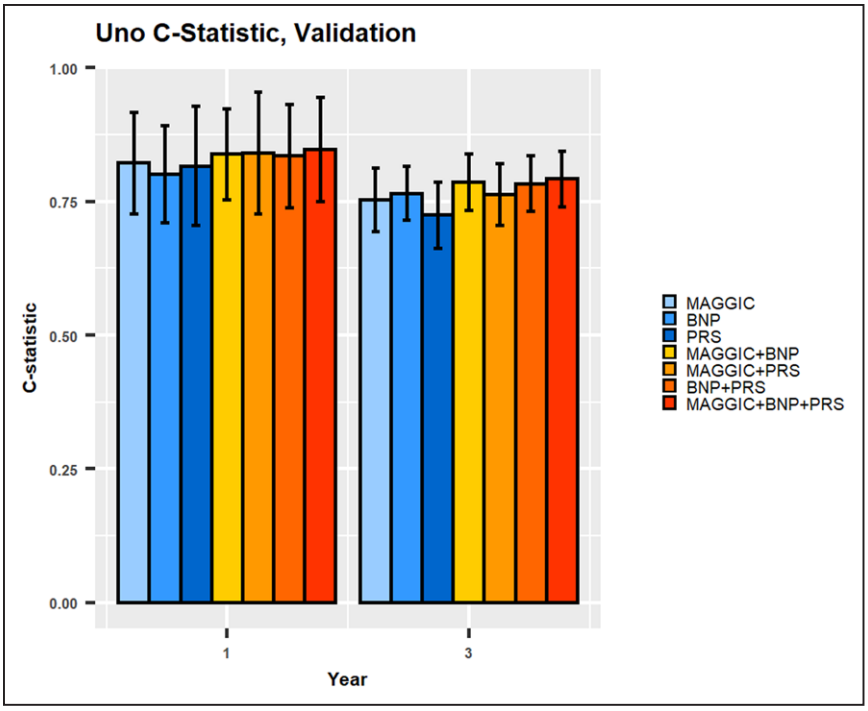

Figure 3. Bar graph of C statistics for each survival predictor and their combinations at 1 y and 3 y in the validation cohort (n=336).
BNP indicates B-type natriuretic peptide; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; and PRS, proteomic risk score.

(via additional validation) as an add-on to existing risk stratification or as a standalone predictor, but the current data gives hope for the future where a convenient multimarker test could enhance or replace current techniques. Another potential path forward is in terms of estimating other outcomes of interest. We focused here on all-cause mortality but additional outcomes could be more actionable and may be modeled as well; these might include progression to transplant or left ventricular assist device, or decrementing EF. There are less established clinical scores or biomarkers for these types of outcomes and the plasma proteome may be very informative towards this end.

Part of the novelty of the current study lies in utilizing the expanded SOMAmer-based proteomics assay from Somologic in the setting of HFrEF. More classical approaches to proteomics involving 2-dimensional gels or mass spectrometry are truly unbiased but are limited in throughput and in the ability to quickly identify specific proteins of interest. On the other hand, antibody based methods of protein quantification provide specificity but are historically limited in terms of multiplexing, though this a dynamic field with recent improvements as well.[26] The SOMA assay leverages chemically modified nucleotides (aptamers) to provide both specificity and extremely high multiplexing, as well as a large range of measurement.[18,21] Although this approach is not strictly unbiased, each of the ≈5000 proteins was known and then targeted, it raises the candidate approach to such a large scale that it eventually approximates an unbiased proteomic approach. Our findings parallel recent studies by Ganz and colleagues which utilized a SOMA-derived protein-based risk score to predict cardiovascular outcomes in patients with stable coronary heart disease.[19] They identified 9 proteins that were associated with adverse outcomes and found that a profile of these proteins was superior in risk prediction to the refit Framingham risk score. The greater increment in prediction attained via proteomics in that study compared to our results could indicate that prognosis in HF may be under broader range of influences compared to atherosclerotic events (HF being such a heterogeneous condition) or that we simply missed the key factors. Related to the heterogeneity of

Table 5. Risk Assessment for PRS Incremental Value in Validation Group

Outcome	Risk stratification assessment*	Improvement	Improvement 95% CI	*P* value
1-y survival	C statistic improvement	0.009†	−0.026 to 0.046	0.612
	IDI	0.041	0.004 to 0.128	0.010
	Continuous NRI	0.391	−0.067 to 0.564	0.078
	Median Improvement in Risk Score	0.039	0.002 to 0.147	0.016
3-y survival	C statistic improvement	0.006†	−0.009 to 0.022	0.441
	IDI	0.022	0.002 to 0.066	0.018
	Continuous NRI	0.150	−0.039 to 0.272	0.114
	Median Improvement in Risk Score	0.012	−0.005 to 0.038	0.172

IDI indicates integrated discrimination index; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NRI, net reclassification improvement; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and PRS, proteomic risk score.

*Discriminations were compared between base model (MAGGIC+NT-proBNP) and full model (MAGGIC+NT-proBNP+PRS).

†The base model C statistic was 0.838 for 1-y survival and 0.785 for 3-y survival.

Table 6. Association of 8-Protein PRS With Cardiovascular and Heart Failure Phenotypes

Phenotype	Measure	Derivation cohort		Validation cohort		Total	
		Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
CAD	OR	1.43	2.07×10^{-5}	1.35	6.9×10^{-3}	1.4	4.62×10^{-7}
AFib	OR	1.29	3.49×10^{-3}	1.51	5.6×10^{-4}	1.37	1.02×10^{-5}
T2DM	OR	1.42	1.89×10^{-5}	1.45	8.76×10^{-4}	1.43	7.01×10^{-8}
6MWD (feet)	beta	−153	$<2.0\times10^{-16}$	−155	2.37×10^{-13}	−152	$<2.0\times10^{-16}$
KCCQ total score	beta	−7.3	5.86×10^{-13}	−4.6	5.74×10^{-4}	−6.3	4.54×10^{-15}
EF baseline (%)	beta	−0.86	8.61×10^{-2}	−2.34	2.99×10^{-4}	−1.36	5.83×10^{-4}
EF future change (%)	beta	−2.5	1.39×10^{-4}	−1.94	2.79×10^{-2}	−2.29	1.49×10^{-5}

Estimates and *P* values are from logistic regression (for CAD, Diabetes, or A Fib) or linear regression (6MWD, KCQQ, EF, and EF change; unit for each variable is shown in Table 1) testing association between each phenotype and HFrEF-PRS score without adjustment. 6MWD indicates 6-min walking distance; AFib, atrial fibrillation; beta, beta coefficient; CAD, coronary artery disease; EF, ejection fraction; HFrEF, heart failure with reduced ejection fraction; KCCQ, Kansas City Cardiomyopathy Questionnaire; LASSO, least absolute shrinkage and selection operator; OR, odds ratio; PRS, proteomic risk score; T2DM, type 2 diabetes mellitus; TFF3, Trefoil factor 3; and TNF sR-II, tumor necrosis factor receptor superfamily member 1B.

HF, future proteomic analyses may benefit from focusing on specific etiologies or subgroups and incorporating change over time. However, the fact that the base model for predicting HF death captured such a greater amount of variability than the Framingham model did in the Ganz study (area under the curve 0.838 compared with 0.64) could also contribute to the differing results.

Examining the specific proteins involved in PRS construction yields some interesting biologic insights. At least one protein has well-known association to HF (renin), whereas others are unrecognized in HF but affect a potentially relevant pathway (eg, EGFR [epidermal growth factor receptor]). Several have no well-established relationship in HF at all. For example, BCHE (butrylcholinesterase, aka pseudocholinesterase), which was the strongest univariate associated protein, is best recognized for breaking down choline esters, organophosphates, and the metabolism of some drugs (such as cocaine or aspirin). Interestingly, it was previously associated with worse survival and ventricular function after myocardial infarction.[27] Another intriguing association is SMPD1 (sphingomyelin phosphodiesterase 1), an important enzyme in the production of cardiac ceramides, which participate in substrate supply to mitochondria and seem to mediate protective anti-fibrotic effects after myocardial infarction in mice.[28] However it was risk-associated in our data. Thus, although this analysis focused on the HFrEF-PRS clinical potential, it can also create a priority list of proteins for further investigation of their role in the pathobiology of HF and as potential targets.

Our study has several advantages and limitations worthy of discussion. We had a diverse patient cohort, utilized high-quality, core-lab experimental data and rigorous statistical techniques (random division of derivation from validation) supporting the veracity of our findings. However, there are certain limitations as well. Regarding the SOMAscan itself, although quite large in scale, it is not truly unbiased, and some plasma proteins are certainly missed. As remarked previously,[29] validation of all SOMA markers on this large array compared to simultaneous alternative measurement techniques has not been completed, and along with the lack of complete internal controls, makes it difficult to rule out all potential measurement error or bias. Despite this, the SOMAscan overall performance and reproducibility has been reasonably demonstrated,[19,21,30–32] and within the current study, the significant markers targeting the same protein showed very similar relationships to risk (ie, TNF [tumor necrosis factor] sR-II and TFF3). The current study focused on circulating proteins measured in plasma, so although this increases potential utility of our work (since peripheral blood is easily obtained), we cannot here gain insight into myocardial protein expression. All of the patients in our study had established HFrEF so that these data inform disease progression/prognostication but not incidence, and there is need for additional investigation of the circulating proteome in people at risk for HF development. Similarly, patients with HF with preserved EF are not represented and insight could be gained from this type of interrogation in HF with preserved EF. Our measurements were at a single time point, so changes over time or in response to medication cannot be assessed from the current data. Repeated measures and correlations to treatments is a critically important future direction, particularly as this could lead to more tailored therapy. Finally, although we created an independent validation set from within our study, all participants were from a single registry in southeast Michigan, thus ideally our findings should be replicated in other external cohorts.

CONCLUSIONS

In >1000 patients with HFrEF, the plasma proteome was highly associated with the risk of death and a multiprotein predictive score can modestly improve risk stratification in patients with HFrEF in addition to a validated clinical score and NT-proBNP level. Several of the key

proteins are known associates of HF in humans or other models, but many are novel and deserve further investigation to determine if they are mechanistically linked to HF. These data support the overarching idea that the proteome can be used to improve clinical care and better understand HFrEF. Additional investigation would be needed to further validate and operationalize the HFrEF-PRS and illuminate the individual proteins and pathways involved. More broadly, the potential value of plasma proteomic profile in terms of risk of incident HFrEF improved understanding of HF with preserved EF or responsiveness to medications is intriguing and maybe worthy of exploration.

ARTICLE INFORMATION

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Supplemental Material

Supplemental Methods
Supplemental Tables I–III
Supplemental Figure I
Supplemental Note I
References [33–41]

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