4-14-2021

Performance of A Metabolomic Biomarker Score Compared to Three Prognostic Scores in Chronic Heart Failure

Anshul Saxena
Baptist Health Medical Group, anshuls@baptisthealth.net

Muni Rubens
Miami Cancer Institute, MuniR@baptisthealth.net

Joseph Salami
Baptist Health South Florida, josephsa@baptisthealth.net

Emir Veledar
Baptist Health South Florida, emirv@baptisthealth.net

Citation
Research Square (2021) 11.

Performance of A Metabolomic Biomarker Score Compared to Three Prognostic Scores in Chronic Heart Failure

Peter McGranaghan

Charité

Anshul Saxena

Baptist Health South Florida

Hans-Dirk Düngen

Charité

Muni Rubens

Baptist Health South Florida

Sandeep Appunni

Government Medical College

Joseph Salami

Baptist Health South Florida

Emir Veledar

Baptist Health South Florida

Philipp Lacour

Charité

Florian Blaschke

Charité

Danilo Obradovic

University Hospital Leipzig

Goran Loncar

University of Belgrade

Elvis Tahirovic

University of Nis

Frank Edelmann

Charité

Burkert Pieske

Charité

Tobias Trippel (✉ tobias_daniel.trippel@charite.de)

Charité

Research Article

Abstract

The Cardiac Lipid Panel (CLP) is a novel panel of metabolomic biomarkers that has previously shown to improve the diagnostic and prognostic value for CHF patients. Several prognostic scores have been developed for cardiovascular disease (CVD) risk, but their use is limited to specific populations and precision is still inadequate. We compared a risk score using the CLP plus NT-proBNP to three commonly used risk scores: The Seattle Heart Failure Model (SHFM), Framingham Risk Score (FRS), and Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) score. We included 280 elderly CHF patients from the Cardiac Insufficiency Bisoprolol Study in Elderly (CIBIS-ELD) trial. Cox Regression and hierarchical cluster analysis was performed. Integrated area under the curves (IAUC) was used as criterium for comparison. The mean (*SD*) follow-up period was 81 (33) months, and 95 (34%) subjects met the primary endpoint. The IAUC for FRS was 0.53, SHFM 0.61, MAGGIC 0.68, and CLP 0.78. Subjects were partitioned into three risk clusters: low, moderate, high with the CLP score showing the best ability to group patients into their respective risk cluster. A risk score composed of a novel panel of metabolite biomarkers plus NT-proBNP outperformed other common prognostic scores in predicting 10-year cardiovascular death in elderly ambulatory CHF patients. This approach could improve the clinical risk assessment of CHF patients.

Introduction

The prevalence of chronic heart failure (CHF) in the western world continues to increase, especially in patients older than 65 years [1]. CHF is a major burden on the health care system and is associated with high morbidity and mortality, including a poor quality of life [2]. An important aspect of CHF management is to ensure that clinicians and patients with CHF have the necessary knowledge and resources to make the best health decisions. A prognostic model is one such resource, defined as a formal combination of multiple predictors from which risks of a specific outcome can be calculated for individual patients.

Prognostic models are abundant in the literature, and the most popular ones include the SHFM (Seattle Heart Failure Model), FRS (Framingham Risk Score), and MAGGIC (Meta-analysis Global Group in Chronic Heart Failure). The SHFM score is the most thoroughly validated and contains the most predictor variables of the three [3]. The MAGGIC score [4] was developed from a dataset of over 39,000 patients across 30 studies and validated on more than 60,000 patients using 2 large CHF cohorts [5, 6]. The FRS score was developed as a sex-specific risk score that can be conveniently used to calculate general cardiovascular disease (CVD) risk and risk of individual CVD events [7]. These models all use common clinical and demographic variables to predict the prognosis of CHF patients and have convenient online calculators. Although these scores have been validated, they have not been widely adopted possibly because they are not routinely calculated in clinical practice [8–10], have poor reliability at the individual patient level [11], suffer from a significant amount of missing data requiring imputation.

Metabolomics is a rapidly growing field in biomarker profiling that could help meet the need for more robust prognostic biomarkers. By applying nuclear magnetic resonance (NMR) spectroscopy and mass

spectrometry (MS), it is now possible to analyze a hundreds of metabolites from human samples such as blood, urine, saliva, and tissue, which can elucidate the outcome of complex networks of endogenous and exogenous biochemical reactions [12]. This approach could provide a more comprehensive signature of biochemical activities that could be associated with diet, medication, disease progression, and thus negative outcomes due to these complex mechanisms [13, 14]. Previous studies have shown that metabolomic biomarkers can be used for risk prediction as well as diagnosis of CHF [15–27].

One promising metabolomic biomarker panel in CHF patients is the cardiac lipid panel (CLP) which is supplemented by N-terminal pro–B-type natriuretic peptide (NT-proBNP). The CLP is consists of three specific metabolomics features: triacylglycerol (TAG) 18:1/18:0/18:0, phosphatidylcholine (PC) 16:0/18:2, and the sum of the 3 isobaric sphingomyelin (SM) species SM d18:1/23:1, SM d18:2/23:0, and SM d17:1/24:1. The diagnostic value of CLP was first discovered in a study by Mueller and colleagues, where they compared CHF patients to healthy controls, and found that CLP was able to improve the diagnostic performance over NT-proBNP alone [28]. The incremental prognostic value of the CLP was first assessed in a recent study which found it improved the discrimination and risk assessment over NT-proBNP and clinical risk factors [29].

The objective of this study was to compare the performance of a risk score composed of the CLP panel plus NT-proBNP to the three commonly used traditional risk scores (SHFM, FRS, and MAGGIC) to predict long-term cardiovascular mortality in ambulatory CHF patients. We hypothesized that the CLP risk score would improve our ability to classify risk of cardiovascular death in comparison to the three validated clinical risk prediction algorithms.

Results

Table 1 shows the baseline characteristics of the total population (n = 280) as well as the variables included in each score. Mean age of this sub-cohort was 72.1 (4.9) years, 26.4% were women, 45% patients had heart failure with reduced ejection fraction (HFrEF) (LVEF < 35%), and most patients were in NYHA functional class II (67.5%) with the remaining in NYHA class III. Hypertension was present in 80% of participants and 45% were current or former smokers; 29% had diabetes and 71% had CAD. During the follow-up period (mean = 81 months, SD = 33; median = 96 months), 95 (34%) patients met the primary outcome. The sample selection criteria as well as the comparison of this sub cohort's baseline characteristics to the source cohort has previously been reported [29], however, this study analyzed 10 year follow up rather than the previously reported 4 year follow up.

Table 1
Baseline characteristics of the study participants and variables included in each prognostic score

Characteristic	Total	Prognostic Score			
	n = 280	SHFM	FRS	MAGGIC	CLP
Age (years), mean ± SD	72 ± 4.9	✓	✓	✓	
NYHA (II/III), n	188/91	✓		✓	
Male, n (%)	206 (74)	✓	✓	✓	
Body mass index (kg/m^2), mean ± SD	26.8 ± 3.4			✓	
Heart rate (bpm), mean ± SD	73 ± 13.0				
Systolic blood pressure (mm Hg), mean ± SD	134 ± 19	✓	✓	✓	
Diastolic blood pressure (mm Hg), mean ± SD	81 ± 11				
Years since first diagnosis of CHF	5.2 ± 5.6		✓		
Laboratory, mean ± SD					
Creatinine (µmol/L)	107 ± 27.9			✓	
Hemoglobin (g/dL)	21.6 ± 2.4	✓			
Sodium (mEq/L)	141.4 ± 3.3	✓			
Uric acid (µmol/L)	356 ± 127	✓			
Total Cholesterol (mmol/L)	5.1 ± 1.6				
HDL cholesterol (mmol/L)	1.2 ± 0.5		✓		
LDL cholesterol (mmol/L)	3.4 ± 1.3				
Triglycerides (mmol/L)	1.7 ± 1.0				
Lymphocytes (%)*		✓			
NT-proBNP (pg/mL)	793 (331−1765)[†]				✓
PC 16:0/18:2 (µg/dl)	36810 (32435−40015)[†]				✓
TAG 18:2 (µg/dl)	121 (76.5-256.4)[†]				✓
SM d18:1/23:1, SM d18:2/23:0, SM d17:1/24:1 (µg/dl)	1342 (1134−1596)[†]				✓
Cardiac Imaging, mean ± SD					

	Total	Prognostic Score	
LVEF (%)	36 ± 9.5	✓	✓
LVDed (mm)	58.8 ± 9.2		
LVDes (mm)	45.5 ± 9.7		
LVVed (mL)	152.7 ± 63.9		
LVVes (mL)	101.1 ± 51.6		
LAes (mm)	45.3 ± 7.2		
E/e'	12 ± 9.2		
E/A	1 ± 0.8		
Deceleration time (ms)	226 ± 80		
Comorbidities, n (%)			
Diabetes	82 (29)	✓	✓
Hypertension	224 (80)		
Coronary artery disease	200 (71)	✓	
Smokers	125 (45)	✓	✓
Hyperlipidemia	162 (58)		
COPD	9 (3)		✓
Medication, n (%)			
ACE inhibitor	247 (88)	✓	✓
Allopurinol	0 (0)	✓	
ARB	115 (41)	✓	
Beta Blocker	203 (73)	✓	✓
Diuretics	219 (78)		
Diuretic Dose mg/kg per day	0.32 ± 0.31	✓	
Glycoside	59 (21)		
Aspirin	216 (77)		
Nitrate	146 (52)		
Antiarrhythmic agent	42 (15)		
Statin	114 (41)	✓	

	Total	Prognostic Score
Caption:		
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CE 18:2, triacylglycerol 18:1/18:0/18:0; COPD, chronic obstructive pulmonary disease; E/A, ratio of the early (E) to late (A) ventricular filling velocities; E/e', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LAes, left atrial end systole; LDL, low-density lipoprotein; NYHA, New York Heart Association; HDL, high-density lipoprotein; LVDed, left ventricular diameter end diastole; LVDes, left ventricular diameter end systole; LVVed, left ventricular volume end diastole; LVEF, left ventricular ejection fraction; LVVes, left ventricular volume end systole; mg/kg, milligrams per kilograms; NTpro-BNP, N-terminal pro−B-type natriuretic; PC 16:0/18:2, phosphatidylcholine 16:0/18:2; SM d18:1/23:1, SM d18:2/23:0, SM d17:1/24:1, the sum of the 3 isobaric sphingomyelin species SM d18:1/23:1, SM d18:2/23:0, and SM d17:1/24:1		
*Imputed using the median, 31%, of the normal range 20−40%		
† Median (Interquartile Range)		

All variables were available for each score except for the lymphocytes (%) variable in the SHFM score, which was imputed as previously described. The SHFM model had the highest number of variables (n = 17), followed by MAGGIC (n = 13), FRS (n = 7), and CLP (n = 4). There were 10 overlapping variables which were included in at least 2 scores. The SHFM score included the most medication (n = 6) and laboratory (n = 5) variables while MAGGIC included the most clinical (n = 7) and demographic variables (n = 3).

Table 2 shows the univariate Cox Regression results. The CLP (HR = 2.38, p < 0.001), SHFM (HR = 2.01, p = 0.002, and MAGGIC (HR = 1.10, p < 0.001) scores were significantly associated with the outcome while FRS was not. Figure 1 shows the AUC change over time (IAUC) for the 4 prognostic scores with the comparison of Uno's concordance statistics for hypothesis testing. The IAUC was 0.53, 0.61, 0.68 and 0.78 for FRS, SHFM, MAGGIC and CLP, respectively. Harrell's c statistics at 10 year follow up show similar results (**Supplemental Table 1**). The three traditional scores were all significantly different (p < 0.001) from the CLP score according to Uno's difference in concordance statistic (**Supplemental table 2**).

<div align="center">

Table 2
Prognostic Scores and Univariate Hazard Ratios
</div>

Score	HR (95% CI)	p value
SHFM	2.01 (1.29−3.13)	0.0022
FRS	1.02 (0.97−1.07)	0.5291
MAGGIC	1.10 (1.05−1.14)	< .0001
CLP	2.38 (1.95−2.92)	< .0001
Caption: Unadjusted Cox proportional hazard models of 10-year outcome for cardiovascular mortality. Total subjects, n = 280. Total events, n = 95. SHFM (Seattle Heart Failure Model), FRS (Framingham Risk Score), and MAGGIC (Meta-analysis Global Group in Chronic Heart Failure), and Cardiac Lipid Panel Risk Score (CLP).		

Figure 2 shows the hierarchical cluster dendrogram mapped to illustrate the assignment of patients into their respective clusters and the associated color map shows the range of each prognostic score and their distribution within each cluster. Hierarchical clustering grouped the patients in separate clusters accounting for the noise between smaller clusters. Each observation was treated as a unique cluster, and this method: (1) identified the two similar or close clusters, and (2) merged the two most similar clusters. Using this clustering technique, similar prognostic score data from participants were grouped together, such that the members in the same group were more similar to each other than the members in the other groups. We can infer from the cluster centres and cluster memberships that CLP risk score was better at grouping patients with respect to their cardiovascular mortality risk and associated clinical characteristics compared to the other three scores. The survival curves for each risk cluster are shown in Fig. 3. Rates of mortality were: low risk cluster (23%), moderate risk cluster (41%) and high-risk cluster (54%). **Supplemental Fig. 1** shows the constellation plot on a two-dimensional plane with nodes and links to describe relationship among component nodes. This plot is an alternate depiction of the dendrogram and illustrates the length between clusters and a balanced structure. **Supplemental Fig. 2** shows the scatterplot matrix of all 4 scores and clusters to illustrate the relationships between each prognostic score and risk cluster assignment.

Table 3 shows the cohort characteristics and the prognostic score distribution for each risk cluster. The three clusters were: low risk, n = 148; moderate risk, n = 74; high risk, n = 58. Most characteristics were different across risk clusters, with 28 out of the 50 cohort characteristics significantly different across the 3 clusters. In particular, patients in the highest risk cluster were older, with lower LVEF, higher NT-proBNP, and experienced a higher frequency of events. All prognostic scores were significantly different across their respective risk clusters. Of the continuous risk scores (FRS, SHFM, MAGGIC), only FRS had its highest mean score in the high-risk cluster. The categorical CLP score showed a skewed distribution of higher risk scores (3–4) in the moderate and high-risk clusters. In the high-risk cluster, the majority of subjects were scored with highest possible CLP score of 4.

Table 3
Comparison of Cohort Characteristics and Prognostic Scores

	Cluster 1 Low Risk	Cluster 2 Moderate Risk	Cluster 3 High Risk	p-value
Characteristic	n = 148	n = 74	n = 58	
Age (years), mean ± SD	71 ± 4.2	73 ± 4.9	74 ± 5.2	< .0001[a]
NYHA (II/III), n	121/26	47/27	20/38	< .0001[c]
Male, n (%)	95 (64)	64 (87)	47 (81)	0.0005[b]
Body mass index (kg/m^2), mean ± SD	27.3 ± 3.5	26.7 ± 3.2	25.9 ± 3.6	0.0620[a]
Heart rate (bpm), mean ± SD	73 ± 12.6	73.1 ± 11.6	74.5 ± 15.4	0.9746[a]
Systolic blood pressure (mm Hg), mean ± SD	138.5 ± 21.5	132.4 ± 14.2	125.2 ± 13.6	< .0001[a]
Diastolic blood pressure (mm Hg), mean ± SD	82.6 ± 12.3	80.1 ± 9.7	77.3 ± 8.5	0.0133[a]
Years since first diagnosis of CHF	5.5 ± 5.8	4.7 ± 4.8	5.1 ± 6.2	0.6249[a]
Cardiac Death, n (%)	34 (23)	30 (41)	31 (54)	0.0001[b]
Laboratory, mean ± SD				
Serum creatinine (µmol/l)	99.4 ± 24	109.4 ± 22.9	122.9 ± 34.9	< .0001[a]
Hemoglobin (g/dL)	21.6 ± 2.2	21.7 ± 2.8	21.2 ± 2.4	0.1927[a]
Sodium (mmol/L)	141.9 ± 3.1	140.5 ± 3.6	141.5 ± 3.0	0.0428[a]
Uric acid (µmol/L)	327.7 ± 116.7	384.6 ± 119	383.4 ± 138.6	0.0007[a]
Total Cholesterol (mmol/L)	5.3 ± 1.6	5.0 ± 1.5	4.7 ± 1.6	0.0271[a]
HDL cholesterol (mmol/L)	1.3 ± 0.5	1.2 ± 0.4	1.1 ± 0.3	0.0441[a]
LDL cholesterol (mmol/L)	3.6 ± 1.3	3.5 ± 1.3	3.0 ± 1.2	0.0105[a]
Triglycerides (mmol/L)	1.8 ± 1.1	1.7 ± 1.9	1.6 ± 0.8	0.5955
Lymphocytes (%)*	31	31	31	N/A
NT-proBNP (pg/mL)	506.3 (307–1228)[†]	985.1 (346–1935)[†]	1453 (609–3704)[†]	< .0001[a]

	Cluster 1	Cluster 2	Cluster 3	p-value
	Low Risk	Moderate Risk	High Risk	
PC 16:0/18:2 (µg/dl)	36975 (33143–40948)[†]	36550 (32575–39015)[†]	34840 (29710–38818)[†]	0.0252[a]
TAG 18:2 (µg/dl)	126 (73–272)[†]	120 (90–182)[†]	97.8 (64–275)[†]	0.5915[a]
SM d18:1/23:1, SM d18:2/23:0, SM d17:1/24:1 (µg/dl)	1432 (1212–1699)[†]	1233 (1080–1490)[†]	1256 (1046–1361)[†]	<.0001[a]
Cardiac Imaging, mean ± SD				
LVEF (%)	39.4 ± 9.2	33.8 ± 7.9	30.0 ± 8.4	<.0001[a]
LVDed (mm)	57.4 ± 8.6	59.7 ± 9.2	61.5 ± 9.9	0.0094[a]
LVDes (mm)	43.5 ± 8.8	47.1 ± 9.7	48.5 ± 10.6	0.0021[a]
LVVed (mL)	140.7 ± 56.2	160.1 ± 74.3	173 ± 62.3	0.0017[a]
LVVes (mL)	88 ± 43.3	111 ± 58.8	120.8 ± 52.6	<.0001[a]
LAes (mm)	44.2 ± 6.7	45.4 ± 7.4	47.7 ± 7.7	0.0146[a]
E/e'	11.3 ± 8.0	11.1 ± 7.6	14.2 ± 13.2	0.2632[a]
E/A	0.9 ± 0.6	1.2 ± 0.9	1.3 ± 0.8	0.0922[a]
Deceleration time (ms)	235.6 ± 80.7	221 ± 63.7	210.8 ± 90.8	0.0572[a]
Comorbidities, n (%)				
Diabetes	28 (19)	34 (46)	20 (35)	0.0001[b]
Hypertension	119 (80)	62 (84)	43 (74)	0.3823[b]
Coronary artery disease	95 (64)	58 (78)	47 (81)	0.0168[b]
Smokers	48 (32)	53 (71)	24 (41)	<.0001[b]
Hyperlipidemia	87 (59)	43 (58)	32 (55)	0.8716[b]
COPD	5 (3)	4 (5)	0 (0)	0.2143
Medication, n (%)				
ACE inhibitor	132 (89)	66 (89)	49 (84)	0.6127[b]

	Cluster 1 Low Risk	Cluster 2 Moderate Risk	Cluster 3 High Risk	p-value
Allopurinol	0 (0)	0 (0)	0 (0)	N/A
ARB	55 (37)	30 (41)	30 (52)	0.1602[b]
Beta Blocker	123 (83)	48 (65)	32 (55)	< .0001[b]
Diuretics	36 (24)	14 (19)	11 (19)	0.5523[b]
Diuretic Dose mg/kg, mean ± SD	0.26 ± 0.26	0.36 ± 0.34	0.41 ± 0.34	0.0033[a]
Glycoside	29 (20)	15 (21)	15 (26)	0.5996[b]
Aspirin	117 (79)	58 (78)	41 (71)	0.4189[b]
Nitrate	74 (50)	41 (55)	31 (54)	0.7307[b]
Antiarrhythmic agent	19 (13)	10 (14)	13 (22)	0.2048[b]
Statin	62 (42)	27 (37)	25 (43)	0.6804[b]
Prognostic Scores				
SHFM, Mean (SD)	0.67 ± 0.45	1.4 ± 0.31	0.60 ± 0.36	< .0001[a]
FRS, Mean (SD)	20.5 ± 4.3	19.5 ± 3.7	21.8 ± 3.8	0.0392[a]
MAGGIC, Mean (SD)	21.3 ± 4.0	28.3 ± 3.3	21.3 ± 4.6	< .0001[a]
CLP, n (%)				< .0001[b]
0	31 (94)	2 (6)	0 (0)	
1	105 (88)	14 (12)	0 (0)	
2	10 (44)	9 (39)	4 (17)	
3	2 (2)	40 (47)	44 (51)	
4	0 (0)	9 (47)	10 (53)	

Caption:

Cohort characteristics and prognostic score distribution across risk clusters. The prognostic scores used for clustering were: SHFM (Seattle Heart Failure Model), FRS (Framingham Risk Score), and MAGGIC (Meta-analysis Global Group in Chronic Heart Failure), and Cardiac Lipid Panel Risk Score (CLP). Each prognostic score was standardized to the same scale (mean = 0; SD = 1). Ward's minimum variance method was used for clustering.

Abbreviations:

	Cluster 1	Cluster 2	Cluster 3	p-value
	Low Risk	Moderate Risk	High Risk	

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CE 18:2, triacylglycerol 18:1/18:0/18:0; COPD, chronic obstructive pulmonary disease; E/A, ratio of the early (E) to late (A) ventricular filling velocities; E/e', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LAes, left atrial end systole; LDL, low-density lipoprotein; NYHA, New York Heart Association; HDL, high-density lipoprotein; LVDed, left ventricular diameter end diastole; LVDes, left ventricular diameter end systole; LVVed, left ventricular volume end diastole; LVEF, left ventricular ejection fraction; LVVes, left ventricular volume end systole; mg/kg, milligrams per kilograms; NTpro-BNP, N-terminal pro−B-type natriuretic; PC 16:0/18:2, phosphatidylcholine 16:0/18:2; SM d18:1/23:1, SM d18:2/23:0, SM d17:1/24:1, the sum of the 3 isobaric sphingomyelin species SM d18:1/23:1, SM d18:2/23:0, and SM d17:1/24:1

[a]Wilcoxon rank sum test, [b]Pearson's chi-square test, [c]Mantel-Haenszel chi-square.

*Imputed using the median, 31%, of the normal range 20−40%

† Median (Interquartile Range)

The correlation of the CLP biomarkers TAG, PC, and SM were most correlated with the clinical characteristics triglycerides (r = 0.531, p < 0.001), total cholesterol (r = 0.431, p < 0.001), and LDL (r=, 0.502, p < 0.001), respectively (**Supplemental Fig. 3**).

Discussion

We found that a risk score based on a novel panel of three metabolite-based biomarkers plus NT-proBNP outperformed commonly used traditional prognostic models for predicting cardiovascular mortality in elderly ambulatory CHF patients. We first measured the association of each risk score with the outcome, followed by discrimination analysis, then cluster analysis to support the discrimination findings, and finally correlation analysis of the individual CLP biomarkers with the clinical characteristics. In our study cohort, CLP score, showed the best discrimination compared to the other 3 scores. This indicates that the biomarker information included in the CLP score could more precisely classify high risk CHF patients than the information included in the 3 other risk scores. On the other hand, the biomarker information from the CLP is not as easily attainable and no convenient calculator exists yet, as these findings should first be validated in larger cohorts. Nevertheless, the other risk scores may be improved with the addition of common biomarkers in their score calculation. For instance, NT-proBNP is a well-established biomarker that is known to be associated with ventricular wall stress [43] and is considered the gold-standard biomarker in CHF diagnosis and prognosis [44]. None of the other prognostic scores included NT-proBNP in their risk score calculation.

We performed cluster analysis to assess how well the risk scores could partition subjects into different risk groups, blinded to the study outcome. A strength of this approach is that clusters could define relevant groups of patients and could mitigate the problems of multicollinearity while determining if the predictive variables are useful in separating these groups. In our study, patients within each cluster varied

considerably along measures of age, comorbidities, laboratory parameters, as well as the prognostic scores. Several prior studies have used similar clustering methods to identify clinically relevant patient subgroups for CHF [41, 42], but we are not aware of previous studies using clustering methods to compare a novel biomarker score to other conventional prognostic scores for CHF.

The combination of the CLP's metabolomic features with NT-proBNP into a risk score may help overcome limitations of using only traditional clinical risk factors. Furthermore, application of a single biomarker such as NT-proBNP for outcome prediction is limited by insufficient specificity (low predictive value or high false positive rate) [45, 46]. Recently, it was reported that the CLP added incremental prognostic value to NT-proBNP in predicting 4-year cardiovascular mortality [29]. We used the same method to calculate the CLP score for this study, as we were not interested in the CLP's incremental value to NT-proBNP, rather the comparison of the CLP score to other established prognostic score. A recent meta-analysis of 18 metabolomic prognostic biomarker studies for CVD found those which incorporated a selection of metabolites into a score (n = 5 studies) had the best prognostic performance rather than using the individual biomarker values [15]. Another systematic review [19] reported 6 studies [20–25] developed a metabolite-based score to predict CVD risk with each score composed between 4 and 16 biomarkers.

In addition to improving risk prediction, developing a biomarker-based risk score could also improve our understanding of the pathophysiology and biological mechanisms involved in CHF. The CLP metabolites can be grouped into three different lipid subclasses, sphingomyelin (SM) phosphatidylcholine (PC), and triglycerides (TAG), which have previously been found to be associated with cardiomyocyte stress/apoptosis [47], intestinal microbial metabolism/inflammation [18], and coronary artery disease [48], respectively. The findings in this study showed that the individual CLP metabolites were correlated with traditional lipid measures; the sphingomyelin metabolite was correlated with LDL, phosphatidylcholine with total cholesterol, and triacylglycerol with total triglycerides. Future studies are needed to establish whether the CLP biomarkers are characteristic of altered biological pathways or are representative of CHF compensation mechanisms.

Study Limitations

The homogeneity of this cohort, elderly patients with stable CHF, may have had an impact on the performance of the prognostic scores. The SHFM score may have been affected by the imputation of lymphocytes % as well as the lack of patients taking allopurinol. The FRS was originally developed for coronary artery disease and not CHF, which may explain its poor performance on this cohort. The CLP biomarker kit was developed for routine use in the clinic; however, it is still a research biomarker panel pending regulatory approval and must be sent to a lab equipped with MS technology. Our findings are limited to this population of elderly CHF patients and future validation studies should be performed to include a more heterogenous cohort such as younger, more women, and early/ asymptomatic patients. Other common biomarkers such as ST2, hs-CRP, and troponins should be compared to the CLP as they are more readily available and do not require samples be sent to a specialized lab. The CLP panel was

originally developed as a diagnostic and early detection biomarker for HFrEF, and clinicians and researchers should be cautious when using it as a prognostic tool, as these are still preliminary findings.

Conclusion

In a cohort of ambulatory CHF patients, we have shown that the prognostic scores included in this study were useful in stratifying patients into risk clusters. Our findings demonstrate that the CLP risk score comprising a panel of 3 novel metabolomic biomarkers and NT-proBNP, could improve the prediction of cardiovascular mortality over traditional prognostic scores. In the future, a broader array of biomarkers should be integrated into a more comprehensive risk score that may improve discrimination potential and risk stratification and the CLP offers a promise. The CLP score is a step in the direction of providing a more precise decision support tool to assist clinicians and patients in managing their CHF treatment.

Methods

Study Population

This study used a sub-cohort randomly selected from the Cardiac Insufficiency Bisoprolol Study in Elderly (CIBIS-ELD) trial, a multi-center, randomized, double-blind trial with ≥ 65-year-old patients being treated for CHF. The original study design and results of the CIBIS-ELD trial have been published previously [30, 31]. Briefly, patients with CHF were randomized in a 1:1 fashion to receive two different beta-blockers, either bisoprolol or carvedilol, and up titrated every fortnight for 12 weeks and then followed at 10 years. From this source cohort (n = 883), there were n = 589 with available blood samples. Patients were randomly selected and included in the analysis only if they passed quality control [32, 33] resulting in a final set of 280 cases. The ethics committees of all participating centers approved the study protocol, and informed consent was signed by all participants prior to study participation. The ethics committees include: Germany: Ethikkommission der Charité on the 13th June 2007 (Amendment 5) (ref: 125/2004), Serbia: Ethics board of the University Hospital on the 31st March 2006 (ref: 6108/18), Slovenia: The national medical ethics committee on the 2nd July 2007 (ref: KME 188/06/07). The investigation conformed to the principles outlined in the Declaration of Helsinki [34].

Biomarker Measurements

Targeted metabolite profiling of the serum samples which passed quality control was performed at a specialized metabolomics lab using a commercially available kit. The kit uses a protocol based on a 1-phase extraction of the blood samples followed by gas chromatography mass spectrometry (GC-MS) (Agilent 6890 GC coupled to an Agilent 5973 MS-System) and liquid chromatography tandem-mass spectrometry (LC-MS/MS) (Agilent 1100 HPLC-System coupled to an Applied Biosystems API4000 MS/MS-System) analysis as previously described [28]. The analytical protocol was designed for routine measurement in the clinical practice setting; however, it is currently only available in specialized labs equipped with MS technology. The samples were stored at − 80 °C and transferred on dry ice prior to

analysis. The three CLP metabolomic features and NT-proBNP measurements, were generated at baseline, only for the previously mentioned samples (n = 280). NT-proBNP was a measured using commercially available assays (Elecsys, Roche Diagnostics).

Calculating Prognostic Scores

Each prognostic score was calculated using the corresponding method proposed by the original authors (3–5). For calculating the SHFM score, % lymphocyte was missing, and the median (31%) of the normal range (20–40%) was imputed for all subjects. The CLP risk score was calculated as the count of biomarkers above the Youden index cut-off [35]. The Youden's index calculates each biomarker's optimal cut-off from the Cox regression. There were 4 cut-off values, since four biomarkers are included in the score: three from the CLP and NT-proBNP. Based on the cut-off, a value of 1 or 0 was assigned if the biomarker value was above/below the cut-off value, or in the direction of greater risk, then all 4 values were summed to generate the final score for each subject. The score ranged from 0 to 4, higher scores indicating higher risk. The primary outcome, cardiovascular death, was defined as death by myocardial infarction, non-responding arrhythmia, asystole, chronic pump failure, or other cardiac cause and verified by a blinded committee of cardiologists.

Statistical Analysis

Power and Sample Size

The sample size was adjusted for an anticipated event rate of 0.34. A Cox regression of the log hazard ratio on a covariate with a standard deviation of 1.5 based on a sample of 257 observations achieves 80% power at a 0.050 significance level to detect a regression coefficient equal to 0.2. Adjusting for an anticipated loss to follow up rate of 10%, the final sample size would be 283.

Discrimination Analysis

Categorical variables were expressed as number (%) and continuous variables were expressed as mean (*SD*). Univariate Cox Regression was performed on each of the prognostic scores, and hazard ratios and 95% confidence intervals were calculated to assess their relationship with the outcome. For the survival models, integrated area under the receiver operator curves (IAUC) and Harrell's c statistic [36] calculated to assess the discrimination of each score in predicting the outcome. Hypothesis testing of the change in discrimination was performed by calculating the differences in Uno's concordance statistics [37]. The IAUC curves are computed as a weighted average of the AUC values at all the event times, with the weights as the jumps of the Kaplan-Meier estimate of the survivor function

Cluster Analysis

Hierarchical cluster analysis was performed using Ward's minimum variance method to assess each prognostic score's ability to separate cases into risk groups. The distance between two clusters is the ANOVA sum of squares between the clusters summed over all variables. Only the 4 risk scores used as

the input variables for the cluster analysis to examine how well they classified patients into a low, moderate, and high-risk of cardiovascular mortality. Data was standardized (mean of 0 and SD of 1), to perform clustering. The clinical characteristics and scores were compared across risk clusters. Comparisons among continuous variables were performed using Wilcoxon rank sum test; and Pearson's chi-square test (or Fisher's exact test) or Mantel-Haenszel Chi-square test for categorical and ordinal data, respectively. Kaplan-Meier curves were used to compare the survival distribution across risk clusters. Survival time was calculated from baseline until cardiovascular death or censoring at 10 year follow up.

Correlation Analysis

To investigate potential relationships between the CLP biomarker values and common clinical parameters, Pearson's correlation coefficients were calculated, significant at the 0.01 level (2-tailed). All analyses were performed using SAS statistical software version 9.4 (SAS Institute, Inc., Cary, North Carolina) and JMP pro software version 14, and R software version 3.6.1 [38–40].

Declarations

Germany) supported the presented analysis by a research grant and performed the measurements without any rights to influence design, data collection, data analysis, and interpretation, or publication of the current manuscript.

References

1. MEMBERS, W. G. *et al.* Heart disease and stroke statistics2012 update: a report from the American Heart Association. *Circulation.* **125** (1), e2 (2012).

2. Bui, A. L., Horwich, T. B. & Fonarow, G. C. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol.* **8** (1), 30–41 (2011).

3. Levy, W. C. *et al.* The Seattle Heart Failure Model: prediction of survival in heart failure. *Circulation.* **113** (11), 1424–1433 (2006).

4. Pocock, S. J. *et al.* Predicting survival in heart failure: a risk score based on 39 372 patients from 30 studies. *Eur Heart J.* **34** (19), 1404–1413 (2013).

5. Allen, L. A. *et al.* "Use of risk models to predict death in the next year among individual ambulatory patients with heart failure." JAMA cardiology 2.4 (2017):435–441.),

6. Sartipy, U. *et al.* "Predicting survival in heart failure: validation of the MAGGIC heart failure risk score in 51 043 patients from the S wedish Heart F ailure R egistry.". *European journal of heart failure.* **16** (2), 173–179 (2014).

7. D'Agostino, R. B. *et al.* General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* **117** (6), 743–753 (2008).

8. Howlett, J. G. "Should we perform a heart failure risk score?." (2013):4–5.

9. Aaronson, K. D. & Cowger, J. Heart failure prognostic models: why bother?. *Circ Heart Fail.* **5** (1), 6–9 (2012).

10. Steyerberg, E. W. *et al.* Prognosis Research Strategy (PROGRESS) 3: prognostic model research. *PLoS Med.* **10** (2), e1001381 (2013).

11. Allen, L. A. *et al.* Use of Risk Models to Predict Death in the Next Year Among Individual Ambulatory Patients With Heart Failure. *JAMA Cardiol.* **2** (4), 435–441 (2017).

12. Albert, C. L. & Tang, W. H. W. Metabolic Biomarkers in Heart Failure. *Heart Fail Clin.* **14** (1), 109–118 (2018).

13. Gupte, A. A. *et al.* Mechanical unloading promotes myocardial energy recovery in human heart failure. *Circ Cardiovasc Genet.* **7** (3), 266–276 (2014).

14. Bedi, K. C. Jr. *et al.* Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation.* **133** (8), 706–716 (2016).

15. McGranaghan, P. *et al.* Predictive value of metabolomic biomarkers for cardiovascular disease risk: a systematic review and meta-analysis. *Biomarkers.* **25** (2), 101–111 (2020).

16. Cheng, M. L. *et al.* Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. *J Am Coll Cardiol.* **65** (15), 1509–1520 (2015).

17. Ahmad, T. *et al.* Prognostic Implications of Long-Chain Acylcarnitines in Heart Failure and Reversibility With Mechanical Circulatory Support. *J Am Coll Cardiol.* **67** (3), 291–299 (2016).

18. Tang, W. H. *et al.* Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *J Am Coll Cardiol.* **64** (18), 1908–1914 (2014).

19. Ruiz-Canela, M. *et al.* Comprehensive Metabolomic Profiling and Incident Cardiovascular Disease: A Systematic Review.J Am Heart Assoc. 2017;6(10).

20. Shah, S. H. *et al.* Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circ Cardiovasc Genet.* **3** (2), 207–214 (2010).

21. Shah, S. H. *et al.* Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease. *Am Heart J.* **163** (5), 844–850 (2012). e1.

22. Rizza, S. *et al.* Metabolomics signature improves the prediction of cardiovascular events in elderly subjects. *Atherosclerosis.* **232** (2), 260–264 (2014).

23. Vaarhorst, A. A. *et al.* A metabolomic profile is associated with the risk of incident coronary heart disease. *Am Heart J.* **168** (1), 45–52 (2014). e7.

24. Kume, S. *et al.* Predictive properties of plasma amino acid profile for cardiovascular disease in patients with type 2 diabetes. *PLoS One.* **9** (6), e101219 (2014).

25. Zheng, Y. *et al.* Associations between metabolomic compounds and incident heart failure among African Americans: the ARIC Study. *Am J Epidemiol.* **178** (4), 534–542 (2013).

26. Lanfear, D. E. *et al.* Targeted Metabolomic Profiling of Plasma and Survival in Heart Failure Patients. *JACC Heart Fail.* **5** (11), 823–832 (2017).

27. Wurtz, P. *et al.* Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation.* **131** (9), 774–785 (2015).

28. Mueller-Hennessen, M. *et al.* A Novel Lipid Biomarker Panel for the Detection of Heart Failure with Reduced Ejection Fraction. *Clin Chem.* **63** (1), 267–277 (2017).

29. McGranaghan, P. *et al.* Incremental prognostic value of a novel metabolite-based biomarker score in congestive heart failure patients.ESC Heart Failure. 2020.

30. Dungen, H. D. *et al.* Bisoprolol vs. carvedilol in elderly patients with heart failure: rationale and design of the CIBIS-ELD trial. *Clin Res Cardiol.* **97** (9), 578–586 (2008).

31. Dungen, H. D. *et al.* Titration to target dose of bisoprolol vs. carvedilol in elderly patients with heart failure: the CIBIS-ELD trial. *Eur J Heart Fail.* **13** (6), 670–680 (2011).

32. Kamlage, B. *et al.* Quality markers addressing preanalytical variations of blood and plasma processing identified by broad and targeted metabolite profiling. Clinical chemistry. 2014 Feb 1;60(2):399–412.

33. Kamlage, B. *et al.* inventors; Metanomics Health GmbH, assignee. Means and Methods for Assessing the Quality of a Biological Sample. United States patent application US 14/767,059. 2016 Jan 7.

34. Rickham, P. P. Human experimentation. Code of ethics of the world medical association. *Declaration of Helsinki. British medical journal.* **2** (5402), 177–177 (1964 Jul).

35. Youden, W. J. Index for rating diagnostic tests. *Cancer.* **3** (1), 32–35 (1950).

36. Harrell, F. E., Califf, R. M., Pryor, D. B., Lee, K. L. & Rosati, R. A. Evaluating the yield of medical tests. Jama. 1982 May 14;247(18):2543-6.

37. Uno, H., Cai, T., Pencina, M. J., D'Agostino, R. B. & Wei, L. J. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Statistics in medicine.* **10** (10), 1105–1117 (2011 May).

38. SAS Institute Inc. 2015. Base SAS 9.4 Procedures Guide. 5th ed. Cary, NC: SAS Institute Inc. Available: http://support.sas.com.

39. JMP®, V. 14. SAS Institute Inc., Cary, NC, 1989–2019.

40. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

41. Ahmad, T. *et al.* Clinical implications of chronic heart failure phenotypes defined by cluster analysis. *J Am Coll Cardiol.* **64** (17), 1765–1774 (2014).

42. Scherzer, R. *et al.* Association of Biomarker Clusters With Cardiac Phenotypes and Mortality in Patients With HIV Infection. *Circ Heart Fail.* **11** (4), e004312 (2018).

43. Weber, M. & Hamm, C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart.* **92** (6), 843–849 (2006).

44. McKie, P. M. & Burnett, J. C. Jr. NT-proBNP: The Gold Standard Biomarker in Heart Failure. *J Am Coll Cardiol.* **68** (22), 2437–2439 (2016).

45. Jensen, J., Ma, L. P., Bjurman, C., Hammarsten, O. & Fu, M. L. Prognostic values of NTpro BNP/BNP ratio in comparison with NTpro BNP or BNP alone in elderly patients with chronic heart failure in a 2-year follow up. *Int J Cardiol.* **155** (1), 1–5 (2012).

46. Maisel, A. *et al.* State of the art: using natriuretic peptide levels in clinical practice. *Eur J Heart Fail.* **10** (9), 824–839 (2008).

47. Borodzicz, S., Czarzasta, K., Kuch, M. & Cudnoch-Jedrzejewska, A. Sphingolipids in cardiovascular diseases and metabolic disorders. *Lipids Health Dis.* **14**, 55 (2015).

48. Do, R. *et al.* Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet.* **45** (11), 1345–1352 (2013).
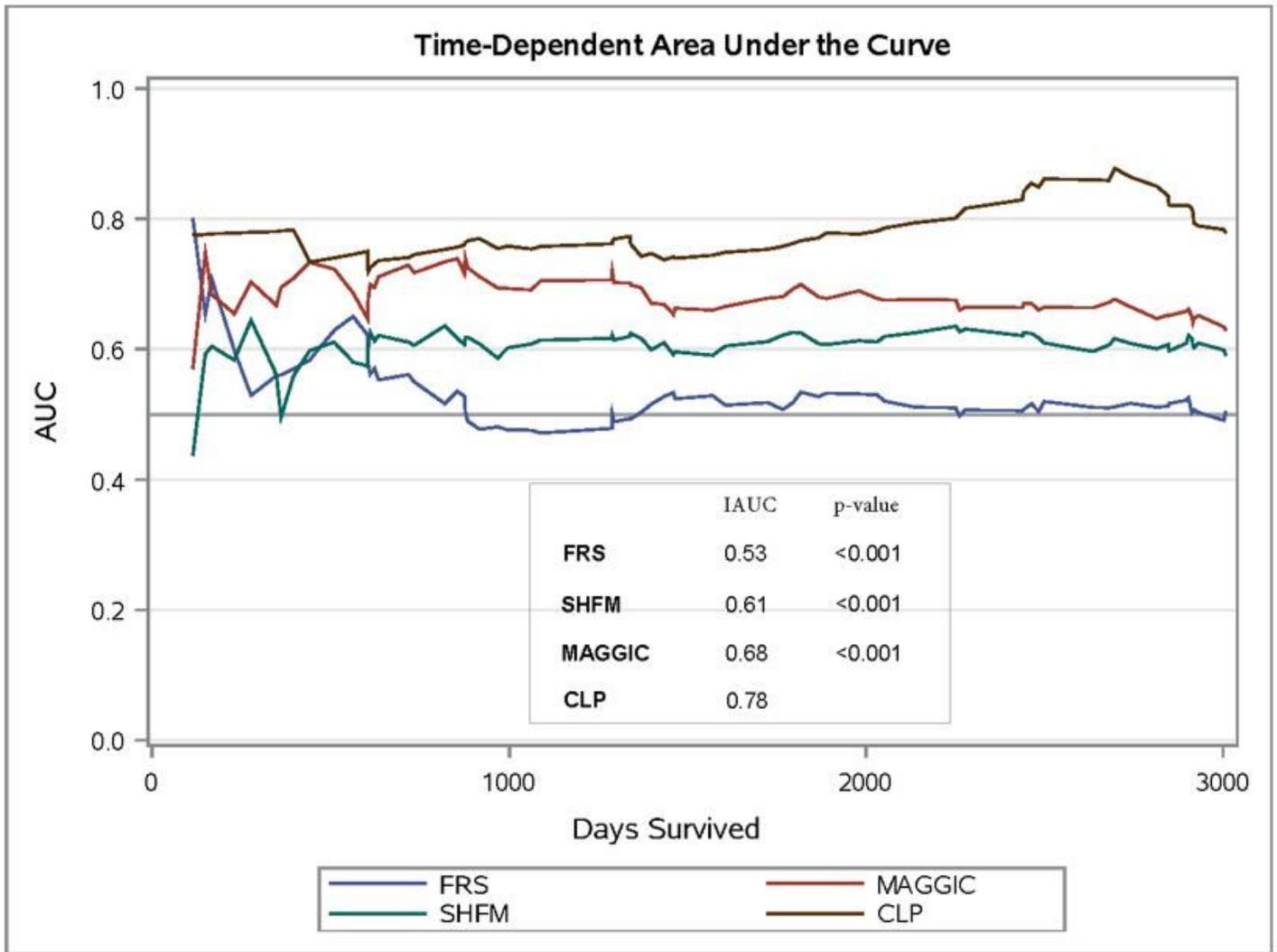
Figures

Figure 1

Discrimination Performance for each Prognostic Score for 10-year Cardiovascular Mortality Caption: Integrated area under the curve (IAUC) for: SHFM (Seattle Heart Failure Model), FRS (Framingham Risk Score), and MAGGIC (Meta-analysis Global Group in Chronic Heart Failure), and Cardiac Lipid Panel Risk Score (CLP). Total subjects, n=280; total events, n=95. P-values were calculated from the differences in Uno's concordance statistic in comparison to the CLP score.
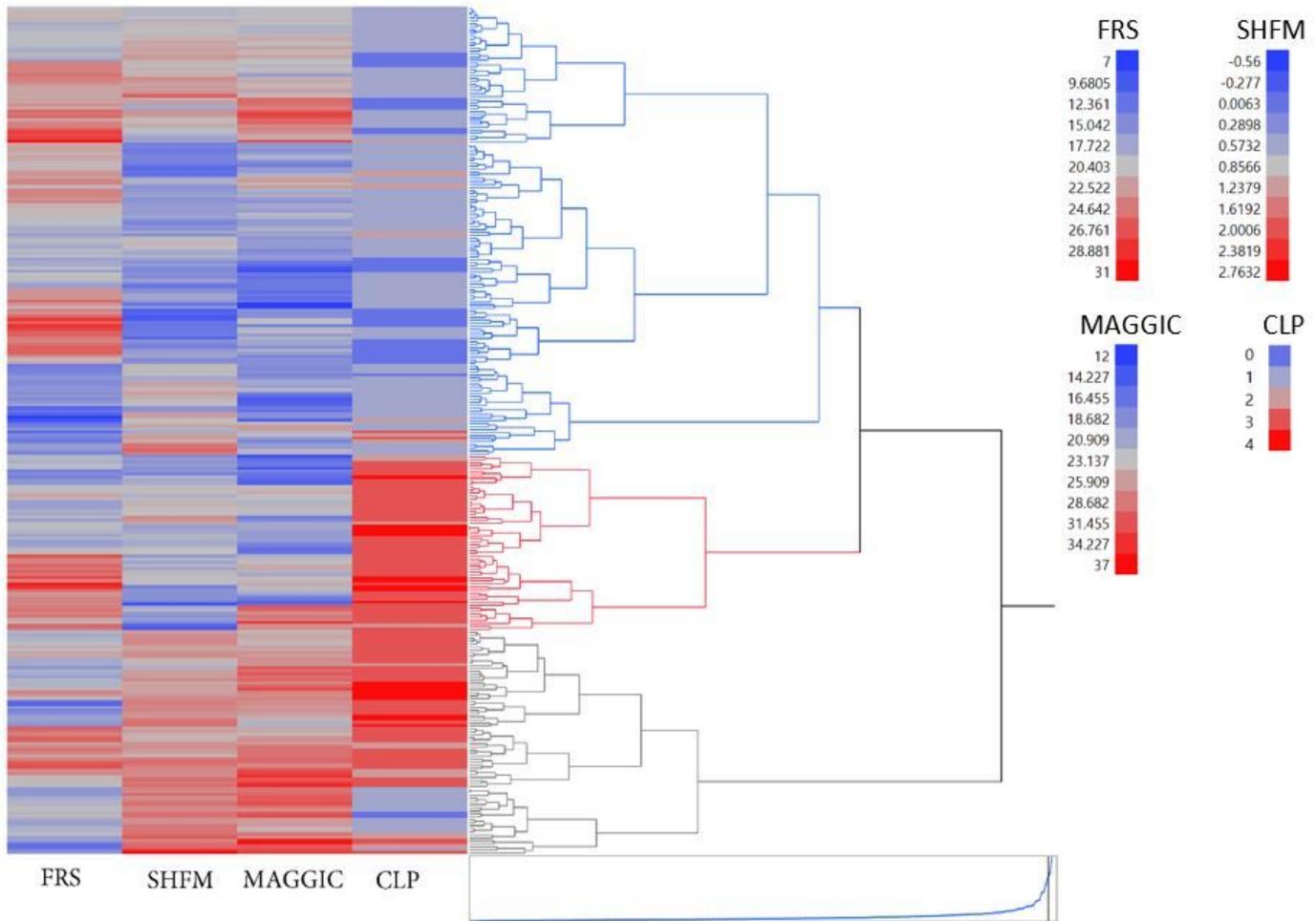
Figure 2

Hierarchal Cluster Dendrogram of Three Risk Clusters Caption: Assignment of patients into risk clusters based on the prognostic scores. The clustering process can be viewed by reading the dendrogram from left to right. Each step consists of combining the two closest clusters into a single cluster. The joining of clusters is indicated by horizontal lines that are connected by vertical lines. The horizontal position of the vertical line represents the distance between the two clusters that are most recently joined to form the specified number of clusters. The prognostic scores used for clustering were: SHFM (Seattle Heart Failure Model), FRS (Framingham Risk Score), and MAGGIC (Meta-analysis Global Group in Chronic Heart Failure), and Cardiac Lipid Panel Risk Score (CLP). Each prognostic score was standardized to the same scale (mean=0; SD=1). Ward's minimum variance method was used for clustering. Blue dendrogram indicates the cluster 1 (low risk), n=148; Grey dendrogram indicates cluster 2 (moderate risk), n= 74. Red dendrogram indicates cluster 3 (high risk), n= 58; Total subjects, n=280

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure2submitSR.docx
- SupplementalTable1submitSR.docx